A Phylogenetic Assessment of the Affinities of the Farasan Islands Flora

A thesis submitted by

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

The Southern Red Sea and its continental archipelagos are a global biodiversity hotspot. The Farasan Islands (Saudi Arabia) form the second-largest archipelago in the Red Sea and have the highest combined marine and terrestrial biological diversity in the basin, which is under threat of loss due to human activities and climate change. The floral diversity of the islands is of particular interest as it represents a transition point between the floristically rich North East Africa and western Asia. This includes well-preserved populations of regionally and nationally rare species, such as *Avicennia marina, Rhizophora mucronata* and *Euphorbia collenetteae.* Very few investigations have sought to explain the floristic relationships between the Farasan Islands and the mainlands. Scientific approaches have involved the comparison with regional floras and inferring the taxonomic distribution patterns of present taxa in the Farasan Islands. The analyses presented in this study used phylogenies and molecular dating to explore the Farasan flora's phylogenetic affinities with the intention of discovering the timescale of colonisation events.

This study is the most comprehensive phylogenetic study to be carried out on the Farasan Islands flora to date. It included ten plant angiosperm genera, including 36 species, which represent the islands' main habitat types. Molecular work included the amplification and sequencing of 20 conventional DNA markers of chloroplasts and nuclear genomes. These data were obtained from 130 samples belong to the Farasan Islands and mainland species of *Avicennia* L., *Convolvulus* L., *Cyperus* L.*, Euphorbia* L.*, Ficus* L., *Heliotropium* L., *Indigofera* L., *Rhizophora* L.*, Suaeda* Forssk. ex J.F.Gmel. and *Tetraena* Maxim*.* The originated data was then incorporated into the worldwide phylogenies of the targeted genera, including a total of 1000 accessions downloaded from the GenBank for ingroups and outgroups.

The phylogenetic evidence showed that the Farasan Islands flora is recent, part of the Red Sea flora, and most of the species are probably more closely tied to the large species pool of adjacent Arabian flora. The islands' populations are not isolated, and the very close phylogenetic links to the mainland populations suggest they might be acting as one population in an integrated area. This creates an important implication for the conservation of the islands' biodiversity in that the planning for the conservation of Farasan endangered lineages needs to be integrated with conservation action on the mainland. The islands' flora appears to have developed through a range of dispersal routes: overland dispersal via land bridges during the Pleistocene and transported by birds, sea currents, winds, and humans.

The significance of the Farasan Islands flora in Saudi Arabia is not in terms of its endemism, which is low, but in being a concentrated pool of individual species that are rare elsewhere in the Arabian Peninsula and the Red Sea area. The conservation assessment of these species according to the IUCN Red List Categories and Criteria reveals a high risk of extinction and, thus, they are of conservation concern both regionally and nationally. These results place a significant conservation responsibility on Farasan Protected Area administrators to include them in conservation policies that should be broadened to include plants, animals and land use.

The approach adopted in this study of combining molecular phylogenies and IUCN Red List assessments provides a complete framework for the identification of conservation priorities for the Farasan Islands flora and stresses the importance of efficient coordination and political cooperation between the Red Sea countries for the protection of the floral diversity of the Red Sea Basin.

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CHAPTER 1: General Introduction

1.1 Introduction

Islands have sparked scientists' attention since Darwin and Wallace's works on the Galápagos and the Malay Archipelagos, respectively (Darwin, 1909; Wallace, 1876). The Red Sea, part of the tropical Indo-Pacific Ocean (Bruckner et al 2012), semi-enclosed separating the eastern coast of North Africa from the western Arabian Peninsula, has over a thousand islands (Rasul & Stewart, 2015). The two largest groups lie in the south: the Farasan Islands (Saudi Arabia) in the east and the more than 350 Dahlak islands (Eritrea) in the west (Rasul & Stewart, 2015). The southern parts of the Red Sea Basin are a unique global hotspot of marine and terrestrial biodiversity and a global priority of *in situ* conservation (Permanent Delegation of the Kingdom of Saudi Arabia to UNESCO, 2019). Their islands form part of Conservation International's Horn of Africa Biodiversity Hotspot (Mittermeier et al., 2004).

The Farasan Islands have the highest combined marine and terrestrial biological diversity of any island in the Red Sea (Permanent Delegation of the Kingdom of Saudi Arabia to UNESCO, 2019). These islands are of international importance (Thouless, 1991), providing safe migratory routes for certain birds of Siberian and Asian origin (Thomas et al., 2010) and comprising breeding colonies of many seabirds and turtles. Major fisheries are based on the surrounding coral banks. The island flora includes several nationally and regionally rare plant species and a good example of mangrove stands in the Red Sea, which are threatened both regionally and nationally (Hall et al., 2010). The islands are also inhabited by an endemic subspecies of mountain gazelle, the Farasan gazelle *Gazella gazella farasani,* and the endemic snake, the Sarso Island racer *Coluber insulanus* (Thouless, 1991; Masseti, 2014).

The Farasan Archipelago is a designated terrestrial and marine reserve (Abuzinada, 2003; Gladstone et al., 2003). It is also listed as an Important Plant Area (**IPA**) in the Arabian Peninsula (Hall et al., 2010) and an Important Bird Area (**IBA**) (Evans, 1994). It also considered a site of international significance as a potential Wetlands of International Importance (RAMSAR wetland) (Scott, 1995).

1.2 Farasan Islands flora

The Farasan Islands are a large near-shore archipelago (600 km²) located between the Jazan coast in Saudi Arabia (40 km) and the Dahlak Islands in Africa (~200 km) (16°20'to 17°20' N and 41°24'to 42°26' E) (Figure 1-1) (Bruckner et al., 2012). The archipelago consists of more than 170 low-lying (0–70 m in altitude) islands and islets that vary in area size from hundreds of kilometres to a few metres. Seven islands are more than 10 km²: Farasan Alkabir (381 km²), Sajid (149 km²), Ad Disan (35.7 km²), Zifaf (33.2 km²), Saswah (19.7 km²), Qummah (15.2 km²) and Dumsuk (12 km²) (Bruckner et al., 2012).

The surface of the Farasan Islands is flat and well-drained, approximately 60% of which is a subtropical desert of fossil limestone. The remainder is divided roughly equally among sabkhah, silty sand and rocky outcrops 10–70 m high (Bruckner et al., 2012). The shore rises gently to be followed by salt marshes and sandy plains, or it is marked by small cliffs emerging from the coralline plateau and covered by coral rubble (Al Mutairi & Al-Shami, 2014).

Figure 1-1. Map of the Farasan Islands. This figure was created using ArcGIS Online (Esri, "Topography") and edited using QGIS 2.10.1-Pisa (QGIS Development Team, 2019).

The climate in the Farasan Archipelago is arid and subtropical with a long hot season from April to October and short mild one from November to March. Since no meteorological station exists on the Farasan islands, all available climatic data are from the nearest one in Jazan city (40 km at 7 m altitude). The highest daily temperature recorded in August around 40.5 °C and the minimum daily temperature is 18.8 °C in January (Ibrahim, 2008). The mean temperature ranges from 26 °C in winter, 30 °C in spring to 33 °C in summer (Figure 1-2, A) (Alfarhan et al., 2005). The humidity is high all the year round, the mean humidity ranges between 61% in July to 77% in February. Maximum humidity is reached in 97% in January while the minimum is 22% in February (Figure 1-2, B) (Ibrahim, 2008). At night, the mean relative humidity may increase to 100% resulting a formation of dew on the plant leaves and hard surfaces so that dew condensation is important for vegetation where it contributes in soil moisture (Alwelaie et al., 1993; Hall et al., 2010). Rainfall is unpredictable in the southern part of the Red Sea; the mean annual rainfall is 150.4 mm per year (Figure 1-2, C) with an erratic distribution year to year (Alwelaie et al., 1993; Ibrahim, 2008). Winds can be constant and strong (Hall et al., 2010; Permanent Delegation of the Kingdom of Saudi Arabia to UNESCO, 2019).

(A)

(B)

(C)
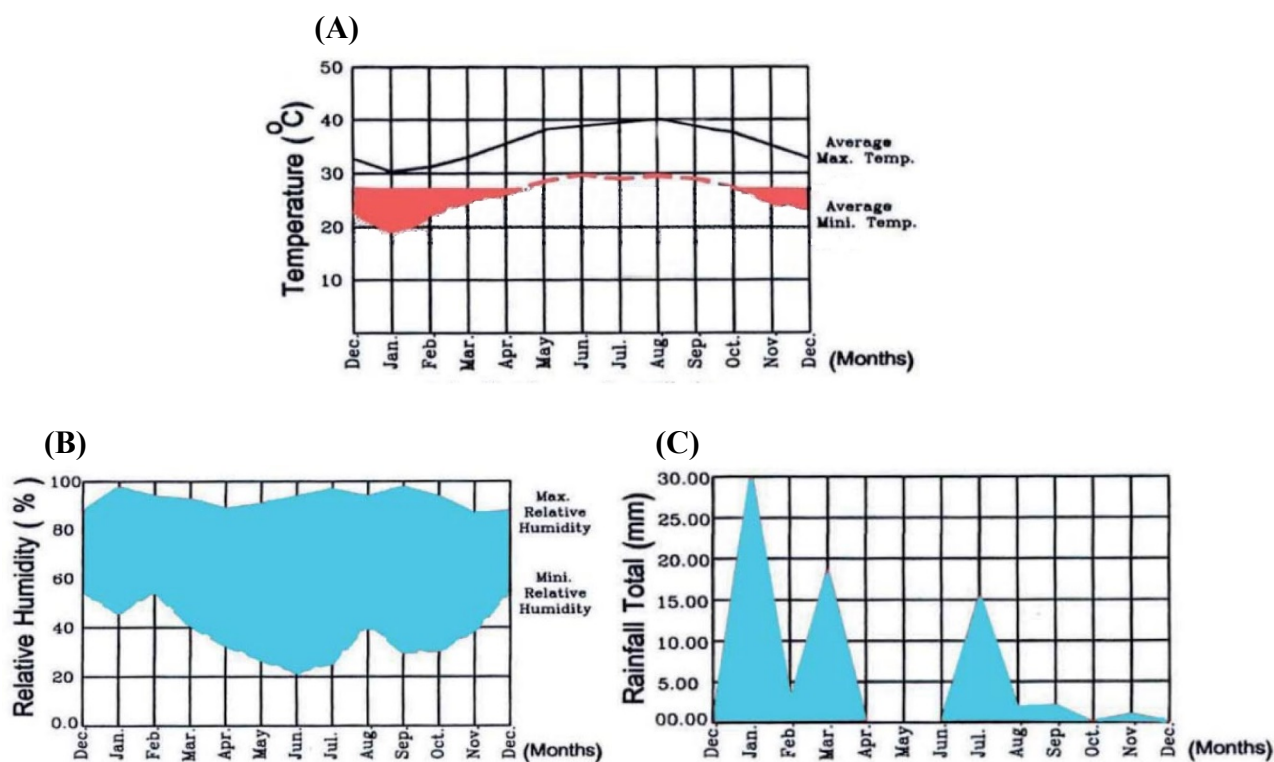
Figure 1-2 Jazan Climate Graphs: **(A)** temperature, **(B)** humidity and **(C)** rainfall in 2002 (modified from Ibrahim (2008)).

The unique location of the Farasan Archipelago between floristically rich Northeast Africa and western Asia, and the existence of a wide range of habitats, namely mangroves, salt marshes, sand formations, wadi channels and coral rocky habitats has resulted in significant floral diversity (Al Mutairi et al., 2012a). Tropical mangroves (*Avicennia marina* and *Rhizophora mucronata*), Nubo-Sindian endemics (e.g., *Vachellia flava* [syn. *Acacia ehrenbergiana*], *Salvadora persica*, *Commiphora gileadensis*, and *Ziziphus spina-christi*), and Arabian regional subzone species (e.g., *Aeluropus logpoides, Arthrocnemum macrostachyum*, and *Capparis cartilaginea*) represent the dominant vegetation communities, which are a mix of East-African and Saharo-Arabian formations (Al Mutairi et al., 2012a).

Geo-morphological diversity, topographic heterogeneity and soil characteristics are mainly responsible for the diverse floral communities in the Farasan Islands (El-Demerdash, 1996; Al Mutairi et al., 2012a; Al Mutairi & Al-Shami, 2014). Shorelines along inlets and bays where the flow of seawater is not strong are dominated by the mangrove species *Avicennia marina* and *Rhizophora mucronata*. *Avicennia marina* is the predominant mangrove species, occurring in pure stands or mixed with *R. mucronata* in extensive patches (Alwelaie et al., 1993). Above the intertidal zone, the vegetation in sandy beaches is usually dominated by halophytes growing in wide or narrow bands, such as *Limonium axillare*, *Suaeda monoica*, *Halopeplis perfoliata*, *Tetraena* ssp., *Aeluropus lagopoides* and *Cressa cretica* (Alfarhan et al., 2005). Inland, vegetation cover is sparse except in ravines between fossil coral outcrops, which have the highest species richness and the greatest number of annuals (Al Mutairi et al., 2012a; Bruckner et al., 2012). The plant groups that dominate the inland are *Vachellia flava*, *Euphorbia collenetteae*, *Salvadora persica*, *Capparis cartilaginea, Indigofera oblongifolia*, *Commiphora gileadensis* and *Ziziphus spina-christi*.

Flora in the Farasan Archipelago comprise about 191 vascular plant species, representing 129 genera, and 53 families (Al Mutairi et al., 2012a). Endemism is very low. *Blepharis saudensis, Commiphora* aff. *kataf* and *Glosonema* sp. aff. *boveanum* are likely endemic to the Farasan Islands (Collenette, 1999; Al-Zahrani, 2010; Basahi & Masrahi, 2019). Eudicots comprise 79.8% of the total number of flora (153 species in 45 families), while the Monocots are represented by 38 species and 8 families. The family Poaceae is the most diverse, representing 14.4% of the total flora (27 spp.), followed by Convolvulaceae (6.9%; 13 spp.), Fabaceae (6.4%; 12 spp.) and Capparaceae (5.9%; 11 spp.) (Al Mutairi et al., 2012a).

The floristic components in the Farasan Islands are not as rich as the flora of oceanic or other continental islands (Table 1-1). Small, low-lying islands often have lower levels of terrestrial and freshwater biodiversity compared with large islands with higher elevation (Thaman, 2009). Major factors such as island size and geomorphology (linked to elevation) could explain higher floristic richness found in other continental islands of relatively similar age to the Farasan Islands; such as Taiwan (Chiang & Schaal, 2006) and the East Aegean Archipelago (Panitsa et al., 2010).

Table 1-1. Floristic richness and endemism of vascular plant species in selected islands and archipelagos. All figures are from Stuessy (2009) except the Farasan data and the marked figures. The marked figures are from [a]Whittaker & Fernández-Palacios (2007); [b]Banfield et al. (2011); [c]Jiang et al. (2019); [d]Triantis & Mylonas (2009); [e]Moody (2009); [f]Panitsa et al. (2010).

Island or archipelago	No. of islands	Area (km^2)	Distance from mainland (km)	Highest Elevation (m)	Age (millions of years)	No. of species	No. of endemics
Oceanic							
Hawaii	8	16,885	3,660	4,250	5	1,180[a]	906[a]
Canary	7	7,601	100	3,710	21	1,300[a]	570[a]
Galápagos	16	7,847	930	1,707	5	529	133
Continental							
Socotra	4[b]	3,791[b]	225[b]	1,550[b]	18[b]	835[b]	308[b]
California Channel Islands	8[e]	905.5[e]	20[e]	753[e]	c. 5[e]	1000[e]	100[e]
Taiwan	1	3,5800	130	3,950	5	3,600[c]	724
East Aegean	20[f]	5,239.9[f]	1.2–49[f]	1,433[f]	5–2[d]	2,238[f]	302[f]
Farasan	170	600	40	70	c. 2	191	c.3

The Farasan Islands lie on the Arabian continental shelf, which off Jazan is less than 200 m deep and about 120 km wide (Bruckner et al., 2012). The bedrock of the Farasan Islands is formed of Pleistocene shallow marine reef limestones deformed by salt diapirs (Bruckner et al., 2012; Dabbagh et al., 1984; Bantan, 1999). Because the islands are close to mainland coasts, they resemble continental archipelagos (Masseti et al., 2015). During the Pleistocene Epoch (2.6 Mya–11.7 ka), approximately 50 glacial cycles were associated with the fluctuation of sea levels, which constantly altered the coastal habitat, influencing the landscape and biota throughout the world (Woodruff, 2010). The culmination of low sea levels was 115–130 m below the current level. The minimum level occurred during the Last Glacial Maximum (LGM)

c. 17–19 ka (Ludt & Rocha, 2015). Because of the shallow continental shelf around the Farasan Islands, the low sea levels (less than 100 m) associated with these cycles likely resulted in recurring land bridges connecting the Farasan Islands and the Arabian Peninsula (Figure 1-3), which may have provided suitable habitats for migrating species (Bailey et al., 2007; Ludt & Rocha, 2015; Masseti et al., 2015). From the LGM to the early Holocene, sea levels rose drastically and rapidly (122 m). The islands assumed approximately their present configuration in the mid-Holocene 6,000 years before the present (**BP**) (Bailey et al., 2007; Ludt & Rocha, 2015).
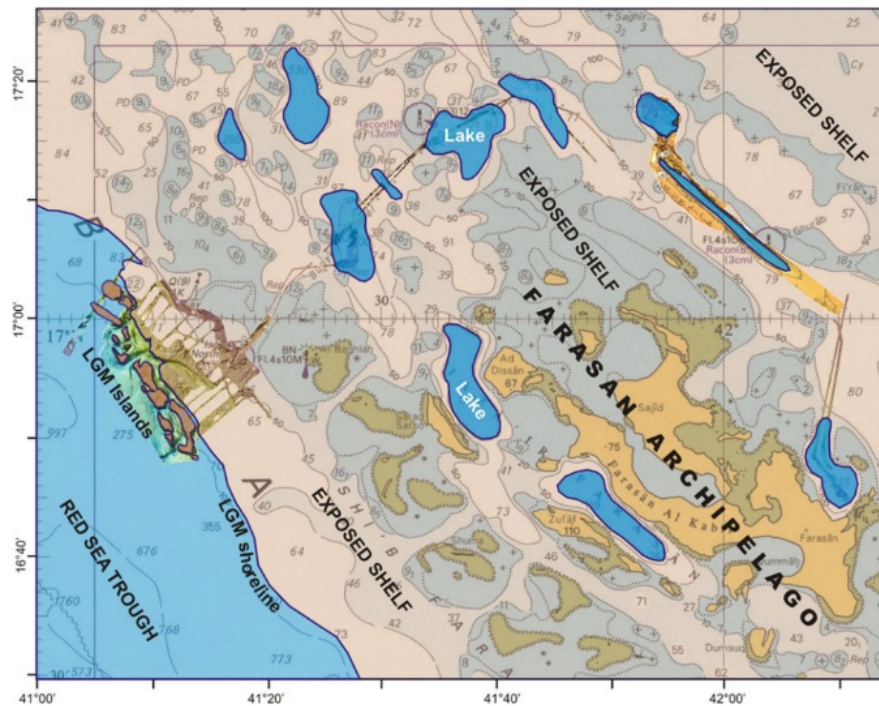
Figure 1-3. Paleogeographic reconstruction of the Farasan Islands shelf during the LGM. Note the islands (brown colour) at a short distance from the LGM shoreline. Reproduced from Sakellariou et al. (2019)

Because of the young age of the Farasan Islands (c. 2 Mya) and the proximity to the continental land mass, the islands' flora is believed to be recent and to have been highly influenced by nearby floras. A previous analysis of Farasan vegetation components compared with the vegetation on the nearest mainlands (the Arabian Peninsula and the north-eastern and northern countries of the African continent) are conducted by Thomas et al. (2010). The results showed that approximately 93% of Farasan species are present in the Tihama Region in southwestern mainland Saudi Arabia, 72.5% occur in Yemen, and 65–70% are found in Sudan, Somalia and Eritrea in northeast Africa (Thomas et al., 2010). Hassan and Al-Hemaid (1996) analysed the perennial flora of Farasan Alkabir Island on the basis of the species world-wide distribution, and concluded that the species appeared to belong to the main Arabian Peninsula flora, which

are mainly of African origin. Hassan and Al-Hemaid argued that plant migration to the islands was facilitated by the short distance between the two areas (40 km).

The Arabian Peninsula, because of its geological history and present location, shares its flora with the African continent in the west and southwest; the Asian Continent in the northeast, east and southeast; and the Mediterranean region in the northwest and north (Chaudhary, 1999). The Arabian Peninsula falls into three main phytogeographical regions (White & Léonard (1991): the Saharo-Sindian regional zone; the Somalia-Masai regional centre of endemism; and the Afromontane archipelago-like regional centre of endemism. The Saharo-Sindian regional zone is further divided into the Arabian regional subzone and the Nubo-Sindian local centre of endemism (Figure 1-4). A brief description of the evolution of the flora of the Arabian Peninsula are in Appendix 1-1.
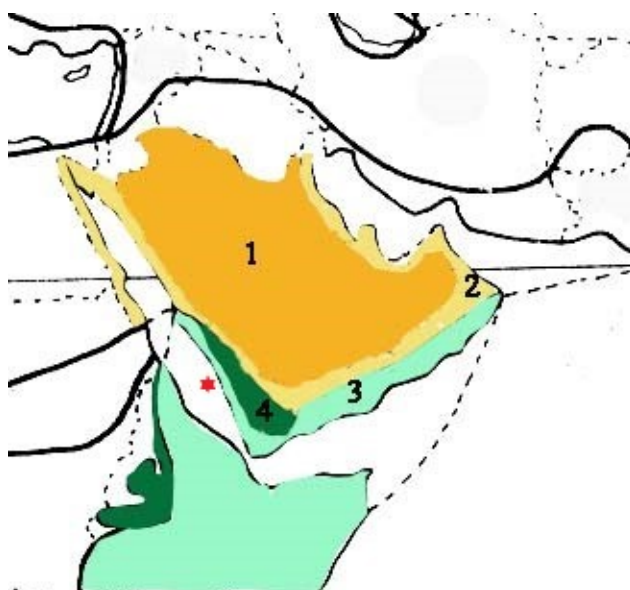
Figure 1-4. Phytogeographical regions in Arabian Peninsula.**(1)** Arabian regional sub zone, **(2)** Nubo-Sindian local centre of endemism, **(3)** Somali-Masai regional centre of endemism, **(4)** Afromontane archipelago-like regional centre of endemism. The Farasan Islands is marked with a red star (modified from White & Léonard (1991) as sited in Thomas, 2011).

Given that the Farasan Islands lie within the Somalia-Masai regional centre of endemism, the greatest association of the flora is within this region (34.7%). The Saharo-Sindian regional zone elements represent 45.44%, in which 27.17% are in the Nubo-Sindian local centre of endemism and 18.27% are in the Arabian regional subzone (Al Mutairi et al., 2012a). The tree component and the majority of the inland species have affinities for the Somalia-Masai phytogeographical region, while the littoral flora have greater affinities for Saharo-Arabian flora (Thomas et al., 2010).

The flora in the Farasan Islands has attracted attention over the past three decades, it has been well-studied compared with the flora in the Dahlak Archipelago, which is still unknown. Previous studies of the Farasan Islands flora mainly relied on quantitative descriptions of the vegetation (Alwelaie et al., 1993; Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Hall et al., 2010), its distribution and structure (El-Demerdash, 1996; Al Mutairi et al., 2012a; Thomas et al., 2010). Also focused on the effects of environmental factors on plant diversity (Al Mutairi & Al-Shami, 2014), the influences of island characteristics on the plant community structure (Al Mutairi et al., 2012b), and the origin and migration trends of perennial vegetation (Hassan & Al-Hemaid, 1996). Vegetation on the Farasan Islands was included in previous studies on the general vegetation of Saudi Arabia and the Jazan region (Chaudhary, 1999, 2001a, 2001b, 2001c, 2001d; Collenette, 1999; Alfarhan et al., 2005).

1.3 Molecular phylogeny and its role in the study of island floras

Islands are of particular importance in a biodiversity context as they have more than 35% of the world's vascular plant species including about 50 000 endemics (Bramwell, 2011). Such species diversity can be the result of all or any of the following factors: adaptive radiation, allopatric speciation, multiple successful colonisations from a continental land mass or neighbouring islands and increased speciation through the bottleneck and founder flush events (Emerson, 2002). The degree of floristic diversity on islands depends largely on their origin. Continental shelf islands, which are located on a continental shelf and separated from the continents by narrow, shallow waters, were mainly created by rising sea levels during the Holocene, isolating the species that were already on these islands from their mainland conspecifics (Whittaker & Fernández-Palacios, 2007). Continental fragments, which are ancient fragments of continental landmasses stranded in the oceans by plate tectonic processes, are separated from the continent by deep and wide waters (Whittaker & Fernández-Palacios, 2007). The long isolation of these islands has allowed both the persistence of some ancient lineages and the development of new species *in situ* (Whittaker & Fernández-Palacios, 2007). On oceanic islands, which originated in submarine volcanic activity, species composition depends mainly on the ability of plant dispersal, the colonisation of new emerged lands, and the subsequent enrichment by speciation (Whittaker & Fernández-Palacios, 2007). Understanding the origins of the islands' organisms can only be addressed with an accurate reconstruction of the phylogenetic relationships within a group of species (Emerson, 2002).

The phylogeny is the representation of the evolutionary history of a group of organisms in the form of bifurcating tree (Baum & Smith, 2013). The founder of the phylogenetic systematic was the German biologist, Willi Hennig (1913–1976) (Brower, 2014), in which the reconstruction of phylogenetic histories was largely dependent on morphological characters (Hennig, 1966). The advancement in sequencing technologies and developments in the phylogenetic analysis methods and software have revolutionised the field, providing strongly supported trees (Yang & Rannala, 2012). DNA sequencing, which allowed for direct assessment of nucleotide differences and interpreted the sequence information with relative ease, has emerged as one of the most utilized of the molecular approaches for inferring phylogenies (Sahu & Kathiresan, 2012). DNA sequences can be used at all taxonomic levels, conserved coding regions, such as the large subunit of ribulose1,5-bisphosphate carboxylase (*rbcL*) gene, are suitable for investigating relationships at family level (Chase et al., 2007). Variable non-coding regions, including many chloroplast introns and spacers (Borsch & Quandt 2009) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Nettel et al., 2008) are appropriate for the analysis at species and population level, respectively.

The study of island floras has enormously improved by using molecular phylogenies, which has transformed the understanding of island lineages and their relationships to continental sister clades. Over the past 30 years, the number of molecular phylogenetic studies that included the use of molecular dating of island flora increased dramatically. These studies were conducted primarily in the Macaronesian Archipelagos (Carine, 2005; Carine et al., 2004; Lledó et al., 2011; Vanderpoorten et al., 2011), the Hawaiian Islands (Keeley & Funk, 2011), the Galápagos Islands (Tye & Francisco-Ortega, 2011), and the Caribbean Islands (Maunder et al., 2011). These previous studies revealed new sources of colonists, clarified the number, direction, and timing of colonisation events, contributed information about the age of several lineages, and provided considerable insight into patterns of adaptive radiation and the role of hybridisation in the evolution of island endemics (Bramwell & Caujapé-Castells, 2011).

The evolution of the floral diversity in the Red Sea islands has attracted less attention compared with those in the Mediterranean Sea (Comes et al., 2008; Crowl et al., 2015; Jaros et al., 2017; Traveset & Navarro, 2018). Only a few investigations (Hassan & Al-Hemaid, 1996; Thomas et al., 2010) have sought to explain the floristic relationships between the Farasan Islands and the mainland. Scientific approaches have involved the comparison of regional floras (Thomas et al., 2010), and inferred the taxonomic distribution patterns of present taxa (Hassan & Al-Hemaid, 1996). The role of Pleistocene land bridges in the formation of the flora on the Farasan Islands have not been discussed in the extant literature (Alwelaie et al., 1993; Hassan & Al-

Hemaid, 1996; Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Al Mutairi et al., 2012a; Al Mutairi et al., 2012b; Al Mutairi & Al-Shami, 2014; Hall et al., 2010; Thomas et al., 2010).

1.4 Molecular clock for dating historical plant dispersal events

Age estimation is a powerful tool for inferring historical plant dispersal events. Knowing the tenure of lineages within a region is key in understanding the evolution of floras, the evolution of biotic interactions, and the evolution of traits (Renner, 2005). The application of this technique in studying the island floras improved our understanding about colonisation events and the evolution of their endemics (Bramwell & Caujapé-Castells, 2011; Gillespie & Clague, 2009; Whittaker & Fernández-Palacios, 2007).

In molecular dating, the divergence time between two species is measured by the number of mutations accumulated in their genomes over time. This hypothesis was first proposed 50 years ago by Zuckerkandl & Pauling (1965) in their study on protein sequences. Molecular dating has been applied in plant biogeography since the 1990s. The number of studies using this technique significantly increased with the increase in computer power and the access to readily implemented molecular evolutionary models (Renner, 2005).

Molecular sequence divergence provides only a relative timescale, therefore, it is required to use information from fossil records to convert relative divergence time into absolute time (Rieux & Balloux, 2016). To date a phylogeny, the genetic distance is calculated between two taxa or sequences (one of which must have a known age or age range that is usually determined from an appropriate fossil). Then the substitution rate is obtained by dividing the genetic distance by the known age (fossil data). Finally, the rate is used to convert genetic distances between taxa of interest into estimates of their absolute ages (Renner, 2005).

The assumption of the clock-like rate of molecular evolution is that the substitution rate of all branches of a phylogenetic tree remain the same (Rutschmann, 2006; Drummond et al., 2006). However, this assumption is biologically unrealistic because rate variations among lineages can seriously mislead the divergence date estimation and phylogenetic inference (Drummond et al., 2006). Consequently, since 1997, several different methods have been developed to relax the assumption of rate constancy among lineages (Sanderson, 1997; Rambaut & Bromham, 1998; Thorne et al., 1998; Aris-Brosou & Yang, 2002; Sanderson, 2002; Thorne & Kishino, 2002; Welch & Bromham, 2005; Drummond et al., 2012).

Currently, two kinds of relaxed molecular clocks are widely used: autocorrelated and uncorrelated. Both permit the rate of evolution to vary from branch to branch but both place limits on the range of rates that are apply (Baum & Smith, 2013). In contrast to the autocorrelated relaxed clock model, which assumes that lineages tend to have a slow rate of evolution, causing the tree branches to share rates of evolution similar to their immediate ancestral and descendant branches, the uncorrelated relaxed clock model assumes no correlation with adjacent branches (Baum & Smith, 2013). The parameters of the uncorrelated models can be estimated by averaging a set of plausible trees using Markov chain Monte Carlo (MCMC) in contrast to previous relaxed clock models, which needed to be applied to a fixed tree topology (Drummond et al., 2006). Thus, uncorrelated models are very useful for estimating evolutionary rates and divergence times in the face of phylogenetic uncertainty (Lemey et al., 2009).

The widely used software, Bayesian Evolutionary Analysis by Sampling Trees 2 (BEAST2), is the only software that can infer phylogenies under a relaxed clock model (Table 1-2). It provides various models of the molecular clock using the MCMC algorithm (Drummond & Bouckaert, 2015). The Bayesian Evolutionary Analysis Utility (BEAUti) is a graphical user interface that is used to create XML input files for BEAST. It allows importing data, specifying prior distributions on individual parameters, and choosing the settings for the MCMC analysis (Drummond et al., 2012). BEAST generates both phylogeny and divergence time simultaneously, thus allowing each to influence the other during the analysis.

Table 1-2. Software for molecular dating that relax the clock assumption of a homogeneous substitution (modified from Renner (2005)).

Method, software	Multidivtime	PhyBase	BEAST	Penalised likelihood in 'r8s'
Reference	Thorne & Kishino (2002); Thorne et al. (1998)	Aris-Brosou & Yang (2002)	Drummond & Bouckaert (2015)	Sanderson (2002)
Platforms	Unix (MacOSX), Linux, Windows	Unix (MacOSX), Linux, Windows	Unix (MacOSX), Linux, Windows requires Java	Unix (MacOSX), Linux
Optimisation	Bayesian Markov chain Monte Carlo MCMC			Smoothing (as in regression analysis)
Input data	Tree topology Sequences Several priors	Tree topology Sequences Several priors	Sequences Several priors	Tree with branch lengths Value for the penalty parameter
Model of rate evolution	Autocorrelation, with rates drawn from a lognormal distribution	Autocorrelation, with rates drawn from six different distributions	Various models are implemented and can be fitted to the data.	Autocorrelation between ancestral and descendant branches
Allows polytomies in input topology	Yes	No	Not applicable	Yes (collapse option)
Handles multiple datasets with different rates	Yes	No	Yes	No
Provides error estimates	Bayesian 95% credibility intervals	Credibility intervals must be calculated by the user	Bayesian 95% credibility intervals	No (separate bootstrapping is required)
Relative speed	Medium	Slow	Fast	Very fast

1.5 Study groups

The current study aims to determine the phylogenetic affinities of the Farasan Islands flora and to discover the timescale for colonisation and extent to which the species present are parts of a more general widespread subtropical flora or whether they are more closely linked to adjacent mainland floras. DNA sequence-based phylogenies and molecular dating approaches will be used on multiple plant groups from the Farasan Islands.

Reconstructing the evolutionary histories of all species of a specific flora is not realistic. However, the phylogeny in habitat-specific plant groups could offer a possible means of investigating the historical construction of the habitat that they characterise. In this study, the choice of study groups was reliant on Al Mutairi et al. (2012a) analysis, which identified five habitat types in the Farasan Islands: mangroves, salt marshes, sand formations, wadi channels and coral rocky habitats. These five habitats were linked with 12 plant groups (Figure 1-5). Because the lowest species richness was in wadi channel habitat that were invaded and indicated by the invasive plant *Prosopis juliflora*, wadi channel was excluded from the sampling.
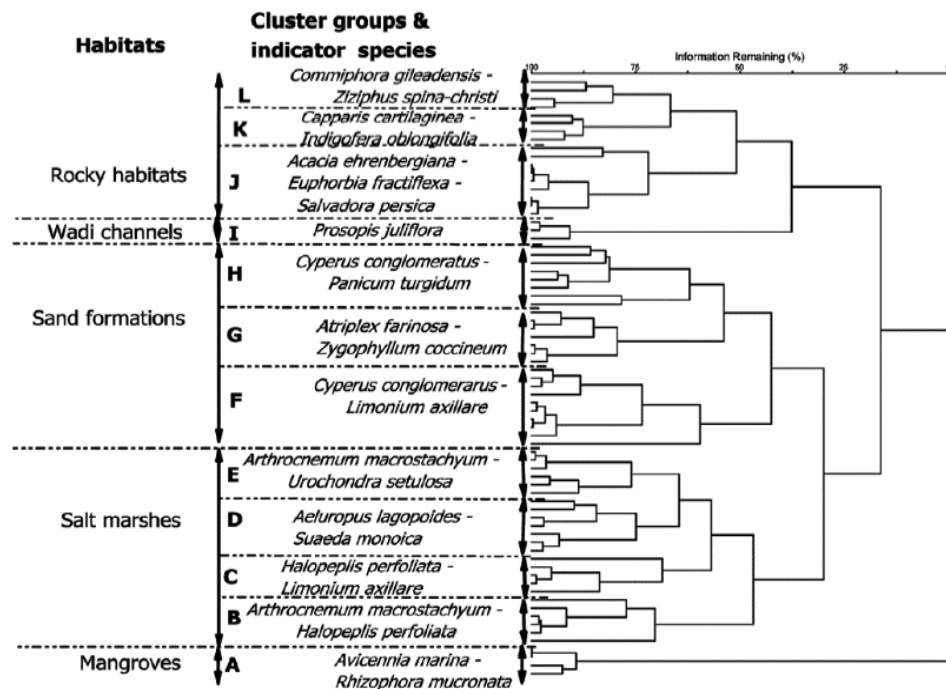
Figure 1-5. Relationships between the 12 vegetation groups in the five main habitats based on a detrended correspondence analysis (DCA) conducted by Al Mutairi et al. (2012a).

Investigate the phylogenetic affinities of any given flora such as the current study need to rely on a large number of plant groups, and comprehensive molecular data on multiple DNA markers are necessary to ensure robust, accurate and well-resolved phylogenies (Heath et al., 2008; Hughes et al., 2005).

Over the past three decades, several thousand molecular-based phylogenetic analyses at all taxonomic levels and in all major groups of plants have been published, which has reshaped views of plant relationships and evolution, which were revolutionised by the DNA sequence data (Soltis & Soltis, 2000; Savolainen & Chase, 2003). Many genera recorded in the Farasan Islands have comprehensive molecular phylogenetics, providing a rich source for ingroups and outgroups that may help in reconstructing accurate phylogenies of Farasan flora. Table 1-3 shows examples of genera in the islands have published molecular studies.

In addition to indicating the habitat type and the availability of molecular data, the study group needed to represent more than one species and to be well-sampled, broadly distributed, and to consist of all dispersal syndromes in the islands. Therefore, ten angiosperm genera represented by 34 species were chosen as exemplars of flora in the Farasan Archipelago: *Avicennia* L. and *Rhizophora* L. represent the mangrove habitat; *Cyperus* L*., Heliotropium* L*., Suaeda* Forssk. ex J.F.Gmel. and *Tetraena* Maxim represent the coastal zone, including salt marshes and sandplains; *Convolvulus* L*., Euphorbia* L*., Ficus* L. and *Indigofera* L. represent the coral rocky habitat in the interior of the islands. *Heliotropium, Convolvulus* and *Ficus* are not among the 12 vegetation groups shown in Figure 1-7, but they are well-represented in the islands by more than two species with available molecular data in contrast to, for example, *Capparis* L., *Ziziphus* Mill. and *Panicum* L. or other genera not included in the study group.

Table 1-3. Examples of the available phylogenetic studies of some genera present in the Farasan Islands.

No.	genus	No. of species in Farasan	DNA markers used	Publications
1	*Aerva* Forssk.	1	*matK, atpB-rbcL*, ITS	Thiv et al. (2006)
2	*Aloe* L.	1	*rbcL, matK, trnH-psbA, trnL* ITS1	Manning et al. (2014)
3	*Amaranthus* L.	2	ITS	Xu & Sun (2001)
4	*Asparagus* Tourn. ex L.	1	*rpl32-trnL, trnQ-rps16, ndhF-rpl32, psbD-trnT, rps, trnK*	Kubota et al. (2012)
5	*Avicennia* L.	1	*trnD-trnT, trnH*, ITS	Nettel et al. (2008), Li et al. (2016)
6	*Blepharis* Juss.	2	*rps16, trnG-S, trnL-F*, ITS	Fisher et al. (2015)
7	*Ceropegia* L.	2	*psbA-trnH, trnL-trnF, trnS-trnG, rps16, rpl32-trnL*, ncpGS, ITS	Bruyns et al. (2017)
8	*Chenopodium* L.	2	*trnL-F, trnk/matK*, ITS	Fuentes-Bazan et al. (2012)
9	*Cissus* L.	1	*rps16, trnL-F, atpB-rbcL, trnH-psbA, trnC-petN*	Liu et al. (2013)
10	*Cleome* L.	3	ITS	Feodorova et al. (2010)
11	*Commelina* Plum. ex L.	2	5S NTS, *trnL-trnF*	Burns et al. (2011)
12	*Convolvulus* L.	5	*rbcL, matK*, ITS	Williams et al. (2014)
13	*Cucumis* L.	2	*trnL-trnF, rpl20-rps12, trnR-atpA, trnG-trnS*, ITS	(Renner et al., 2007)
14	*Cyperus* L.	5	*rpl32-trnL, trnH-psbA*, ETS1f	Larridon et al. (2011); Larridon et al. (2013)
15	*Euphorbia* L.	2	*NdhF, matK/trnK*, ITS	Dorsey et al. (2013)
16	*Ficus* L.	3	ITS,ETS, G3pdh, ncpGS	Ronsted et al. (2007) Chantarasuwan et al. (2015); Renoult et al. (2009)
17	*Heliotropium* L.	4	*trnL* LUAA intron, ITS$_1$	Hilger & Diane (2003)

Table 1-3. Continued.

No.	genus	No. of species in Farasan	DNA markers used	Publications
18	*Indigofera* L.	7	ITS	Schrire et al. (2009)
19	*Gymnosporia* (Wight & Arn.) Hook.f.	3	*matK, trnL–F,* 26S rDNA, ITS	McKenna et al. (2011)
20	*Kickxia* Dumort.	2	ITS, *rpl32-trnL*	Yousefi et al. (2016)
21	*Kohautia* Cham. & Schltdl.	1	*atpB-rbcL, petD, rpsl6, trnL-F,* ETS, ITS	Groeninckx et al. (2010)
22	*Polycarpaea* Lam.	2	*rpsl6, ndhF,* RPB2, ITS	Kool et al. (2007)
23	*Rhizophora* L.	1	*trnG-trnS, trnH-rpl2*, ITS	Lo et al. (2014)
24	*Suaeda* Forssk. ex J.F.Gmel.	3	*atpB-rbcL, psbB-psbH,* ITS	Schutze et al. (2003); Schütze (2008)
25	*Tetraena* Maxim.	5	*rbcL, trnLF*	Sheahan & Chase (2000); Bellstedt et al. (2008); Bellstedt et al. (2012); Alzahrani & Albokhari (2017)
26	*Vachellia* Wight & Arn.	2	*matK/trnK, trnL-trnF, psbA-trnH*	Kyalangalilwa et al. (2013)
27	*Ziziphus* Mill.	1	*trnL-F*, ITS, 26S rDNA	Islam & Simmons (2006)

1.6 International Union for the Conservation of Nature (IUCN) Red Listing for conservation assessment

Island organisms have always been vulnerable to human activities and climate change (Bramwell, 2011; Heywood, 2011). There is abundant evidence that island floras are currently more vulnerable than they were 30 years ago (Heywood, 2011). In the future, these factors will cause a massive increase in the number of threatened island plants and will lead to the extinction of many (Bramwell, 2011). The increase in tourism has led to massive urban and tourist-related development with accompanying infrastructural effects, such as desalinisation, drainage, irrigation and large-scale transport infrastructures (Heywood, 2011). The growth of the local population on the islands has led to the destruction of natural vegetation due to agricultural and urban exploitation and the introduction of alien species (Bramwell, 2011). Biological invasions are main drivers of biodiversity change, it is well known that islands are often much more susceptible to invasion by exotic species than mainland sites are (Heywood, 2011). Because of human-induced climate change, islands worldwide face further threats and challenges, including a rise in sea level between 2 and 15 metres in the next 100 years (Bramwell, 2011). Rising sea levels in some cases, such as low-lying islands and coastal areas, will be catastrophic, which may lead to changes in coastal ecosystems, such as mangroves, dunes and halophyte communities. The effects of global change on islands include declines in forest cover due to floods, droughts, or the increased incidence of pests, pathogens or fires, all of which will affect island endemics (Bramwell, 2011).

The Farasan Islands are not an exception. The rapid expansion of the petroleum-based economy in Saudi Arabia led to extensive development in the islands, particularly in the main islands: Farasan Alkabir and Sajid. The construction of the main seaport and highway resulted in the massive destruction of the *Avicenna marina* population in Khor Farasan, which is on the east side of Farasan Alkabir (Mandura & Khafaji, 1993). Waste dumping, localised oil pollution, litter from the ferry, removal of beach sand, coastal development and tourism combined with global climate change and rising sea levels could lead to an increase in the loss of biodiversity and the erosion of the islands' coasts (Gladstone, 2000; AlRashidi et al., 2012). The local population in the Farasan Islands continues to increase (GaStat, 2019) and the main island, Farasan Alkabir, is becoming increasingly urbanised. Moreover, excessive grazing and the expansion of agriculture have increased the threat to the local biodiversity of the island's interior. The invasive *Prosopis juliflora* has spread into the densest areas of *Vachellia flava*

woodland, which is the main source of the endemic gazelle diet (Wronski & Schulz-Kornas, 2015). In Saudi Arabia, the Farasan Islands have long been a popular holiday destination, and the number of tourists is expanding (Hall et al., 2010). The 2030 vision of Saudi Arabia aims to develop the Farasan Islands and increase the number of tourists (SCTH, 2017). Therefore, it is important to ensure that tourism remains sustainable.

The flora of the Farasan Islands in Saudi Arabia is significant not because of endemism, which is low, but because they are the home of several regionally and nationally rare species that are known only in the Farasan Islands (i.e., Farasan-restricted species). In 2010, the Farasan Islands were designated an Important Plant Area in the Arabian Peninsula because of these plant groups and mangrove stands (Hall et al., 2010). However, awareness of the need to conserve and to manage threatened flora is increasing slowly. Despite the anthropogenic and ecological stresses, none of these rare species is represented in the current conservation framework, which is currently focussed upon protecting Idmi gazelle (Abuzinada, 2003) and the mangroves (Gladstone et al., 2003).

The IPA framework was originally developed by Plantlife International (Anderson, 2002; Plantlife International, 2004) in response to the Global Strategy for Plant Conservation (GSPC) in contributing to achieve GSPC targets (Targets 2, 4, 5, 7, 13–16) (Hall & Miller, 2011). The GSPC was first approved at the Conference of the Parties (COP) to the Convention on Biological Diversity in 2002 (CBD, 2003). The Global Strategy includes 16 outcome-oriented targets aimed at achieving a series of measurable goals (CBD, 2003). One of the most critical and ambitious goals of the GSPC is Target 2, which is "a preliminary assessment of the conservation status of all known plant species, at national, regional and international levels" (CBD, 2003). None of the Farasan-restricted species were subject to the Red List assessment until recently (Personal communication with Abdul Wally Al-Khulaidi, a member of the IUCN Arabian Plant Specialist Group [APSG]).

The IUCN Red List of Threatened Species™ has become a major and well-established tool for guiding conservation on the species level (De Grammont & Cuarón, 2006; Sodhi & Ehrlich, 2010). The IUCN Red List Categories and Criteria are accepted widely as the most objective and reliable system available for assessing species global extinction risk (De Grammont & Cuarón, 2006; Mace et al., 2008; Hoffmann et al., 2008; Rodrigues et al., 2006; Lamoreux et al., 2003). The list of threatened species has been used to influence and inform conservation legislation and policies, identify priority areas for biodiversity conservation, increase public awareness of human impacts on biodiversity and regulate development and exploitation (Possingham et al., 2002; Miller et al., 2007; Hoffmann et al., 2008). National Red Lists are

influential in the protection and recovery of endangered species (Miller et al., 2007). They also play a valuable role in informing global conservation efforts, especially when the information they contain is incorporated into the global IUCN Red List (Rodríguez et al., 2000).

Conservation assessment and Red listing the endemic and non-endemic rare species of the Farasan Islands in global and regional context are the first steps required to highlight the threat of the loss of floral biodiversity in the Farasan Islands and to contribute to achieving Target 2 of the Global Strategy. The objective of this research is to provide up-to-date, scientifically based information about the distribution, status, trends and threats to endangered species in order to inform policymakers and catalyse actions to conserve floral diversity in the Farasan Islands.

1.7 Aims and Outlines

Using phylogenies and molecular dating has disentangled the biogeographic history with disjunct distributions on a mainland and adjacent islands, which has provided insights into the evolution of continental island floras and can guide the conservation of island plants. Since the biogeographic history of the Farasan Islands flora has, to date, received limited scientific attention and the rare native species are neglected in the conservation legislation, the principle aims of this project are the following:

1. Determine the phylogenetic affinities of the Farasan Islands flora.

2. Assess the conservation status and produce red list data for the Farasan Islands flora, with particular focus on endemic and non-endemic restricted species.

This thesis contains seven chapters as follows:

Chapter 1 is a general introduction.

Chapter 2 presents the sample collection procedures and field observations in two main parts. The first part describes the methods used for supplementing the phylogenetic study groups of materials for DNA extraction from the field and herbaria. The second part presents the main observations of the habitat situations of the eight rare species (Farasan restricted species) to facilitate later conservation assessment.

The phylogenetic affinities and divergence-time estimates for the Farasan Islands flora have been divided into three chapters based on the distribution of the habitat in the islands.

Chapter 3 presents the phylogenies and molecular dating of two genera representing the mangrove habitat that dominates the sheltered coastal areas (*Avicennia* and *Rhizophora*).

Chapter 4 presents the phylogenies and molecular dating of four genera representing the sandplain and salt marshes habitats that dominate coastal areas (*Cyperus, Heliotropium, Suaeda* and *Tetraena*).

Chapter 5 presents the phylogenies and molecular dating of four genera representing the rocky coral fossil habitats that dominate the islands' interiors (*Convolvulus, Euphorbia, Ficus* and *Indigofera).*

Chapter 6 presents a conservation assessment and Red Listing of the Farasan Islands' endemic and non-endemic restricted species.

Chapter 7 discusses the main findings and presents the conclusions.

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Appendix

Appendix 1-1. Vegetation history of the Arabian Peninsula

- **The late Cretaceous-early Eocene (c.80 – 50 Mya)**

During this period, Arabia was part of Africa and the Tethys Sea covered the majority of central Arabia (the Arabian Platform). Probably the oldest section of the Arabian flora is the semidesert and desert flora of the Arabian regional subzone that was derived from a Cretaceous Mesogean floral stock located along the coasts of the Tethys sea (Kürschner, 1998). By this time, especially in the mid-Paleocene (figure 1-1, A) the arid regions in northern and southern Africa already existed (Liu et al. 2019).

- **The middle-late Eocene and Oligocene (c.38-23 Mya)**

The African-Arabian Shield was not divided by the Red Sea yet, a palaeo-African vegetation from the southwest extended eastwards into the future Arabian Peninsula throughout this period. This palaeotropical vegetation persisted in the western and southwestern highlands as a precursor of today's Nubo-Sindian vegetation (Kürschner, 1998). In the late-Oligocene (figure 1-1, B) the arid region in northern Africa (including northern part of Arabian Peninsula) expanded rapidly as the African continent continued to move northward (Liu et al. 2019).

- **The early to Late Miocene (c. 22-6 Mya)**

Major geological events took place in the eastern and southern Africa during this period including the maximum widening of the Red Sea, uplift of much of southern and eastern Africa and closing of the Tethys Sea (Hegazy & Lovett-Doust, 2016). Arabia experienced its main floro-historical events during Miocene. According to the Arabian fossil pollen record a proto-Sudanian flora existed in Arabia during the Mid Miocene. Families of tropical origin were
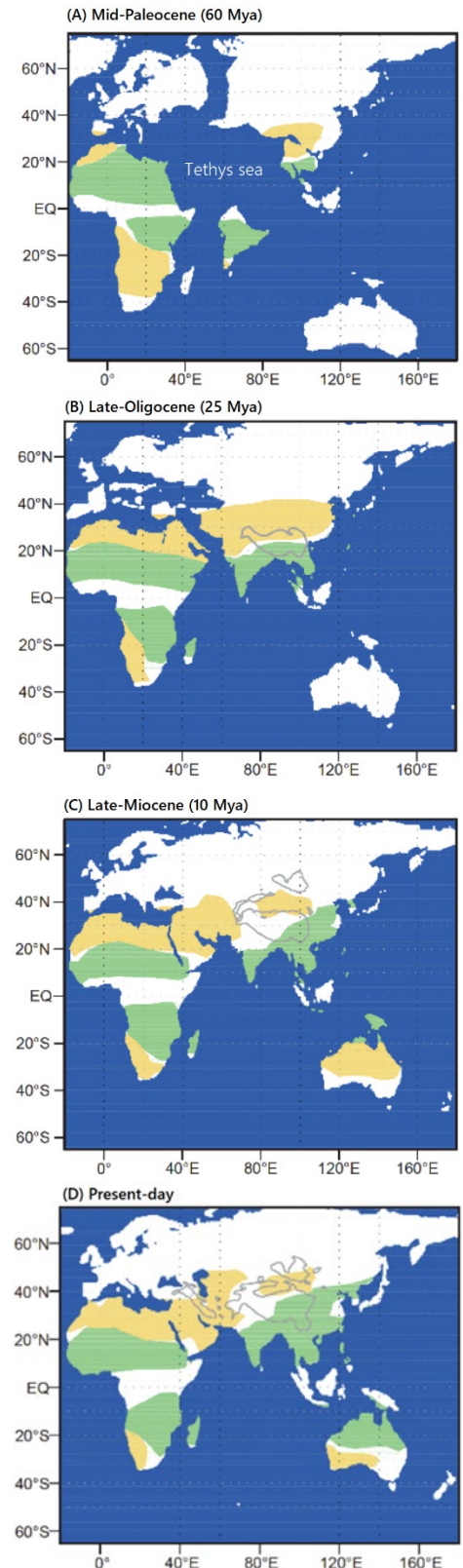
Appendix figure 1-1. Distributions of the Asian-African-Australian monsoon regions (green) and arid regions (yellow) in four periods during the Cenozoic (modified from Liu et al. (2019)).

present that are today rare or absent such as Arecaceae, Combretaceae, Meliaceae, Myrtaceae and Sapotaceae. In the intertidal zone of the Gulf coast a coastal environment of broad alluvial flood plains with swamps, open savanna grasslands, streams and mangroves existed from Upper to Late Miocene. In the eastern Rub' al Khali, a hyper arid area today, pollen of the extinct genus *Psilatricolporites* of the families Arecaceae, Myrtaceae, and of the water fern *Ceratopteris* have been found 150-200 m below the present surface. This indicating freshwater marshlands of a humid, tropical to subtropical climate (Kürschner, 1998). During the late-Miocene (figure 1-1, C), the Sahara Desert and other deserts in the Middle East and Arabian Peninsula significantly expanded (Liu et al., 2019) and became progressively more arid, reaching desert conditions during this time (Sepulchre et al., 2006). The vegetation was replaced by a more drought-adapted Saharo-Arabian flora (Kürschner, 1998).

- **Late Pliocene or Early Pleistocene (c. 4-1.8 Mya)**

The former Nubo-Sindian flora partly recovered its former area by migration via the large trans-Tuwayq wadi systems from refuge areas in the mountains of western Arabia, and this flora is still present within this Arabian regional subzone. During the more humid period of 3.5-1.2 million years ago, these great wadi systems provided routes for a limited and selective reintroduction of an arborescent Sudanian flora from the west to the east. After this pluvial episode central and eastern Arabia became more arid, only interrupted by increased precipitation during the Pleistocene. These hotter and dryer conditions favoured many Chenopodiaceae and led to the development of the present pre-adapted, Saharo-Arabian desert flora derived from the Mesogean stock (Kürschner, 1998).

Due to the relatively late separation of the Arabian Peninsula from the African continent in the Early Miocene, some 25 million years ago, the southwestern and southern mountainous part of Arabia exhibit close relationships with the Somalia Masai regional zone and the Afromontane archipelago-like regional centre of endemism (Kürschner, 1998).

CHAPTER 2: Sample collection and field observation

2.1 Introduction

The unique location of the Farasan Archipelago in the Red Sea, between East Africa and Western Asia and between four phytogeographical regions: Tropical, Saharo-Arabian, Mediterranean and Sudanian, has resulted in rich biological diversity and a wide range of habitats (Al Mutairi et al., 2012). One hundred and forty-five genera were recorded in the islands distributed in four main habitat types namely mangroves, salt marshes, sand formations, and coral rocky habitats (Al Mutairi et al., 2012; Alfarhan et al., 2002; Alfarhan et al., 2005; Alwelaie et al., 1993; Atiqur Rahman et al., 2002; Chaudhary, 1999, 2001a, 2001b, 2001c, Collenette, 1999; El-Demerdash, 1996; Hall et al., 2010; Thomas et al., 2010). This study aims to assess the floristic affinities of the Farasan Islands flora using a molecular phylogenetic approach. Such an investigation cannot be built from scratch since it needs to rely on many plant groups and comprehensive molecular data of multiple DNA markers. Ten genera have been chosen primarily as an exemplar of the Farasan Archipelago vegetation based on the criteria summarised in Chapter 1. Those genera are: *Avicennia* and *Rhizophora* representing the Mangroves habitat; *Cyperus, Heliotropium, Suaeda* and *Tetraena* represent coastal zone including salt marshes and sandplains; and *Convolvulus, Euphorbia, Ficus* and *Indigofera* for coral rocky habitat, the interior part of the large islands. The study group comprises 34 species and requires supplementation with plant materials for DNA extraction, since none of the Farasan lineages had been sampled in earlier phylogenetic studies of any given genus (Hilger & Diane, 2003; Schütze, 2008; Schrire et al., 2009; Dorsey, 2013; Larridon et al., 2013; Lo et al., 2014; Williams et al., 2014; Chantarasuwan et al., 2015; Li et al., 2016; Alzahrani & Albokhari, 2017). Therefore, multiple plant collecting expeditions were necessary to collect these species from around the archipelago.

The nature and flora of the Farasan Islands have attracted the botanists over the past three decade and their efforts have resulted in many collections deposited in both local and international herbaria. Sheila Collenette, the famous plant collector of the flora of the Kingdom of Saudi Arabia, has housed her specimens from the Farasan Islands in various herbaria: Royal Botanic Garden, Edinburgh (**E**), Royal Botanic Garden, Kew (**K**), UK and in the National Herbarium of the National Agriculture and Water Research Centre (**RIY**), Riyadh, Saudi Arabia. In addition, the voucher specimens of two hundred and two species recorded in Alfarhan et al. (2005) have been deposited in E, RIY and the Department of Botany and Microbiology, King Saud University (**KSU**), Riyadh, Saudi Arabia. There are also a group of

one hundred and thirty-three species of Atiqur Rahman et al.'s (2002) collection preserved at the herbarium of the College of Pharmacy, King Saud University (**KSUP**) Riyadh, KSA. These herbarium collections represent an invaluable source of DNA in case that the targeted species could not be collected from the wild.

The Farasan Islands is an Important Plant Area in the Arabian Peninsula due to the presence of regionally and nationally rare species that are known only in the Farasan Islands (i.e., Farasan-restricted species); further details about the group are outlined in Chapter 6. Despite the rapid increase of the anthropogenic and ecological stresses on the Farasan Islands flora, the awareness of the need to conserve and to manage threatened species is growing slowly. The second aim of the thesis is to conduct a Red List assessment to the Farasan restricted species (eight species) at the global and regional context in accordance to the IUCN categories and criteria (IUCN, 2012, IUCN Standards and Petitions Subcommittee, 2017). Accurate assessment of conservation status necessitates field observation of the habitat and recording of the distribution data.

Thus, the project's aims are to:

1. Supplement the exemplar generic phylogenies with samples from the Farasan Islands.
2. Conduct herbarium sampling to augment fieldwork sampling.
3. Observe and record the distribution and habitat of the species of interest to facilitate later conservation assessment.

2.2 Materials and Methods

Before starting fieldwork, the regional floras of the islands (El-Demerdash, 1996; Alwelaie et al., 1993; Collenette, 1999; Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Alfarhan et al., 2005; Hall et al., 2010; Thomas et al., 2010; Al Mutairi et al., 2012) were studied and used to compile a checklist of all native recorded species of the targeted species, including distribution and habitat type. They were also used to gain familiarity with key species' identifying characteristics.

The necessary collecting permits were obtained from the Saudi Wildlife Authority (SWA), which controls the Farasan Islands Protected Area, and the fieldwork health and safety risk assessment was submitted to the University of Reading.

Three field trips were carried out over the course of this PhD project, in 2016 and 2017. The second and third plant expeditions were not funded; consequently, they were shorter in duration than the first. This due to the regulations regarding field trips imposed by the Saudi Culture Bureau, whereby the PhD student is entitled to undertake a scientific trip once during the entire academic stage. The itineraries for each expedition centred on visits to areas of high vegetation density, all habitat types and the locations of the rare species.

Field observations were recorded, including distribution data for Farasan restricted species, habitat conditions and threats where evident.

Leaf samples from each of the species were collected in silica-gel in small polythene bags for DNA extraction. Voucher specimens were also prepared, by wrapping a branch from each species in newspaper and then pressing it flat using a wooden press. The specimens were taken to the University of Reading, where the lab work was carried out, and the voucher specimens were deposited in the university's herbarium (RNG), the duplicate specimens will be deposited in the herbarium of Umm-Al-Qura University, Makkah, Saudi Arabia. All data regarding the site and plants were recorded with accompanying photographs and coordinates, using the Global Positioning System (GPS).

The initial identification followed Chaudhary (1999; 2000) and Collenette (1999). Identifications were confirmed by comparison with authentic herbarium material at RNG and with pictures of herbarium specimens provided by the website of the Royal Botanic Garden, Edinburgh. Images of some specimens were posted on the Flora and Vegetation of Yemen Facebook group, which includes experts on the flora of the area. The nomenclature and

taxonomy were updated in accordance with the Angiosperm Phylogeny Group classification APG IV (2016) for plant families, and the Catalogue of Life for species (Roskov et al., 2019).

International and local herbaria were approached to sample the taxa that were not found during the expeditions. Those herbaria are E, K, RIY, KSU, KSUP , King Abdul-Aziz City for Science and Technology herbarium (**MUZ**), Riyadh, KSA, Department of Biology, King Abdul-Aziz University (**KAUH**), Jeddah, KSA, and Jazan University Herbarium (**JAZUH**), Jazan, KSA.

2.3 Results

2.3.1 Sample collection

2.3.1.1 Field trips

- First field trip

The first plant collecting expedition to the Farasan Archipelago was undertaken between 15 June and 15 July 2016, and was conducted across six islands: Farasan Alkabir, Sajid, Qummah, Zifaf, Dumsuk and Dawshak. A guide from SWA and Rahmah Al-Qthanin, a PhD student in the Culham research group, accompanied us on this trip. The locations visited are shown in (Figure 2-1, A).

Farasan Alkabir and Sajid were explored extensively, as they are connected by a bridge, which facilitated transport between them by car. Qummah, Zifaf, Dumsuk and Dawshak islands were accessed by boat, and explored on foot. Camping on these islands was unsafe, due to the ongoing war in Yemen. In total, 18 species of the targeted genera were collected during this visit; these are detailed in Table 2-1.

- Second field trip

The second plant collection expedition was undertaken from 12 to 22 December 2016; it was conducted across the main islands of the Farasan group: Farasan Alkabir and Sajid (Figure 2-1, B). Strong winds impeded safe sailing to the other islands. During this expedition, we had the opportunity to collect plant materials from the Arabian mainland, from Jazan, Sabia, Wadi Baish and Wadi Ash Shahd in the southwestern region of Saudi Arabia and Makkah in the western region (Figure 2-2). This expedition's goals were (1) to sample the species of genera under investigation that had not been sampled in the first trip and (2) to collect plant materials from the Saudi Arabian mainland to supplement the available molecular data of the chosen

genera that lack complete geographical coverage worldwide, particularly from the adjacent Arabian mainland.

A total of 32 samples of 10 species belonging to 7 genera were collected from the islands, one of which is a new record (*Indigofera spiniflora*). The sampled materials were limited in terms of new species for the incomplete genera and more samples of the same species from the first trip. Species of *Cyperus, Suaeda* and *Convolvulus* were extensively sampled, due to the difficulty of identification in the field. Collection details are presented in Table 2-1.

From the Saudi Arabian mainland, 11 samples of 8 species and 6 genera were collected. The details are presented in Table 2-2.

- Third field trip

The third expedition to the Farasan Archipelago was undertaken between 25 and 28 December 2017. The fieldwork was conducted across two islands only—Farasan Alkabir and Sajid—as it was unsafe to sail to the other islands due to the intensification of the war at the border with Yemen during the visit. The site locations are shown in Figure 2-1, C.

The objective of this trip was to complete the collection of targeted species. Eleven species were collected, four of which had not been collected on the previous trips, among them (*Cyperus rotundus*) is a new record of the Farasan flora. The details are listed in Table 2-1. It was found that Sajid Island and the north-western region of Farasan Alkabir Island had experienced rain, resulting in numerous green patches of ephemeral vegetation (Figure 2-3). While the eastern, north-eastern and southern regions of Farasan Al Kabir Island had suffered from severe drought (Figure 2-6, E & F).
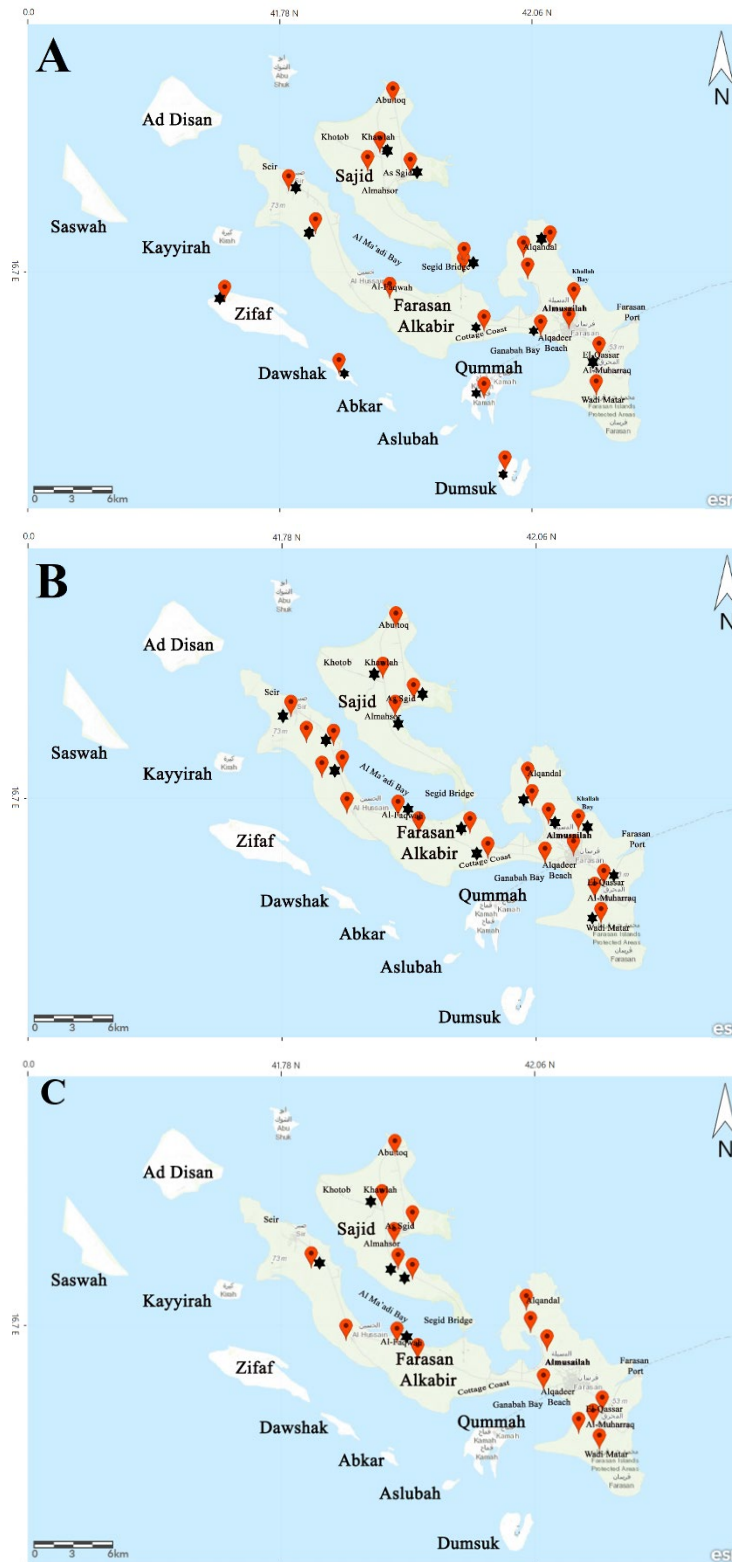
Figure 2-1. Map of the Farasan Islands showing the locations visited (red marks) and the collection sites (marked with stars). **(A)** First trip, June/July 2016; **(B)** second trip, December 2016; **(C)** third trip, December 2017. Maps were created using ArcGIS Online (Esri, "Topography") and edited using QGIS 2.10.1-Pisa (QGIS Development Team, 2019).
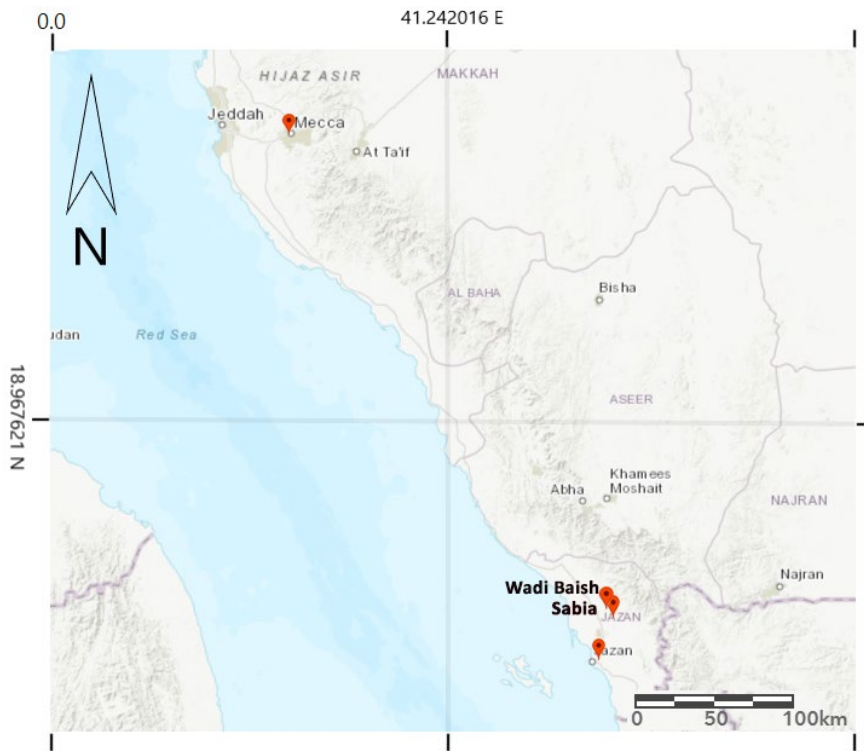
Figure 2-2. Collection sites on the Saudi Arabian mainland (red marks), December 2016. Maps created using ArcGIS (Esri, "Topography") and edited using QGIS 2.10.1-Pisa (QGIS Development Team, 2019).
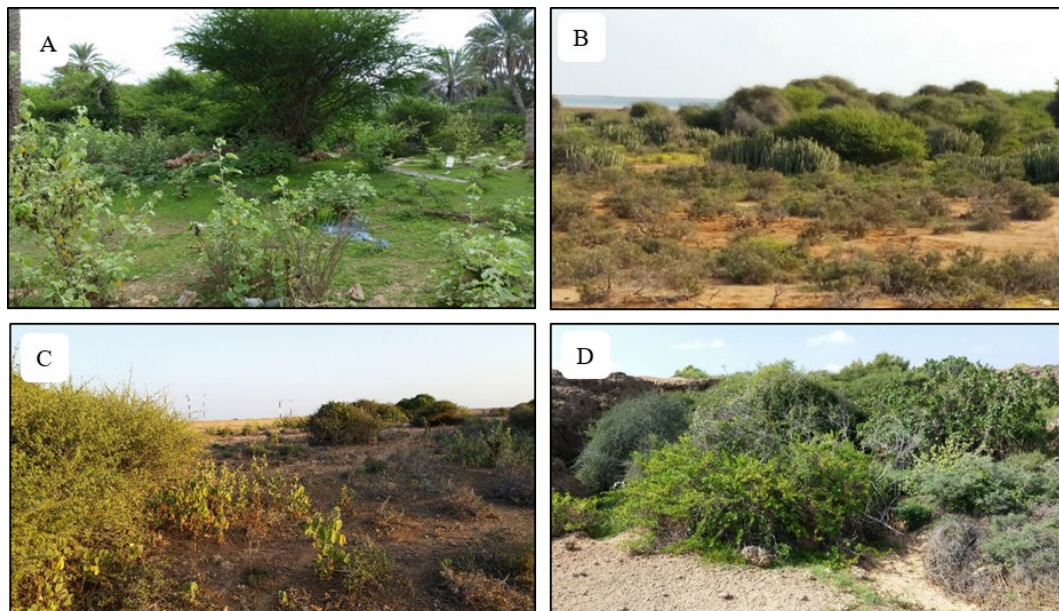
Figure 2-3. Photos taken of the Farasan Islands after the rainy season during the third trip. **(A & B)** green patches on Sajid Island; **(C& D)** northern part of Farasan Alkabir Island. Scale bar equals one metre.

Table 2-1. Details of samples collected during the three field trips; samples indicated with * used in the phylogenetic study. Grey shaded cells in the second and third trips = newly collected species from these trips. T. = Trip.

No.	T.	Collector name & No.	Species	Location	Coordinates
1	1st	S. Alharbi & R. Al Qthanin 7	*Indigofera oblongifolia* Forssk.	Near Coral Resort, Farasan Akabir Island	16°40'53.4"N 42°06'43.8"E
2	field	S. Alharbi & R. Al Qthanin 11	*Tetraena coccinea* (L.) Beier & Thulin*	Alghadeer Beach, Farasan Akabir Island	16°39'30.8"N 42°06'54.9"E
3	trip	S. Alharbi 13	*Suaeda fruticosa* Forssk. ex Gmel.*	Alghadeer Beach, Farasan Akabir Island	16°39'30.8"N 42°06'54.9"E
4		S. Alharbi & R. Al Qthanin 18	*Tetraena simplex* (L.) Beier & Thulin	Near Coral Resort, Farasan Akabir Island	16°40'53.4"N 42°06'43.8"E
5		S. Alharbi & R. Al Qthanin 23	*Cyperus conglomeratus* Rottb.	Alghadeer Beach, Farasan Akabir Island	16°39'30.8"N 42°06'54.9"E
6		S. Alharbi & R. Al Qthanin 29	*Euphorbia granulata* Forssk. var. *granulata**	Sajid Island	16°45'33.7"N 42°00'00.9"E
7		S. Alharbi & R. Al Qthanin 32	*Avicennia marina* (Forssk.) Vierh. var. *marina**	Sajid Island	16°45'25.4"N 42°00'07.9"E
8		S. Alharbi & R. Al Qthanin 36	*Avicennia marina* var. *marina*	Khor Alqandal, Farasan Akabir Island	16°47'23.0"N 42°05'52.7"E
9		S. Alharbi & R. Al Qthanin 38	*Rhizophora mucronate* Lam.*	Khor Alqandal, Farasan Akabir Island	16°47'23.0"N 42°05'52.7"E
10		S. Alharbi 49	*Euphorbia collenetteae* Al-Zahrani & El-Karemy*	2.5 kilometres before Seir Dis. Farasan Akabir Island	16°43'32.4"N 41°58'02.0"E
11		S. Alharbi & R. Al Qthanin 51	*Indigofera coerulea* Roxb. var. *coerulea* *	2.5 kilometres before Seir Dis. Farasan Akabir Island	16°43'32.4"N 41°58'02.0"E
12		S. Alharbi & R. Al Qthanin 61	*Ficus populifolia* Vahl*	Seir District, Farasan Akabir Island	16°50'58.7"N 41°49'01.0"E
13		S. Alharbi & R. Al Qthanin 64	*Tetraena coccinea*	Seir District, Farasan Akabir Island	16°50'58.7"N 41°49'01.0"E
14		S. Alharbi & R. Al Qthanin 74	*Convolvulus glomeratus* Hochst. ex Choisy	Khawlah, Sajid Island	16°54'31.9"N 41°54'23.5"E
15		S. Alharbi & R. Al Qthanin 80	*Indigofera coerulea* var. *coerulea**	Sorghum farm, Farasan Akabir Island	16°46'48.5"N 41°50'56.4"E
16		S. Alharbi & R. Al Qthanin 89	*Convolvulus glomeratus*	Sorghum farm, Farasan Akabir Island	16°46'48.5"N 41°50'56.4"E
17		S. Alharbi & R. Al Qthanin 95	*Heliotropium longiflorum* Hochst. & Steud.*	Sorghum farm, Farasan Akabir Island	16°46'51.1"N 41°50'57.7"E
18		S. Alharbi & R. Al Qthanin 102	*Indigofera* sp.	Dumsuk Island	16°33'07.8"N 42°03'23.7"E
19		S. Alharbi & R. Al Qthanin 101	*Convolvulus glomeratus*	Dumsuk Island	16°33'07.8"N 42°03'23.7"E
20		S. Alharbi & R. Al Qthanin 103	*Tetraena alba* (L.f.) Beier & Thulin var. *alba**	Dawshak Island	16°39'16.3"N 41°52'27.8"E
21		S. Alharbi & R. Al Qthanin 104	*Cyperus conglomeratus**	Dawshak Island	16°39'16.2"N 41°52'27.8"E
22		S. Alharbi & R. Al Qthanin 37	*Avicennia marina* var. *marina**	Zifaf Island	16°43'49.3"N 41°52'20.8"E
23		S. Alharbi & R. Al Qthanin 39	*Rhizophora mucronata**	Zifaf Island	16°43'49.3"N 41°52'20.8"E

Table 2-1. Continued.

No	T.	Collector name & No.	Species	Location	Coordinates
24	1st field	S. Alharbi & R. Al Qthanin 107	*Tetraena coccinea**	The Beach, near Algermin castle, Qumah Island	16°39'19.0"N 42°01'51.7"E
25	trip	S. Alharbi & R. Al Qthanin 108	*Suaeda aegyptiaca* (Hasselq.) Zoh.*	The Beach, near Algermin castle, Qumah Island	16°39'19.0"N 42°01'51.7"E
26		S. Alharbi & R. Al Qthanin 112	*Ficus cordata* subsp. *salicifolia* (Vahl) C.C.Berg	Almuharaq, Farasan Akabir Island	16°39'06.5"N 41°08'56.5"E
1	2ed	S. Alharbi 115	*Indigofera oblongifolia*	Wadi Matar, Farasan Akabir Island	16°38'23.6"N 42°08'55.9"E
2	field	S. Alharbi 119	*Indigofera oblongifolia*	Alqesar, Farasan Akabir Island	16°40'12.4"N 42°08'51.7"E
3	trip	S. Alharbi 122	*Heliotropium ramosissimum* (Lehm.) Sieb. ex DC.*	Alhases Beach, Farasan Akabir Island	16°43'10.9"N 42°04'50.8"E
4		S. Alharbi 123	*Indigofera semitrijuga* Forssk.*	Alhases Beach, Farasan Akabir Island	16°43'10.9"N 42°04'50.8"E
5		S. Alharbi 125	*Cyperus conglomeratus*	Alhases Beach, Farasan Akabir Island	16°43'10.9"N 42°04'50.8"E
6		S. Alharbi 127	*Indigofera semitrijuga*	Alhases Beach, Farasan Akabir Island	16°43'10.9"N 42°04'50.8"E
7		S. Alharbi 128	*Suaeda fruticosa*	Alhases Beach, Farasan Akabir Island	16°43'10.9"N 42°04'50.8"E
9		S. Alharbi 130	*Cyperus conglomeratus*	Farasan Akabir Island	16°45'19.4"N 42°03'59.5"E
10		S. Alharbi 132	*Suaeda fruticosa*	Farasan Akabir Island	16°45'19.4"N 42°03'59.5"E
11		S. Alharbi 133	*Convolvulus glomeratus*	Farasan Akabir Island	16°45'19.4"N 42°03'59.5"E
12		S. Alharbi 134	*Euphorbia collenetteae**	Alhussain, Farasan Akabir Island	16°44'48.3"N 41°52'33.1"E
13		S. Alharbi 135	*Convolvulus glomeratus*	Alhussain, Farasan Akabir Island	16°45'04.6"N 41°52'17.1"E
14		S. Alharbi 136	*Indigofera oblongifolia*	Alhussain, Farasan Akabir Island	16°45'07.0"N 41°52'15.0"E
15		S. Alharbi 137	*Convolvulus glomeratus**	Alhussain, Farasan Akabir Island	16°45'07.0"N 41°52'15.0"E
16		S. Alharbi 138	*Indigofera spiniflora* Boiss. (new record) *	Al-Faqwah Coast, Farasan Akabir Island	16°48'01.8"N 41°51'37.1"E
17		S. Alharbi 139	*Cyperus conglomeratus*	Al-Faqwah Coast, Farasan Akabir Island	16°48'01.8"N 41°51'37.1"E
18		S. Alharbi 140	*Indigofera coerulea* var. *coerulea*	Al-Faqwah Coast, Farasan Akabir Island	16°48'01.8"N 41°51'37.1"E
19		S. Alharbi 142	*Euphorbia collenetteae*	Almahsor, Sajid Island	16°50'24.3"N 41°55'15.7"E
20		S. Alharbi 143	*Indigofera coerulea* var. *coerulea*	Sajid Island	16°51'28.8"N 41°55'52.5"E
21		S. Alharbi 145	*Heliotropium ramosissimum**	Khawlah, Sajid Island	16°54'34.2"N 41°54'26.1"E
22		S. Alharbi 146	*Suaeda fruticosa*	Khawlah, Sajid Island	16°54'34.2"N 41°54'26.1"E

Table 2-1. Continued.

No	T.	Collector name & No.	Species	Location	Coordinates
23	2^{ed} field trip	S. Alharbi 147	*Convolvulus glomeratus*	Khawlah, Sajid Island	16°54'35.6"N 41°54'25.1"E
24		S. Alharbi 148	*Ficus populifolia*	Seir District, Farasan Akabir Island	16°50'17.6"N 41°48'49.7"E
25		S. Alharbi 150	*Convolvulus glomeratus*	Seir District, Farasan Akabir Island	16°51'27.8"N 41°48'57.4"E
26		S. Alharbi 153	*Indigofera spiniflora*	Almusailah, Farasan Akabir Island	16°41'46.2"N 42°08'34.4"E
27		S. Alharbi 154	*Suaeda fruticosa*	Cottage Coast, Farasan Akabir Island	16°41'44.4"N 42°03'16.7"E
28		S. Alharbi 155	*Suaeda fruticosa*	Cottage Coast, Farasan Akabir Island	16°41'44.4"N 42°03'16.7"E
29		S. Alharbi 156	*Suaeda fruticosa*	Cottage Coast, Farasan Akabir Island	16°41'39.4"N 42°02'39.2"E
30		S. Alharbi 157	*Suaeda fruticosa*	Farasan Akabir Island	16°42'37.8"N 42°03'42.3"E
31		S. Alharbi 158	*Cyperus conglomeratus*	Farasan Akabir Island	16°42'27.1"N 42°03'39.8"E
32		S. Alharbi 160	*Indigofera coerulea* var. *coerulea*	2km SE Alhussain	16°44'57.9"N 41°54'04.9"E
1	3rd field trip	S. Alharbi 162	*Suaeda fruticosa*	Farasan Alkabir Island	16°45'20.3"N 42°03'43.2"E
2		S. Alharbi 163	*Convolvulus glomeratus*	Farasan Alkabir Island	16°43'35.1"N 42°05'02.8"E
3		S. Alharbi 164	*Ficus glumosa* Del.*	Al-Faqwah Coast, Farasan Akabir Island	16°43'35.1"N 42°05'02.8"E
4		S. Alharbi 166	*Cyperus conglomeratus*	Al-Faqwah Coast, Farasan Akabir Island	16°43'35.1"N 42°05'02.8"E
5		S. Alharbi 169	*Heliotropium longiflorum*	Northwest Farasan Akabir Island	16°48'57.2"N 41°51'08.5"E
6		S. Alharbi 170	*Indigofera spiniflora**	Northwest Farasan Akabir Island	16°48'57.0"N 41°51'08.2"E
7		S. Alharbi 171	*Heliotropium ramosissimum* (Lehm.) Sieb. ex DC.	Sajid Islands	16°49'38.0"N 41°55'16.0"E
8		S. Alharbi 172	*Convolvulus glomeratus*	Sajid Islands	16°49'38.0"N 41°55'16.0"E
9		S. Alharbi 175	*Indigofera linifolia* (L.f.) Retz.*	Sajid Islands	16°50'21.4"N 41°54'58.2"E
10		S. Alharbi 176	*Cyperus rotundus* L. (new record)*	Sajid Islands	16°52'27.9"N 41°54'43.2"E
11		S. Alharbi 180	*Convolvulus rhyniospermus* Choisy*	Sajid Islands	16°53'44.0"N 41°54'33.0"E
12		S. Alharbi 184	*Ficus populifolia*	Al-Faqwah Coast, Farasan Akabir Island	16°44'50.7"N 41°54'24.2"E

Table 2-2. Details of samples collected from Saudi Arabian mainland during the second field trip, samples indicated with * used in the phylogenetic study.

No.	Collector name & No.	Species	Location	Coordinates
1	S. Alharbi 1	*Avicennia marina* (Forssk.) Vierh. var. *marina*	Jazan City, Saudi Arabia	16°49'38.1"N 2°37'05.6"E
2	S. Alharbi 2	*Suaeda monoica* Forssk.*	Jazan City, Saudi Arabia	16°49'38.1"N 2°37'05.6"E
3	S. Alharbi 3	*Cyperus conglomeratus* Hochst. ex Choisy*	Jazan City, Saudi Arabia	16°49'38.1"N 2°37'05.6"E
4	S. Alharbi 4	*Heliotropium pterocarpum* (DC.) Hochst. & Steud. ex Bunge	Jazan City, Saudi Arabia	16°52'54.0"N 2°37'50.4"E
5	S. Alharbi 5	*Suaeda monoica*	Jazan City, Saudi Arabia	16°52'54.0"N 2°37'50.4"E
6	S. Alharbi 6	*Heliotropium pterocarpum**	Wadi Baish, Saudi Arabia	17°20'04.5"N 2°35'04.7"E
7	S. Alharbi 7	*Convolvulus* sp.	Wadi Baish, Saudi Arabia	17°20'04.5"N 2°35'04.7"E
8	S. Alharbi 8	*Heliotropium longiflorum* Hochst. & Steud.*	Sorghum farm in Wadi Ash Shahd, Saudi Arabia	17°16'07.3"N 2°36'41.8"E
9	S. Alharbi 9	*Suaeda monoica**	Jazan, Saudi Arabia	16°56'34.4"N 2°36'37.0"E
10	S. Alharbi 10	*Indigofera argentea* Burm.f. *	Jazan, Saudi Arabia	16°56'34.4"N 2°36'37.0"E
11	S. Alharbi 11	*Heliotropium arbainense* Fresen*	Makkah, Saudi Arabia	21°24'07.2"N 9°45'57.5"E

2.3.1.2 Herbarium sampling

Five samples in Collenette's collection were obtained from E and K, the former representing four species, while one species was taken from RIY (Table 2-5). Unfortunately, a significant proportion of the voucher specimens that were supposed to be available from RIY, KSU and KSUP were not found.

Table 2-5. Summary of voucher specimens obtained from the international and local herbaria. H= herbarium, Coll.= collector, Y. Col.=year of collection.

H. code	No.	Species	Locality	Coll. No.	Coll. name	Y. Col.
E	1	*Heliotropium pterocarpum* (DC.) Hochst. & Steud. ex Bunge	Sarso Island	9254		1995
	2	*Zygophyllum boulosii* Hosny	Farasan Islands	6378		1987
	3	*Zygophyllum boulosii*	Farasan Islands	5598	I.S. Collenette	1986
	4	*Cyperus bulbosus* Vahl	Farasan Islands	8981		1994
K	5	*Rhizophora mucronate* Lam.	N. Solain Island	6835		No data
RIY	6	*Indigofera hochstetteri* Bak.	Farasan Islands	s.n.	s.coll.	No data

2.3.1.3 Summary of all collections from the Farasan Islands

All habitat types were visited on Farasan Alkabir and Sajid Islands during the trips, and roughly 80% of the area of these two islands was covered. It was possible to sail to four islands—Qummah, Zifaf, Dumsuk and Dawshak—during the first visit. The intensification of the war in Yemen (the Archipelago is in the border area) and the lack of funding affected the duration and the number of visited islands during the second and third trips. The field expeditions resulted in two new records for the Farasan flora *Indigofera spiniflora* and *Cyperus rotundus*, increasing the total number of study group from 34 to 36 species. Approximately 63.8% (23 species of 36) of the targeted species were collected from the field during these three expeditions. This group is composed of 21.73% trees, 4.35% shrub, 30.43% undershrub and shrublet, 8.69% perennial herb, 4.35% perennial sedges, 30.43% annual herb. Photographs of the species are shown in Figure 2-4. From the herbaria, 5.5% were obtained, and only two new species were found: *Cyperus bulbosus* and *Indigofera hochstetteri*. Specimens of *Heliotropium pterocarpum* and *Zygophyllum boulosii* that obtained from (E) were misidentified samples of *H. ramosissimum* and *Tetraena coccinea*, respectively. Therefore, 25 out of 36 species of the study group were sampled from the field and herbaria for phylogenetic study. A list of the study group species and sample sources is presented in Table 2-4.

Table 2-4. Study group species, and their availability. Grey shaded cells = unavailable.

No.	Family and Species	Field	Herb.
	1. Acanthaceae		
1	*Avicennia marina* (Forssk.) Vierh. var. *marina*	√	
	2.Amaranthaceae (included Chenopodiaceae).		
2	*Suaeda aegyptiaca* (Hasselq.) Zoh.	√	
3	*Suaeda fruticosa* Forssk. ex Gmel	√	
4	*Suaeda monoica* Forssk.		
	3. Boraginaceae		
5	*Heliotropium longiflorum* Hochst. & Steud.	√	
6	*Heliotropium pterocarpum* (DC.) Hochst. & Steud. ex Bunge		
7	*Heliotropium ramosissimum* (Lehm.) Sieb. ex DC.	√	
8	*Heliotropium zeylanicum* (Burm.f.) Lam.		
	4. Convolvulaceae		
9	*Convolvulus arvensis* L.		
10	*Convolvulus glomeratus* Hochst. ex Choisy	√	
11	*Convolvulus pilosellifolius* Desr.		
12	*Convolvulus prostratus* Forssk.		
13	*Convolvulus rhyniospermus* Choisy	√	
	5. Cyperaceae		
14	*Cyperus bulbosus* Vahl		√
15	*Cyperus conglomeratus* Rottb.	√	
16	*Cyperus jeminicus* Rottb.		
17	*Cyperus rubicundus* Vahl		
18	*Cyperus rotundus* L. (new record)	√	
	6. Euphorbiaceae		
19	*Euphorbia granulata* Forssk. var. *granulata*	√	
20	*Euphorbia collenetteae* Al-Zahrani & El-Karemy	√	
	7. Fabaceae		
21	*Indigofera coerulea* Roxb. var. *coerulea*	√	
	Indigofera coerulea Roxb. var. *occidentalis* Gillet & Ali		
22	*Indigofera hochstetteri* Bak.		√
23	*Indigofera linifolia* (L.f.) Retz.	√	
24	*Indigofera oblongifolia* Forssk.	√	
25	*Indigofera semitrijuga* Forssk.	√	
26	*Indigofera spinosa* Forssk.		
27	*Indigofera spiniflora* Boiss. (new record)	√	
	8. Moraceae		
28	*Ficus cordata* subsp. *salicifolia* (Vahl) C.C.Berg	√	
29	*Ficus glumosa* Del.	√	
30	*Ficus populifolia* Vahl	√	
	9. Rhizophoraceae		
31	*Rhizophora mucronata* Lam.	√	
	10. Zygophyllaceae		
32	*Tetraena alba* (L.f.) Beier & Thulin var. *alba*	√	
33	*Tetraena propinqua* (Decne.) Ghaz. & Osborne		
34	*Tetraena coccinea* (L.) Beier & Thulin	√	
35	*Tetraena hamiensis* (Schweinf.) Beier & Thulin var. *mandavillei* (Hadidi ex Beier & Thulin) Alzahrnai & Albokhari		
36	*Tetraena simplex* (L.) Beier & Thulin	√	
Total		23	2
		25	
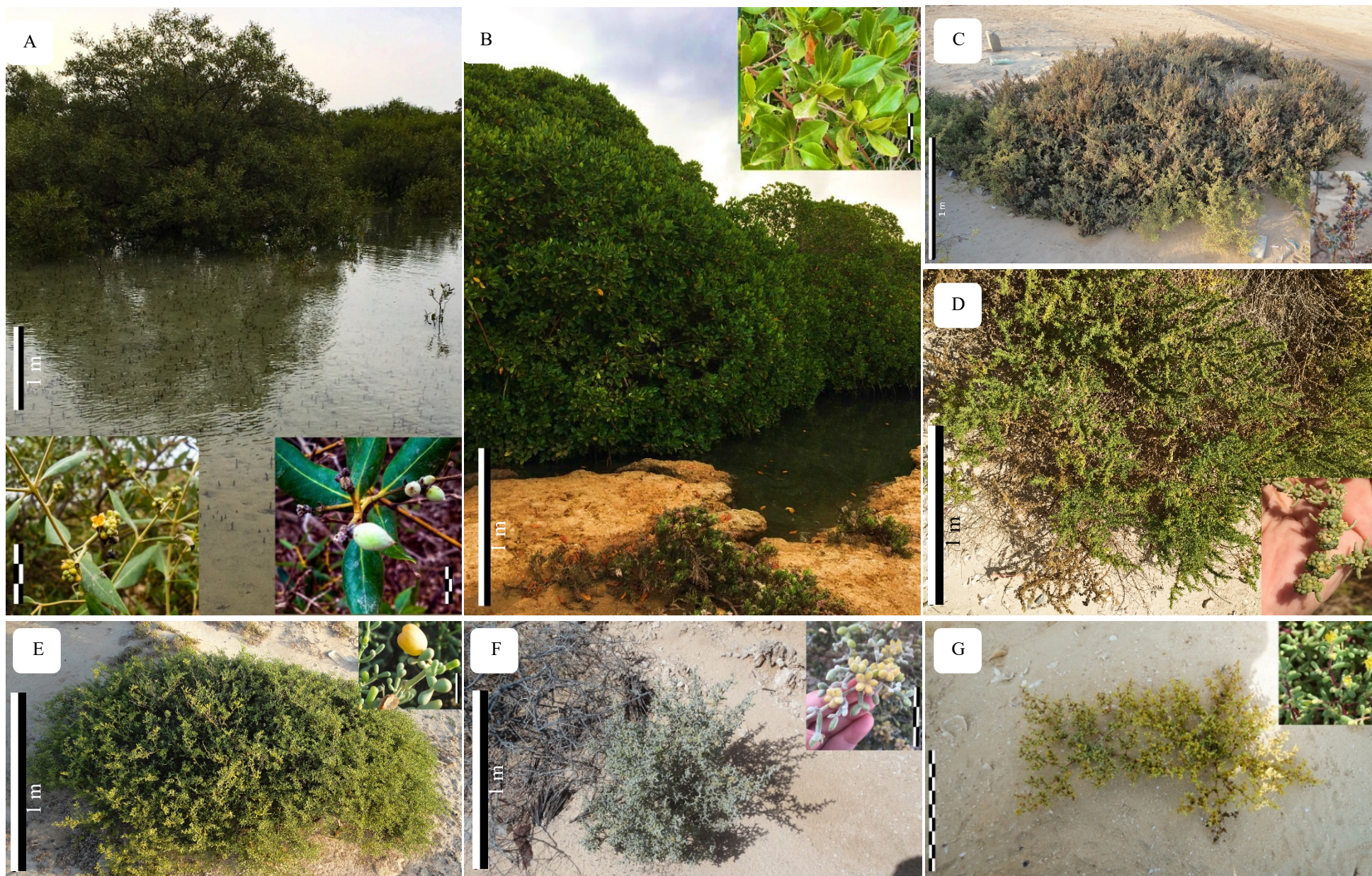
Figure 2-4a. Photographs of the study group species sampled from the field, growth habit, flowers and/or fruits (insets). **(A)** *Avicennia marina*; **(B)** *Rhizophora mucronata*; **(C)** *Suaeda fruticosa*; **(D)** *Suaeda aegyptiaca*; **(E)** *Tetraena coccinea*; **(F)** *Tetraena alba* var. *alba*; **(G)** *Tetraena simplex*. One unit of the scale bar = 1cm (except where marked)
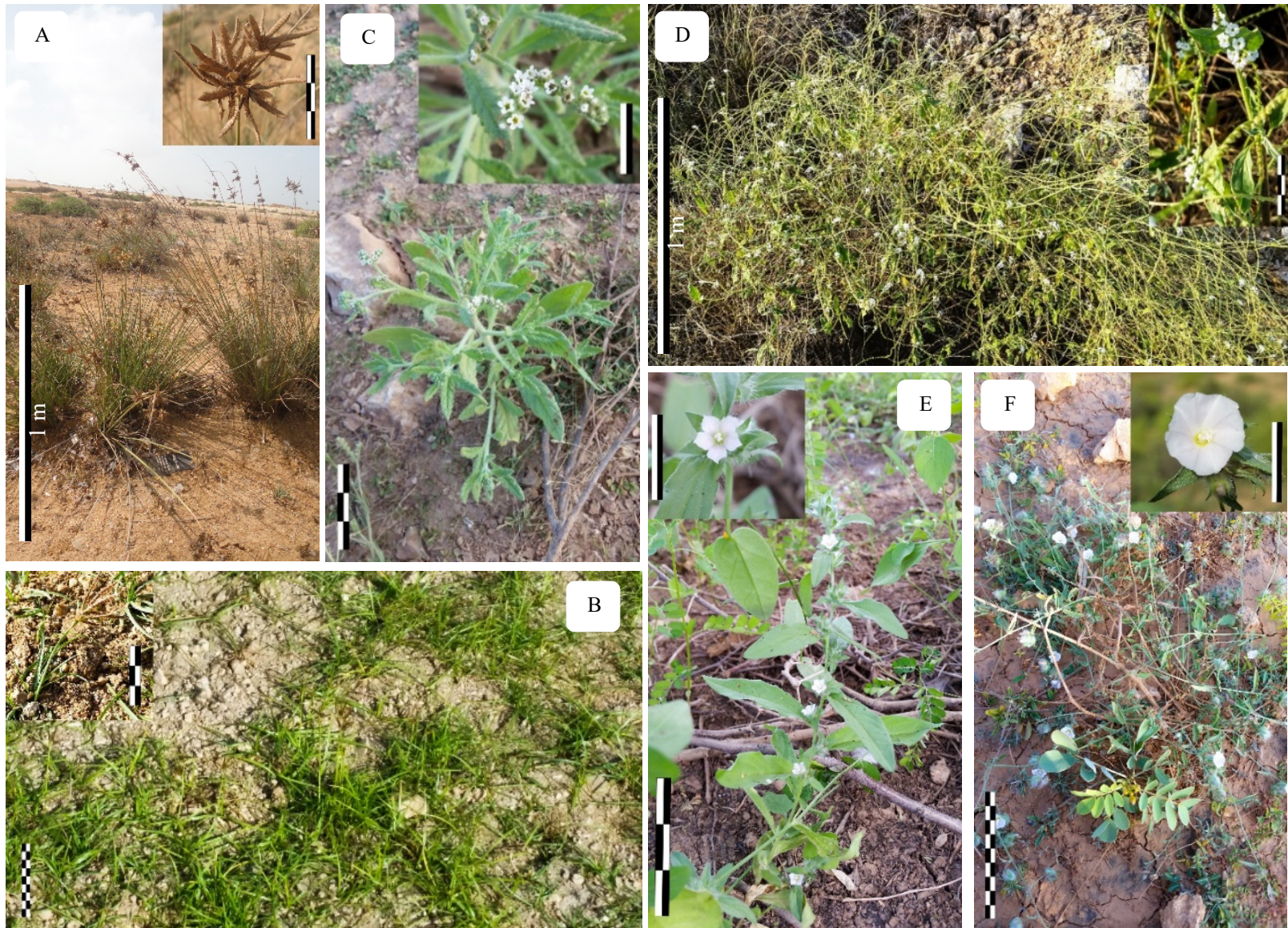
Figure 2-4b. Photographs of the study group species sampled from the field, growth habit, flowers and/or fruits (insets). **(A)** *Cyperus conglomeratus*; **(B)** *Cyperus rotundus*; **(C)** *Heliotropium ramosissimum*; **(D)** *Heliotropium longiflorum*; **(E)** *Convolvulus rhyniospermus*; **(F)** *Convolvulus glomeratus*. One unit of the scale bar = 1 cm (except where marked).

Figure 2-4c. Photographs of the study group species sampled from the field, growth habit, flowers and/or fruits (insets). **(A)** *Indigofera coerulea*; **(B)** *Indigofera linifolia*; **(C)** *Indigofera semitrijuga*; **(D)** *Indigofera oblongifolia*; **(E)** *Indigofera spiniflora* (fruit circle with red). One unit of the scale bar = 1 cm (except where marked).
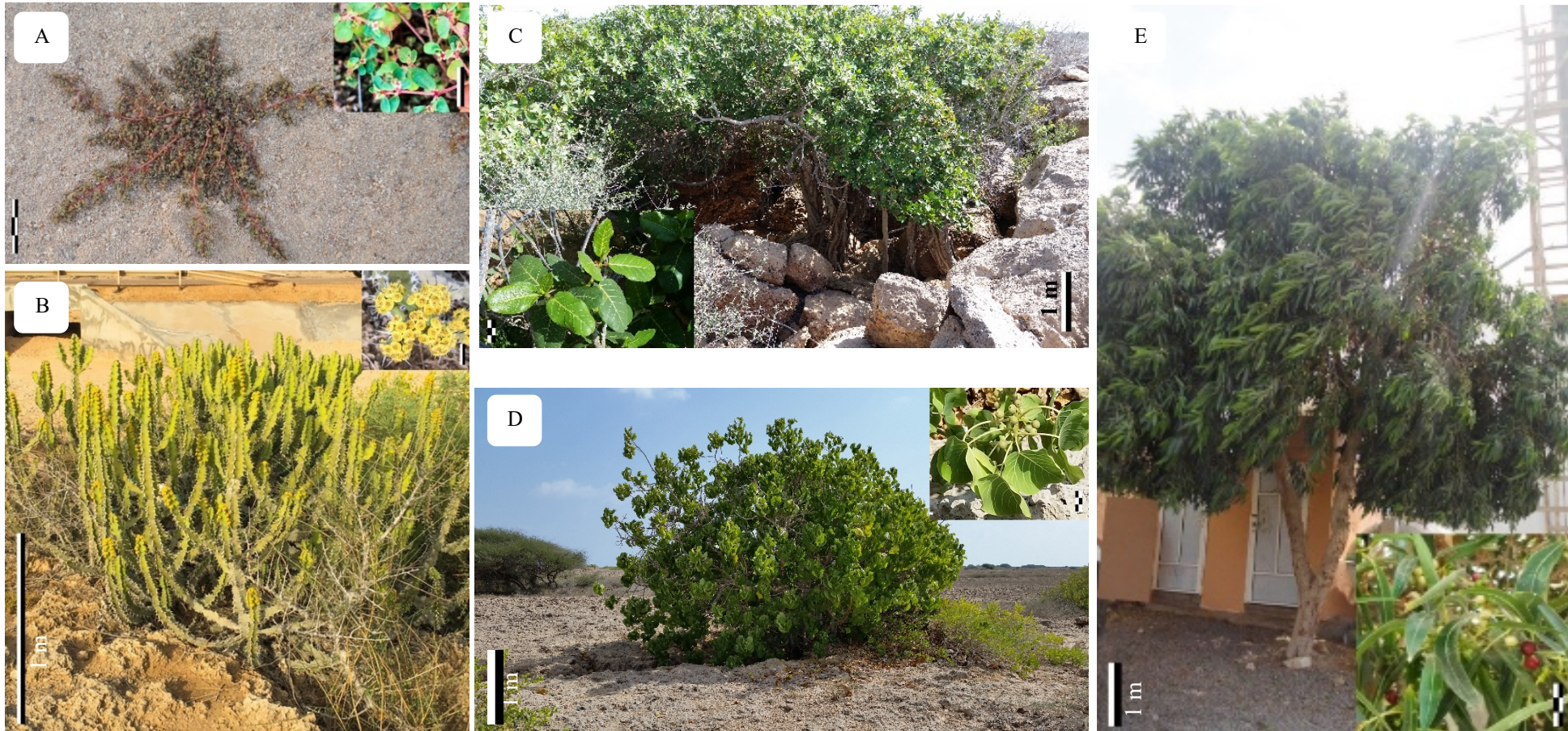
Figure 2-4e. Photographs of the study group species sampled from the field, growth habit, flowers and/or fruits (insets). **(A)** *Euphorbia granulata*; **(B)** *Euphorbia colenetteae*; **(C)** *Ficus glumosa*; **(D)** *Ficus populifolia*; **(E)** *Ficus salicifolia* .One unit of the scale bar = 1 cm (except where marked).
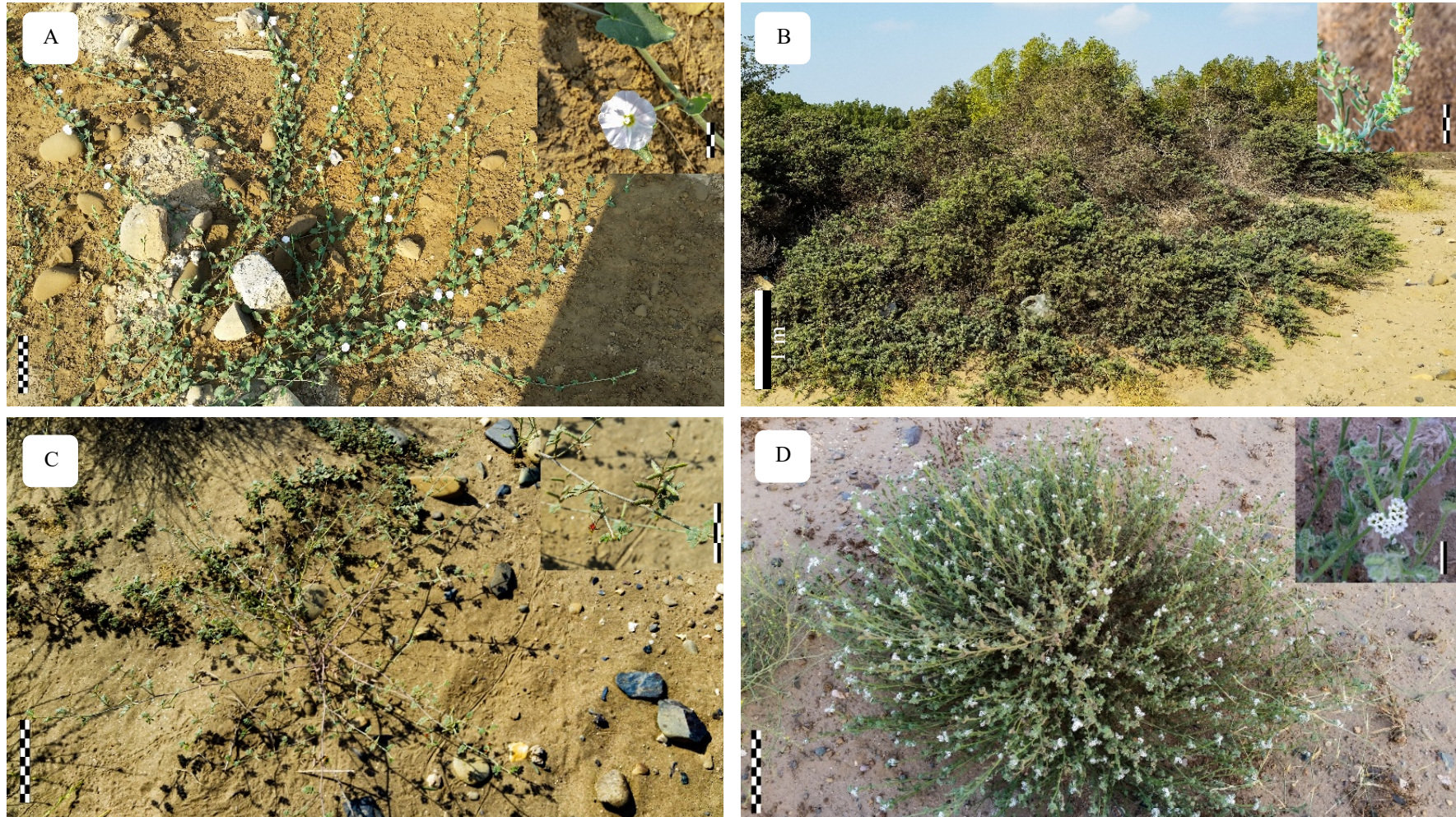
Figure 2-5. Photographs of species collected from Saudi Arabian mainland, growth habit, flowers and/or fruits (insets). **(A)** *Convolvulus* sp.; **(B)** *Suaeda monoica*; **(C)** *Indigofera argentea*; **(D)** *Heliotropium pterocarpum.* One unit of the scale bar = 1 cm (except where marked).

2.3.2 Field observations

The vegetation of the Farasan Islands faces biological and anthropogenic pressures, which have increased significantly in recent years, particularly on the main islands, Farasan Alkabir and Sajid (Hall et al., 2010; Thomas et al., 2010). The major threats that were observed during the field trips are off-road traffic, invasive species, such as *Prosopis juliflora*, urbanisation, development projects and drought (Figure 2-6).

Figure 2-6. Major threats to plant wildlife in the Farasan Islands observed during the field trips. **(A & B)** off-road traffic; **(C)** invasive species such as *Prosopis juliflora* (photos were taken at Al-Muharraq area; **(D)** development projects (the photo for the collage construction in Farasan Alkabir Island); **(E & F)** drought in Wadi Matar (**E**), locality of *Dinebra somalensis* N.W Farasan Village, Farasan Alkabir Island (**F**).

The targeted species for the conservation assessment occur mainly on Farasan Alkabir Island (Al-Muharraq area, Khallah Bay, the north-western region of the island), Sajid Island, Dumsuk Island and Dawshak Island (Table 2-5). All these locations were visited, with the exception of Khallah Bay (locality of *Rorida brachystyla*), due to the rugged terrain and the site's remoteness. Although several visits were made to the Wadi Matar and Al-Muharraq area—the locality of *Basilicum polystachyon*, *Micrococca mercurialis* and *Vahlia digyna*— in the southern region of Farasan Al Kabir Island, none of these species were found. *Dinebra somalensis*, which was recorded from the clay pan to the north-west of Farasan village, was not found either, as the location had suffered severe drought (Figure 2-6, F). Limited exploration was conducted on Dumsuk and Dawshak Islands (the locality of *Commiphora* aff. *kataf)*. Species of *Euphorbia collenetteae* and *Glossonema* sp. aff. *boveanum*, which occur on Sajid and Farasan Alkabir Islands, were found in good condition and were the most frequently recorded and observed species within the group.

Table 2-5. Farasan-restricted species under investigation.

No.	Species name	Species locality based on Collenette (1999)
Endemic		
1	*Commiphora* aff. *kataf*	Dumsuk Island and Dawshak Island.
2	*Glossonema* sp. aff. *boveanum*	Farasan Alkabir Island and Sajid Island
Species not known from any part of Arabian Peninsula		
3	*Basilicum polystachyon* (L.) Moench	*Vachellia* woodland, Al-Muharraq area, Farasan Alkabir Island.
4	*Dinebra somalensis* (Stapf) P.M.Peterson & N.Snow	Salty clay pan among *Salvadora persica* 10km N.W. of the Farasan village, Farasan Alkabir Island
5	*Euphorbia collenetteae* D.Al-Zahrani & El-Karemy	Northwest Farasan Alkabir Island, Sajid Island, Dumsuk Island and Dawshak Island.
6	*Vahlia digyna* (Retz.) Kuntze	*Vachellia* woodland, Wadi Matar near Al-Muharraq area, Farasan Alkabir Island.
Species not known from any part of Saudi Arabia		
7	*Micrococca mercurialis* (L.) Benth.	Damp sand among the palm trees, Al-Muharraq area, Farasan Alkabir Island.
8	*Rorida brachystyla* (Deflers ex Franch.) Thulin & Roalson	Kallah Bay, west Farasan Alkabir Island.

2.4 Discussion

The field trips were conducted with the aim of sampling materials for DNA extraction of the study group species from all habitat types throughout the islands, and observation of potential threat and recording the distribution and habitats of eight rare species (Farasan restricted species) to facilitate later conservation assessment. It was particularly valuable to conduct the field trips during different seasons, in terms of collecting as many species of interest as possible. The annual species were mostly collected during the second and third trips, which took place during the winter, and particularly during the third trip, which was conducted after the rainy season. The first trip's collection, having been conducted during the summer, was composed primarily of trees, shrubs, semi-shrubs and perennial herbs. The severe drought that was observed in the southern part of the Farasan Alkabir Island during all three trips may account for the difficulty in finding *Basilicum polystachyon, Micrococca mercurialis* and *Vahlia digyna* (further discussion about rare species and the current threat observed during the trips will be included in Chapter 6, which focuses on IUCN Red listing).

Thirteen out of 36 species were not found in the wild, among which were perennial herbs (4 spp.), annual herbs (4 spp.), shrublet (4 spp.), and shrubs or small trees (1 spp.). This may be due to various reasons: the targeted species may grow on other islands in the archipelago that were not visited. For example, the island localities of the following species have not been determined in the literature: *Convolvulus piloselifolius* (Wood et al., 2015), *Tetraena propinqua* (syn. *T. boulosii*) (Hall et al., 2010), *Tetraena hamiensis* var. *mandavillei* (Alzahrani & Albokhari, 2018) and *Indigofera coerulea* var. *occidentalis* (Alfarhan et al., 2005). In general, no coordinate data were provided for any of the Farasan Islands species. Some of these may be rare species; for example, *Tetraena hamiensis* var. *mandavillei* and *Indigofera coerulea* var. *occidentalis* are each recorded only by a single author, which may indicate these species' rarity. Another possible reason may be that the Farasan Alkabir and Sajid Islands are experiencing rapid development and increasing urbanisation, which may negatively influence the presence and abundance of the species in these islands. *Suaeda monoica*, which is a shrub or a small tree, was recorded on the beaches of Farasan Alkabir Island in all earlier studies of the islands' vegetation. (El-Demerdash, 1996; Alwelaie et al., 1993; Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Hall et al., 2010; Thomas et al., 2010; Al Mutairi et al., 2012). However, the species was not found on the beaches that were visited, including Cottage, Alghadeer, Al-Faqwah and Husais. These beaches are experiencing high anthropogenic pressure from land clearance for the development of tourism projects and off-road traffic that has severely damaged the salt marches. Another reason for missing these species may be that

the timing of the visits did not coincide with the species' growth season, as was the case with *Indigofera hochstetteri*, which flowers and fruits from August to October (Tropicos. org. Missouri Botanical Garden, 2011).

Herbaria are a valuable source of rare species, endangered local endemics, and species collected from places that are currently difficult to access (Zeng et al., 2018). It is disappointing that the collections of Farasan plants reported in Alfarhan et al. (2002) and Atiqur Rahman et al. (2002), which were supposed to be available in KSU, RIY and KAUS, were not found. In this study, four out of 14 missing species were found and obtained from local and international herbaria: *Cyperus bulbosus, Heliotropium pterocarpum, Indigofera hochstetteri* and *Tetraena boulosii* (Table 5-2). However, the specimens of *T. boulosii* and *H. pterocarpum* were found to have been misidentified; the correct identifications are *Tetraena coccinea* and *Heliotropium ramosissimum*. *Tetraena coccinea* (Chaudhary, 2001) and *Heliotropium ramosissimum* (Akhani & Förther, 1994) are taxonomically challenging because they are very morphologically diverse. According to Chaudhary (2001), the samples in Saudi Arabia that were identified as *T. boulosii* are often other forms of *Tetraena coccinea*. Thus, in this study herbaria played a modest role in completing study group.

2.5 Summary

The field expeditions resulted in two new records for the Farasan flora *Indigofera spiniflora* and *Cyperus rotundus*. Twenty-five out of 36 species of the phylogenetic analysis study groups were sampled for DNA extraction: 23 from the field and two from the herbaria. Despite the importance of herbarium specimens in enhancing fieldwork sampling, in this study they played a modest role in completing study group species, since a significant portion of the specimens that were supposed to be available in the local herbaria (KSU, RIY and KAUS) were not found. The reasons that the group was not completed may include that we failed to visit during the growth season that some species grow on unvisited islands or are negatively affected by the threats observed during the three expeditions. Off-road traffic, invasive species, such as *Prosopis juliflora*, urbanisation, development projects and drought are the ma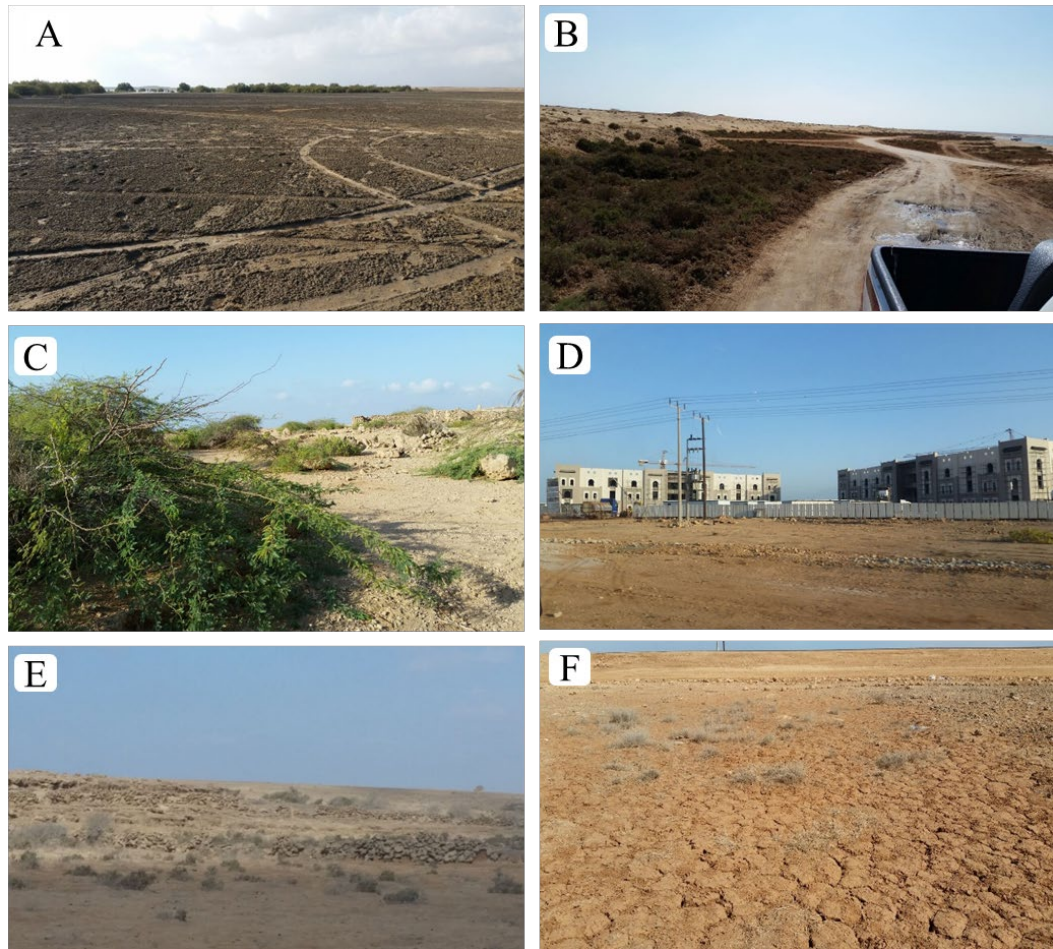jor threats to plant wildlife in the Farasan Islands that were observed during the field trips. Distribution data and the population conditions of *Commiphora* aff. *kataf, Euphorbia collenetteae* and *Glossonema* sp. aff. *boveanum* were the best recorded and observed among the species of interest for conservation status assessment.

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CHAPTER 3: Phylogenetic affinities and divergence time of the Farasan Islands mangroves: *Avicennia marina* **(Forssk.) Vierh.** **and** *Rhizophora mucronata* **Lam.**

3.1 Introduction

Mangrove is one of the most productive ecosystems in the world, supporting coastal fisheries, yielding commercial forest products, protecting coastlines and serving as nursery habitats for many coastal species (Alongi, 2002; Kathiresan & Bingham, 2001). It occupies the tropical and subtropical intertidal zones of the marine coastal environment, covering 60% -75% of the shores in the tropics with a total area of 152,000 km^2 (Tomlinson, 2016; Spalding et al., 2010). Mangroves exhibit highly specialised morphological and physiological adaptations for coping with salinity, regular inundation by tides and saturated soils, such as foliage salt excretion, exposed breathing roots, extensive support roots, high intercellular salt concentrations and water-borne propagules (Duke, 2017). It is troubling, then, that this ecosystem is 'one of the world's threatened major tropical environments,' having experienced a 35% loss in the past two decades due to anthropogenic activities (Valiela et al., 2001). Mangrove vegetation comprises a diverse group of shrub and tree species, with approximately 69 species and 11 hybrids belonging to 18 families and 32 genera (Duke, 2017). They are distributed between 30°N and 30°S (Giri et al., 2011) and are restricted to two major floral realms: the Indo West Pacific (IWP) and the Atlantic East Pacific (AEP). The IWP extends from East Africa to the central Pacific Ocean, and the AEP includes the Americas and West Africa (Spalding et al., 2010). The extent of the distributional range is limited by many factors that prevent water-borne gene dispersal, such as land barriers, cold temperatures (20° C isotherm of seawater in winter) and broad water expanses (Alongi, 2009; Duke, 2017).

The Red Sea is the northern-most limit of the distribution range of the IWP mangroves (Figure 3-1). The harsh conditions prevailing in the sea, such as high salinity (36–40 ppt), extreme water temperatures (12°C – 40°C), low rainfall and no permanent freshwater inputs (Bruckner et al., 2012, Mandura et al., 1987), have led to habitat fragmentation and low population density (Kumar et al., 2010). The southern part of the Red Sea is more favourable for mangrove stands than the northern part due mainly to more wadis, higher precipitation and nutrient levels, less saline waters and tropical climate (Khan et al., 2010; Saifullah, 1996). Two species of mangroves occur in the Red Sea: *Avicennia marina* (after Duke (1991) Arabian populations assigned to *A. marina* var. *marina* (Dodd et al., 1999)) and *Rhizophora mucronata* (El Shaer, 2014). *Avicennia marina*, a pioneer species adapted to extreme environmental conditions (MacNae, 1969), is the dominant species found in all coastal swamps in the area, while *R. mucronata* is less widespread and is reported to coexist with *A. marina* in 11 locations (Bruckner et al., 2012).
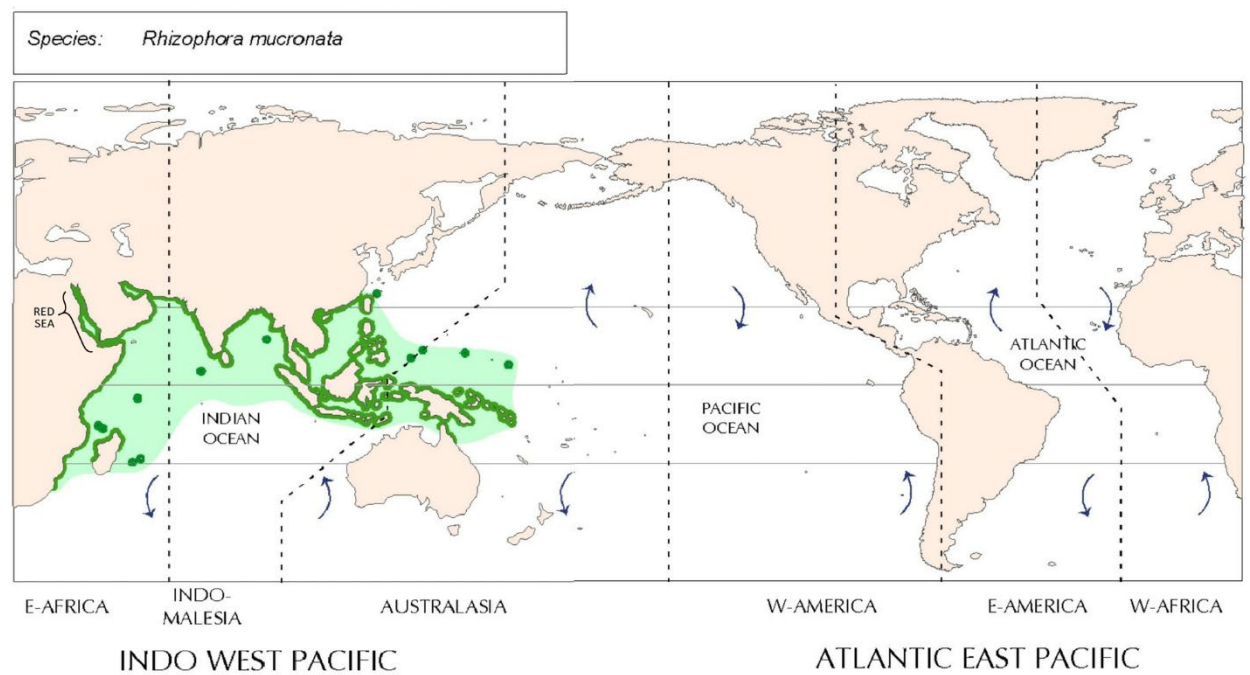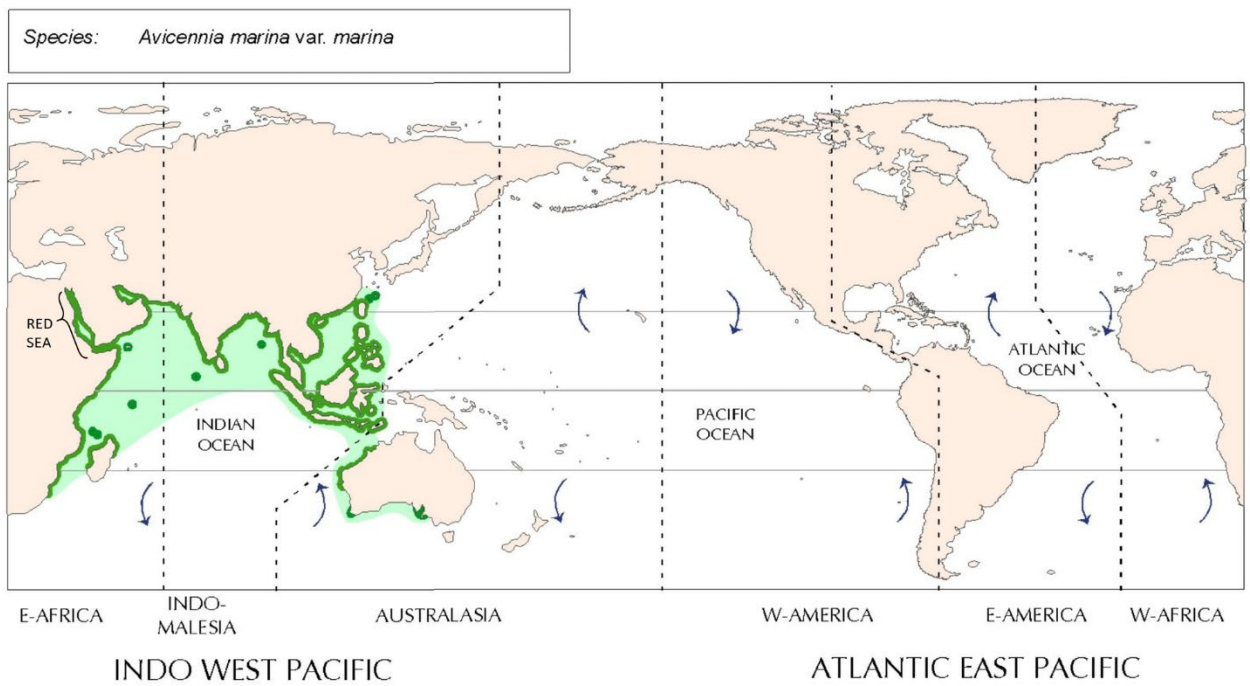
Figure 3-1. Distribution of mangrove species *A. marina* var. *marina* and *R. mucronata* (modified from Duke (2014))

Historically, the occurrence of mangroves in the Red Sea has likely been affected by sea level fluctuation following climatic oscillations in the Quaternary (2.6 – 0 million years ago). The Red Sea is a marginal water mass whose current circulation pattern through the narrow and shallow sill Bab-el-Mandeb is driven by the winter and summer monsoons (Siddall et al., 2004; Biton et al., 2008). During the glacial cycles of the Pleistocene, low sea levels culminated in coastlines 115–130 m below the current level, reaching a minimum during the Last Glacial Maximum (LGM) c. 17,000 –19,000 years BP. As a result, the Red Sea's shallow marine habitats deteriorated to an extreme degree during the glacial cycles due to insufficient water flow through Bab-el-Mandeb, the only natural gateway to the Red Sea; this deterioration is associated with shifts in salinity (>50‰) and temperature (Ludt & Rocha, 2015). Evidence from dated molluscan shells accompanied by characteristic mangrove molluscs *Terebralia palustris* indicate that mangrove was present on the Egyptian shores in the Last Interglacial and likely disappeared during the climatic deterioration of the LGM; modern mangrove analogues were found only outside the Red Sea, in the Gulf of Aden and near Muscat (Oman) (Ludt & Rocha, 2015; Plaziat, 1995). Mangroves, then, flourished in the Red Sea after the postglacial sea level rise in the middle Holocene c. 6000 years BP (Mayer & Beyin, 2009; Plaziat et al., 1995; Plaziat, 1995; Tosi, 1986; Vermeersch et al., 2005).

The Farasan Islands in the south-eastern part of the Red Sea hosts the best examples of mangrove habitat in Saudi Arabia (Hall et al., 2010), which is the foremost factor in qualifying the islands as a Marine Protected Area (MPA) (Gladstone, 2000) and an Important Plant Area in the Arabian Peninsula (Hall et al., 2010). However, the accelerating infrastructure development in the archipelago has led to many healthy mangrove areas becoming endangered communities (Mandura & Khafaji, 1993). This pressure, coupled with global climate change and rising sea levels (AlRashidi et al., 2012), may lead to an increase in the loss of the islands' mangrove populations which play a significant role in the conservation of biological diversity on the islands. The aerial roots of mangroves (Figure 3-2, A & B) are ideal sites for the breeding of fishes, crabs, prawns and shrimps (figure 3-2, C & D) and are a refuge for many small animals and rich communities of algae and microorganisms (Mandura et al., 1987; Alfarhan et al., 2002; Mohamed & Al-Shehri, 2015; Ameen et al., 2016). Mangrove also serves as an important breeding and nesting site for both shorebirds and seabirds (Figure 3-2, E) (Newton & Symens, 1996; PERSGA, 2004), and provides shelter for gazelles (Assaeed et al., 1995). These mangrove forests are also valuable economic resources of timber and camel fodder during drought seasons (Muftah, 1997).

Avicennia marina is the most abundant mangrove species in the Farasan Islands and occupies relatively large areas, such as channels, sheltered coastal flats and creeks (Hall et al., 2010), forming pure stands or mixed with *R. mucronata*. The extensive mixed patches of *A. marina* and *R. mucronata* show clear zonation (Figure 3-2, F) and occur around the large lagoons in Khawr Al-Qandal in northeastern Farasan Alkabir Island (Figure 3-2, G) and north-western Zifaf Island. The likely reason for the limited unique distribution of *R. mucronata* is the nature of the lagoons. They are almost entirely enclosed, with only a small opening to the outside environment, providing an extremely sheltered environment with soft muddy substratum that forms a suitable protected area for germination of *Rhizophora* seedlings (Mandura et al., 1987). *Avicennia* and *Rhizophora* produce water-borne propagules, namely cryptoviviparous and viviparous respectively (Duke, 2006). The flowering and fruiting periods are confined to spring and summer seasons. The flowers and young fruits of *A. marina* appear in March and bloom during June, July and August, by October, all flowers and fruits disappear from the trees. In *R. mucronata*, flowers and fruits are copious in August; by January, viviparous seedlings are abundant on the trees, but no flowers remain (Mandura et al., 1987).

The study of marginal populations of mangrove is of great importance because they may harbour local adaptation (Zhou et al., 2011; Arnaud-Haond et al., 2006). Previous mangrove studies of the Red Sea region focused on population genetics (Sabri et al., 2018), identification and analysis of microRNAs (Khraiwesh et al., 2013), and many were ecologically driven (Mandura et al., 1987; Mandura & Khafaji, 1993; Saifullah, 1996; Mandura, 1997; Ahmed, 2007; Khan et al., 2010; Kumar et al., 2010, 2011; El Shaer, 2014; Khalil, 2015; Al-Hammad, 2016; Almahasheer et al., 2016; Eid et al., 2016; Alzahrani et al., 2018). The origins and affinities of the Mangal vegetation in the area have received less attention; Hassan & Al-Hemaid (1996) assumed that the Farasan Islands mangroves have a closer link with the populations of the Arabian mainland, which are mostly of African origin. This biogeographic hypothesis was inferred based on the short distance between the islands and the western coast of the Arabian Peninsula (c. 40 km) but has not yet been investigated using modern molecular methods. Although comprehensive phylogenies have been carried out for *Avicennia* and *Rhizophora* (Lo et al., 2014; Li et al., 2016), no samples from the Farasan Islands or the Red Sea basin were included.

This study is the first to incorporate *A. marina* and *R. mucronata* from the Farasan Islands in global phylogenies and to infer molecular divergence age estimates for the Red Sea mangroves. The study aims to determine the placement of *A. marina* and *R. mucronata* from the Red Sea among the IWP region and to obtain a more detailed picture of the origin and timing of diversification events of the Red Sea mangroves after the opening of the basin.
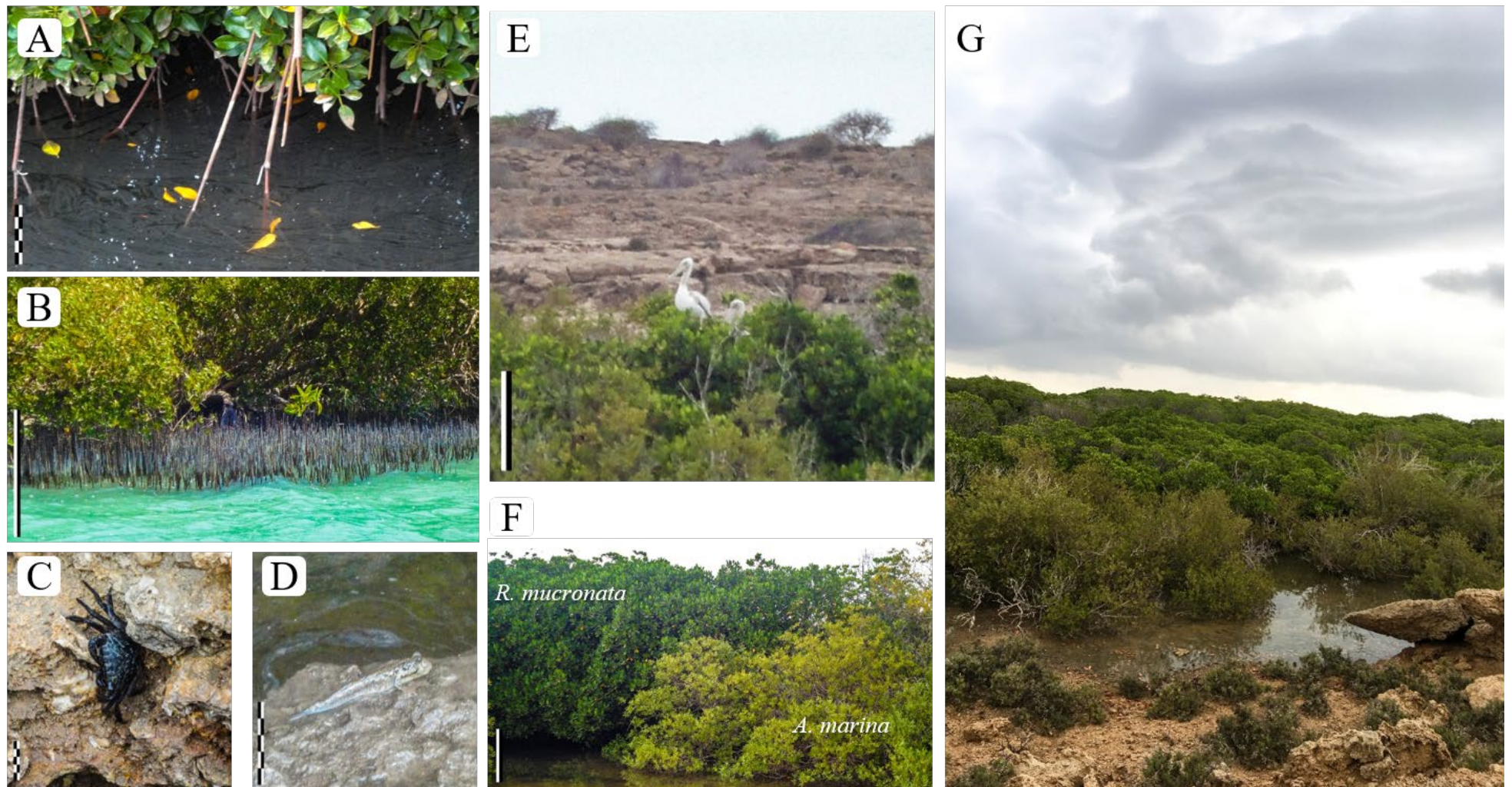
Figure 3-2. Mangrove ecosystem in the Farasan Islands (A) Stilt root of *R. mucronata*; **(B)** The pneumatophores of *A. marina.* Associated fauna of mangrove habitat **(C)** Crab, **(D)** Mudskipper & **(E)** Pelican nested in mangrove forest in Khawr Al-Qandal; **(F)** Clear zonation in mangrove belt *A. marina* toward the sea *R.mucronata* landward; **(G)** Dense and rich mixed stand of *A.marina* and *R.mucronata* in in Khawr Al-Qandal, NE of Farasan Alkabir. One unit of the scale bar = 1cm in A, C, D/ 1 m in B, E, F.

3.2 Materials and Methods

3.2.1 Taxon sampling

Multiple accessions of *A. marina* and *R. mucronata* were sampled from the Farasan Islands and adjacent areas (where possible), whether from field collection or herbarium specimens. Eight individuals of *A. marina* were sampled: three from the Farasan Islands, three from other areas of the Red Sea (Egypt, Eritrea and Saudi Arabia) and two from the Arabian Gulf (Kuwait and Al-Quatif). *Rhizophora mucronata* was sampled only from the Farasan Islands (three accessions) and no samples were available from other sites in the Red Sea; voucher information is listed in Appendix 3-2. Newly generated sequences in this study were added to previously published data from Nettel et al. (2008), Li et al. (2016) and Sahu et al. (2016) for *Avicennia* and in Lo et al. (2014) for *Rhizophora*. Seventy and fifty-four DNA accessions of *Avicennia* and *Rhizophora*, respectively, were downloaded from GenBank, representing all species for each genus and the outgroup. Details of taxon names, accession numbers and publications are shown in Appendix 3-1.

3.2.2 DNA region selection

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA, *trnD-trnT* and *trnH* intergenic spacers of cpDNA were widely used in the molecular studies of *Avicennia*, providing a rich source of data of the extant species worldwide. The phylogeny of *Avicennia* was reconstructed based on these three markers with *Thunbergia grandiflora* serving as the outgroup in order to investigate the placement of the Red Sea populations. In addition, six nuclear DNA regions of the 25 genes used by Li et al. (2016), were chosen for further phylogeny building among IWP taxa, with *A. germinans* as the outgroup. This was done to test the utility of multiple nuclear data in distinguishing between the populations in the Red Sea. Gene names and IDs are as follows: Serine-threonine kinase receptor-associated protein gene (0256), Shaggy-related protein kinase alpha gene (0259), Abscisic acid receptor PYL9 gene (0347), Heat shock 70 kda protein 14 gene (c099), Alpha-glucan phosphorylase H isozyme gene (c121) and Thioredoxin H-type 1 gene (c138).

For *Rhizophora*, intergeneric spacers of chloroplast DNA (*trnH-rpl2* and *trnS-trnG)* and ITS were chosen to reconstruct the phylogeny with *Bruguiera gymnorrhiza* serving as the outgroup following Lo et al. (2014) as it is the most extensive study that samples the entire geographical distribution of all *Rhizophora* species.

3.2.3 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 350 mg of silica-dried leaves and herbarium-dried leaves using modified cetyltrimethylammonium bromide (CTAB) extraction protocol based on Sahu et al. (2012). For the herbarium specimens, the extraction protocol has been modified to include one-week isopropanol precipitation. Plant tissues were ground two or more times in a 2 ml Eppendorf tube with a small amount of sand and two tungsten beads (QIAGEN 69997) using the Qiagen TissueLyser II (QIAGEN, Hilden, Germany) at 30 Hz for 45 seconds. DNA extractions were stained with GelRed™ (Biotium, Fremont, CA, USA) and visualised under UV light using T:Genius gel documentation (Synoptics Ltd, Cambridge, UK) following gel electrophoresis on a 0.7% agarose gel (Fisher Scientific Midi Submarine Gel) in 1x TAE buffer PH 8.0; photographs were taken to record the extractions. DNA size and concentration were determined using HyperLadder™ 1kb (Bioline Reagents Ltd., London, UK) for marking. Concentration and quality were also determined using spectrophotometry (NanoDrop™ Lite, Thermo Fisher Scientific Inc., Waltham, MA, USA). Fifty-microliter (50 µl) aliquots of 2–10 ng/µl were prepared for polymerase chain reaction (PCR) amplification and stored at -20°C until required.

PCR was done using the Applied Biosystems Thermal Cycler SimpliAmp™ (Thermo Fisher Scientific Inc.), and Gradient PCRs were performed for some samples in order to gather optimum annealing temperatures using the Eppendorf Mastercycler® gradient (Eppendorf, Hamburg, Germany). ITS in the degraded herbarium materials of *A. marina* amplified in two segments using internal primers. Tables 3-2 and 3-3 show the details of primer sequences, PCR reactions and cycling conditions for each marker for *A. marina* and *R. mucronata*, respectively. Ten percent (10%) DMSO (dimethyl sulfoxide, Sigma-Aldrich) was added to nrDNA region reactions, and 0.5 µl of 100 mg/ml BSA (bovine serum albumin, Sigma-Aldrich) was added to cpDNA regions to enhance the PCR reaction. The samples that failed to amplify were repeated at lower stringency (the annealing temperatures lowered by 2–4°C); whereas samples that produced multiple PCR products were repeated at 2–4°C higher than the original annealing temperature. Restorase® DNA Polymerase (Sigma-Aldrich), an enzyme designed to repair damaged DNA, was also investigated with unsuccessful samples, following the supplier's instructions.

The PCR products were separated on 1% agarose gels in 1x TAE buffer stained with GelRed™, visualised under UV light. Approximate size and concentration of the PCR products were determined by comparison using Bioline's HyperLadder™ 1kb as a marker. PCR products were

purified and sequenced by Eurofins Genomics (Cologne, Germany); each region was sequenced
bi-directionally using the same primers as the amplification primers.

Table 3-2. List of Polymerase Chain Reaction (PCR) Primers, Master Mix recipe and cycling conditions in
Avicennia.

Region ID	Primer sequences 5'-3'	Master Mix recipe	cycling conditions (Li et al., 2016)
trnD-T	Forward trnD : ACCAATTGAACTACAATCCC	**20 µl volume using:**	94°C for 4 min;
	Reverse trnT : CTACCACTGAGTTAAAAGGG	1 µl of genomic template DNA (10-50 ng)	30 cycles of 94°C for 30 sec, 55°C
	(Demesure et al., 1995)	10 µl 2x BioMix Red	for 30 sec, and 72°C for 2 min;
		1 µl of each primer (10 mM)	final extension 72°C for 8 min.
		6.5 µl Milli-Q water	
		0.5 µl BSA	
trnH	Forward trnH: ACGGGAATTGAACCCGCGCA	**20 µl volume using:**	94°C for 4 min;
	Reverse trnK: CCGACTAGTTCCGGGTTCGA	1 µl of genomic template DNA (10-50 ng)	30 cycles of 94°C for 30 sec, 53.5 °C
	(Demesure et al., 1995)	10 µl 2x BioMix Red	for 30 sec, and 72°C for 2 min;
		0.75 µl of each primer (10 mM)	final extension 72°C for 8 min
		7.5µl Milli-Q water	
ITS	ITS-I: GTCCACTGAACCTTATCATTTAG	**15 µl volume using:**	95°C for 2 min;
	(Urbatsch et al., 2000)	2 µl of genomic template DNA (10-50 ng)	30 cycles of 95°C for 20 sec, 55°C
	ITS4: TCCTCCGCTTATTGATATGC	7.5 µl 2x BioMix Red	for 30 sec, and 70°C for 1 min;
	(White et al., 1990)	0.5 µl of each primer (10 mM)	final extension 72°C for 7 min.
	Internal primers:	4 µl Milli-Q water	
	ITS-A: GAAGGAGAAGTCGTAACAAGG	0.5 µl BSA	
	ITS-B: CTTTTCCTCCGCTTATTGATATG		
	ITS-C: GCAATTCACACCAAGTATCGC		
	ITS-D: CTCTCGGCAACGGATATCTCG		
	(Blattner, 1999)		
	In one part: A+B		
	In two parts: A+C/D+B		
0259	F: TCTCGCCAGGAAACAGAGGC	**15 µl volume using:**	94°C for 4 min;
	R: CTTTGTCGTATGTCCATCGTGGTA	2 µl of genomic template DNA (10-50 ng)	30 cycles of 94°C for 30 sec, 62.3°C
	(Li et al., 2016)	7.5 µl 2x BioMix Red	for 30 sec, and 72°C for 2 min;
		0.4 µl of each primer (10 mM)	final extension 72°C for 8 min
		4.7 µl Milli-Q water	
0347	F: CACGCTTCCAATCTTTAGATCACCCT	**15 µl volume using:**	94°C for 4 min;
	R: GGAGACACCACAGGCACCAACC	2 µl of genomic template DNA (10-50 ng),	30 cycles of 94°C for 30 sec, 60.6°C
	(Li et al., 2016)	7.5 µl 2x BioMix Red	for 30 sec, and 72°C for 2 min;
		0.4 µl of each primer (10 mM),	final extension 72°C for 8 min
		4.7 µl Milli-Q water	
c099	F: GCAGCAAGTCCTCCATAGACAACCT	**15 µl volume using:**	94°C for 4 min;
	R: GATACTGGTCCACCTGCCACAAA	2 µl of genomic template DNA (10-50 ng)	30 cycles of 94°C for 30 sec, 56°C
	(Li et al., 2016)	7.5 µl 2x BioMix Red	for 30 sec, and 72°C for 2 min;
		0.4 µl of each primer (10 mM)	final extension 72°C for 8 min
		4.7 µl Milli-Q water	

Table 3-2. Continued.

Region ID	Primer sequences 5'-3'	Master Mix recipe	cycling conditions (Li et al., 2016)
c121	F: GTGCTGAGTATCGACATCTTTATCC R: ATGAAGTTCCTCGGTTGCGTA (Li et al., 2016)	**15 µl volume using:** 2 µl of genomic template DNA (10-50 ng), 7.5 µl 2x BioMix Red 0.4 µl of each primer (10 mM), 4.7 µl Milli-Q water	94°C for 4 min; 30 cycles of 94°C for 30 sec, 51°C for 30 sec, and 72°C for 2 min; final extension 72°C for 8 min
c138	F: TGGCACCAACAAGCCTGTCAAT R: TCCAGAAGGGCGTCGAGTCTAA (Li et al., 2016)	**15 µl volume using:** 2 µl of genomic template DNA (10-50 ng), 7.5 µl 2x BioMix Red 0.4 µl of each primer (10 mM), 4.7 µl Milli-Q water	94°C for 4 min; 30 cycles of 94°C for 30 sec, 51°C for 30 sec, and 72°C for 2 min; final extension 72°C for 8 min

Table 3-3. List of Polymerase Chain Reaction (PCR) Primers, Master Mix recipe and cycling conditions in *Rhizophora*.

Region ID	Primer sequences 5'-3'	Master Mix recipe	Thermocycler conditions (Lo et al., 2014)
trnH-rpl2	F: CGGATGTAGCCAAGTGGATC R: GATAATTTGATTCTTTCGTCGCC (Vaillancourt & Jackson, 2000)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng), 10 µl 2x BioMix Red 0.6 µl of each primer (10 mM), 7.3 µl Milli-Q water 0.5 µl BSA	94°C for 5 min; 35 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 90 sec; final extension 72°C for 7 min.
trnG-trnS	*trnS*: GCCGCTTTAGTCCACTCAGC *trnG*: GAACGAATCACACTTTTACCAC (Hamilton, 1999)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng), 10 µl 2x BioMix Red 0.75 µl of each primer (10 mM), 7.3 µl Milli-Q water 0.2 µl MgCl$_2$	94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 45sec, and 72°C for 90 sec; final extension 72°C for 7 min.
ITS	ITS5: GGAAGTAAAAGTCGTAACAAGG ITS4: TCCTCCGCTTATTGATATGC	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng), 10 µl 2x BioMix Red 0.6 µl of each primer (10 mM), 7.8 µl Milli-Q water	95°C for 3 min; 35 cycles of 95°C for 30 sec, 52.3°C for 30 sec, and 72°C for 1min; final extension 72°C for 10 min.

3.2.4 Sequence editing and alignment

Sequence trace files for forward and reverse sequences were checked for quality, assembled and edited using SeqMan Pro™ 15.2.0 (DNAStar, Inc., Madison, WI, USA). Contigs were examined using the Basic Local Alignment Search Tool (BLASTn) in GenBank to detect any potential problems of contamination. Multiple sequence alignments were then built for each genus using the MUSCLE algorithm (Edgar, 2004) implemented in AliView version 1.19 for

windows (Larsson, 2014). The beginning and end of the alignment were excluded from the analyses where base callings were ambiguous. The newly generated sequences were deposited in the GenBank, the accession numbers shown in Appendix 3-2.

3.2.5 Phylogenetic analysis and Bayesian divergence time estimation

Phylogenetic trees were constructed through Bayesian inference analysis (BI) using MrBayes v. 3.2.6 (Huelsenbeck & Ronquist., 2001; Ronquist & Huelsenbeck, 2003) in the CIPRES Science Gateway 3.3 (Miller et al., 2010). For each marker, the best fitting model of evolution was calculated with jModelTest 2.1.10 (Darriba et al., 2012) and selected using Akaike's information criterion (AIC) (Akaike, 1974). All BI analyses were conducted with two separate runs, each of four Markov chain Monte Carlo (MCMC) chains. The analysis for individual partition and the combined dataset were run for 5 million and 10 million generations respectively, sampling every 10,000th replicate based on tests for autocorrelation of tree length using the Excel CORREL function. Trees from the first 25% of the sampled generations were discarded as burn-in. Convergence of runs was tested by inspecting whether the standard deviation of split frequencies of the runs was <0.01 and by using the effective sample sizes (ESS) as calculated with Tracer v1.7.1 (Rambaut et al., 2018), considering ESS values greater than 200 for all parameters as good evidence. The Combinable component consensus tree, which shows the best supported clades, including those with low (<50%) support, was generated in BayesTrees v.1.3 (Meade, 2011). The incongruence among different partitions was assessed with the incongruence length difference (ILD) test (the partition homogeneity test) (Farris et al., 1994) in PAUP* 4 (Swofford, 2002).

Bayesian dating analysis was conducted in BEAST2 v 2.5.0 (Drummond & Bouckaert, 2015) on the CIPRES portal (Miller et al., 2010). BEAST2 package bModelTest (Bouckaert & Drummond, 2017) was used to explore substitution model space while simultaneously estimating model parameters and the phylogeny. Model parameters were averaged over visited substitution models and weighted given the support of each model. An uncorrelated lognormal (UCLN) relaxed clock model was used to allow rate variation/independence across branches. A Calibrated Yule tree prior was specified to model speciation that assumes a constant lineage birth rate for each branch in the tree. Four independent runs of 50 million generations sampling every 10,000th generations were carried out. Log files were first analysed individually with Tracer v1.7.1 to examine the effective sample size (ESS) for each of the parameters which was adequate, greater than 200, the tree files then combined with LogCombiner v 2.4.7 after the burn-in was removed (set at 25%). The combined postburn-in trees from the independent runs

were then analysed with TreeAnnotator v 2.4.7, and a maximum clade credibility tree (MCC) was chosen which gives the maximum value when the posterior probability of all nodes is summed, the mean node heights is reported for the tree which visualized with FigTree 1.4.3 (Rambaut & Drummond, 2012). The tree was plotted against stratigraphy using the strap package (Bell & Lloyd, 2015) in RStudio v. 1.1.463 (RStudio Team, 2016). Diagram summarizing all estimated lineage divergence times indicated in the MCC chronograms were plotted using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California USA; www.graphpad.com).

3.2.6 Fossil constraints and secondary calibrations

List of calibrated points that assigned to date *Avicenna* and *Rhizophora* phylogenies are shown in Table 3-4.

Table 3-4a. List of fossils priors utilized in BEAST analysis. (Mya= millions of years ago, dis. = distribution).

Constrained node	Fossil name (Organ)	Age (Mya)	Source	Prior dis.	Offset (Mya)	Mean	Log (SD)
Avicennia							
1. *Avicennia* crown	*Avicennia* sp. (pollen)	The early Eocene (c.50)	Plaziat et al. (2001)	Lognormal	50.0	0.5	0.6
Rhizophora							
Rhizophoreae (root)	*Rhizophora* sp. (pollen)	The early Eocene (47.8–56)	Collinson (1983); Graham (2006)	Lognormal	56	1.5	0.5

Table 3-4a. List of secondary priors utilized in BEAST analysis. (Mya= millions of years ago, dis. = distribution).

Constrained node	Age (Mya)	Source	Prior dis.	Mean	Log (SD)
Avicennia					
2. *A. marina* crown	The Pleistocene (2.8)	Duke et al. (1998b); Li et al. (2016)	Normal	2.8	0.85

3.3 Results

3.3.1 Phylogenetic analyses

All silica-dried samples of *Avicennia* were successfully amplified and sequenced for all regions, except the 0256 region, which failed in sequencing. In contrast, none of the herbarium specimens were amplified, except ITS, *trnD-trnT* and *trnH* for the Egyptian accession. In *Rhizophora,* DNA regions for all samples have been amplified and sequenced successfully, except the chloroplast *trnG-trnS*, which failed to amplify and was subsequently treated as missing data (Appendix 3-2). A Summary statistic of datasets, including amplicon length, alignment length, number of taxa, number of variable sites and the best-fit model of nucleotide substitution, are shown in Table 3-5.

Table 3-5. Descriptive statistics of nuclear and chloroplast datasets of *Avicennia* and *Rhizophora.* CP= Chloroplast region, NU= nuclear region.

Genus	Gene ID	Amplicon length	Total aligned length (bp)	No. of taxa	Variable sites	Best-fit model of nucleotide substitution
Avicennia						
Data set 1	*trnD-trnT*	840	814	19	82 (10.7%)	GTR+G
	trnH	767	860	16	96 (11.1%)	GTR
	ITS	700	659	18	159 (24.12%)	GTR+G
	CP + NU	-	2333	21	337 (14.4%)	GTR+G
Data set 2	0259	848	819	11	102 (12.5%)	SYM+G
	0347	711	718	12	89 (12.3%)	SYM+G
	C099	624	575	12	80 (13.9%)	SYM+G
	c121	1129	1171	10	137 (11.6%)	SYM+G
	c138	852	1132	11	139 (12.2%)	SYM+G
	ITS	720	648	11	25 (3.1%)	GTR
	Combined NU	-	5063	13	627 (12.3%)	HKY+G
Rhizophora	*trnH-rpl2*	488	594	22	57 (9.5%)	SYM+G
	trnG-trnS	680	790	18	38 (4.8%)	SYM+G
	ITS	700	640	22	91 (14.2%)	SYM+G
	CP + NU	-	2024	22	186 (9.1%)	HKY+I+G

3.3.1.1 *Avicennia marina*

Two datasets were analysed, in data set one the partition-homogeneity test indicated no significant difference between the sequence data of nuclear ITS and chloroplast intergenic spacers *trnD-trnT* and *trnH* (P>0.05); therefore, the datasets were combined to give a total-evidence phylogeny. A Bayesian inference tree of the whole dataset of *Avicennia* species (Figure 3-3) supported the extant geographical grouping of *Avicenna* species of AEP (*A. bicolor, A. germinans and A. schaueriana*) and IWP (*A. rumphiana, A. alba, A. officinalis, A. integra* and the *A. marina* complex). Among the *A. marina* complex group, the Red Sea and the East African populations formed a monophyletic group without significant support (posterior probability (PP) of 0.65). Within this group, the Red Sea populations (from the Farasan Islands, Saudi Arabia (Jazan) and Egypt) were resolved as monophyletic, but without statistical support (PP=0.46) sister to a strongly supported clade (PP=0.98) consisting materials from Kenya and Madagascar. The African/Red Sea clade was closely related to populations from Malaysia, India and Australia and altogether formed a low supported clade (PP=0.55).

In dataset two, ITS and the nuclear DNA coding genes are congruent (P>0.05), so the nuclear markers concatenated for further analysis of *Avicennia* species of the IWP region. This analysis increased the resolution within the clades (Figure 3-4) and strongly supported the monophyly of the Red Sea lineages (PP=1.00). However, the intraspecific relationships among the Red Sea populations were not fully resolved.
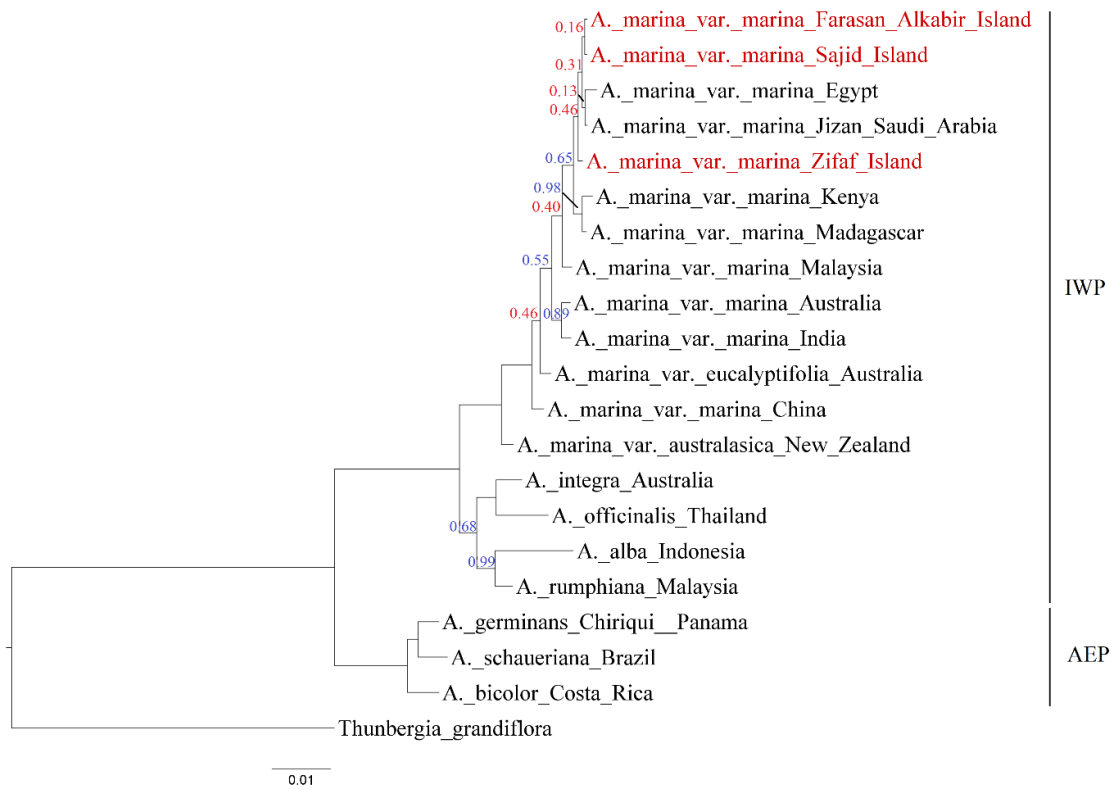
Figure 3-3. Combinable component consensus tree of *Avicennia* based on combined nuclear ITS and chloroplast data set. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red <0.50. Red text signifies the Farasan Islands lineages. Scale bar shows the number of substitutions per site.
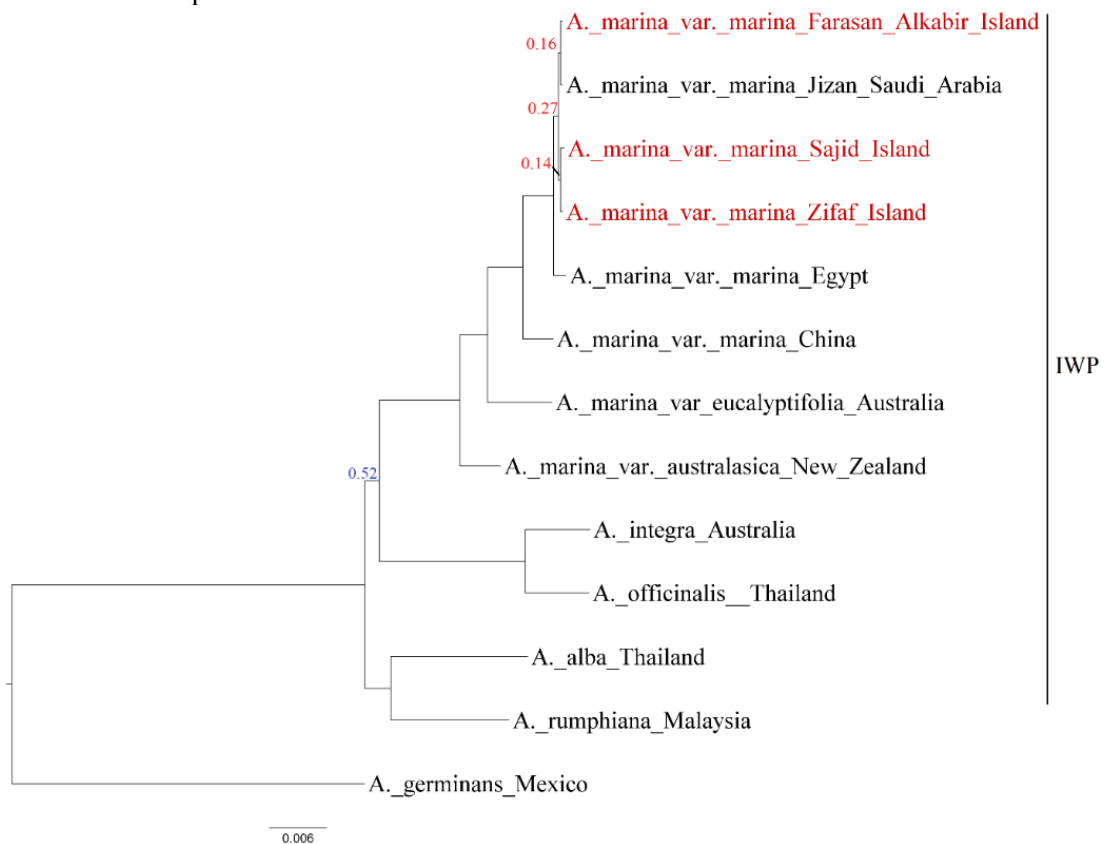
Figure 3-4. Combinable component consensus tree of *Avicennia* based on the combined six nuclear genes. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red <0.50. Red text signifies the Farasan Islands lineages. Scale bar shows the number of substitutions per site.

3.3.1.2 *Rhizophora mucronata*

The partition-homogeneity test indicated no significant difference between the nuclear ribosomal ITS and chloroplast data (P>0.05), and therefore the two datasets were combined for phylogenetic analyses. The combined data BI tree (Figure 3-5) provided better resolution and well-supported clades compared to separate sequence analyses. *Rhizophora* species were divided into two main groups: the IWP clade (PP=0.77) and the AEP clade (PP=1.00). Within the IWP clade, *R. apiculata* diverged first (PP=0.96); *R. stylosa* and *R. mucronata* are sister species (PP=1.00). *Rhizophora mucronata* and *R. stylosa* were not clearly distinguished from one another; instead, they divided based on their geographical distribution. The relationships among this clade were not strongly supported (<0.50). The Farasan Islands and Kenyan populations of *R. mucronata* constituted a monophyletic clade, although without statistical support (PP=0.40), materials from Australia, Japan and the islands of northwest Pacific (Micronesia) were located at the base of this clade. The former clade was sister to a clade that included *R. mucronata* and *R. stylosa* from southeast Asia, Sri Lanka and the Pacific islands (PP=0.5).
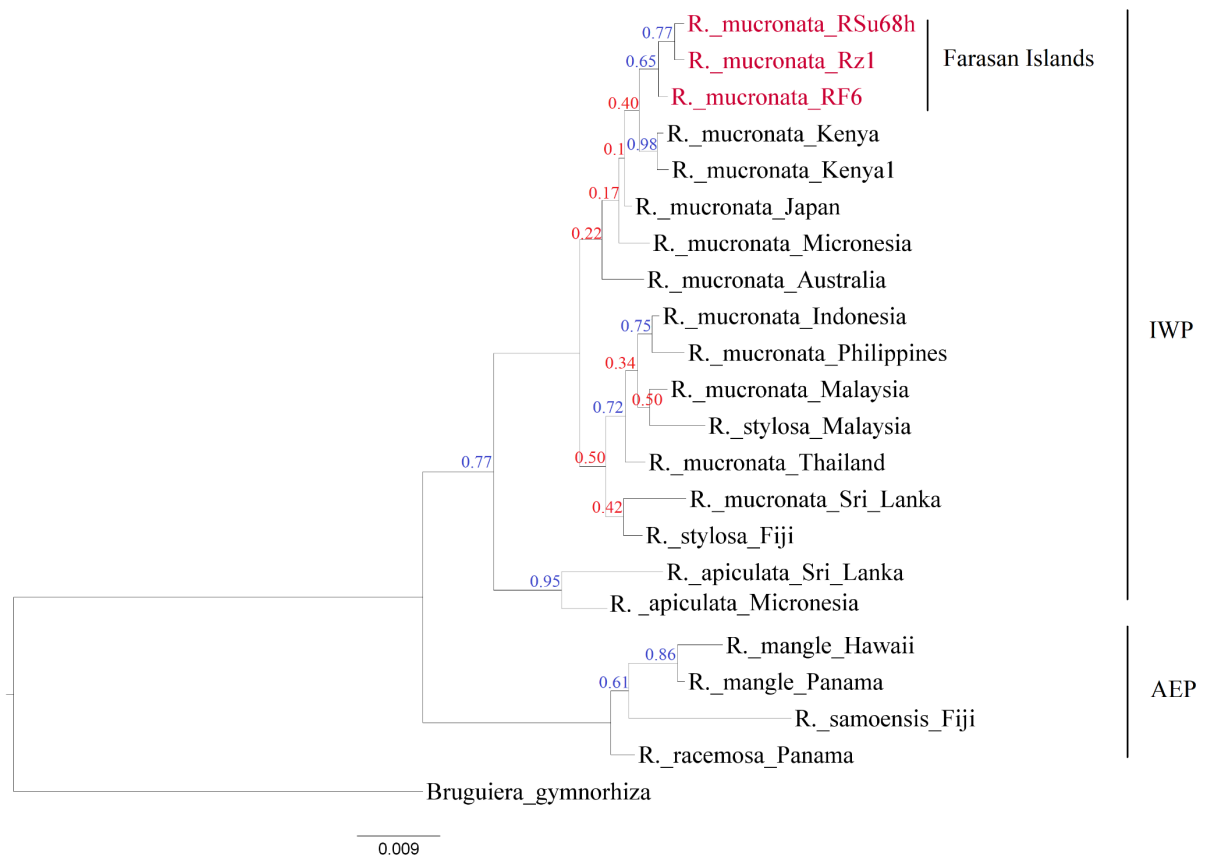
Figure 3-5. Combinable component consensus tree of the combined chloroplast and nuclear genes in *Rhizophora*. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red <0.50. Red text signifies the Farasan Islands lineages. Scale bar shows the number of substitutions per site.

3.3.2 Divergence time estimates

The dated trees produced in the BEAST analysis (Appendix 3-3) for any given genus are topologically the same as that produced by MrBayes for all nodes with a posterior probability >0.50, while clades weakly supported with a posterior probability <0.50 differed in their topological ordering. Given the lack of genetic variation among the *A. marina* accessions from the Red Sea, two individuals were included in the divergence estimation analysis: Af1 from the Farasan Islands, and Am1, from Saudi Arabia where they have a complete data set exists. In *Avicennia* molecular dating were conducted for dataset one that included the complete sampling sorting for the whole genus. The divergence time estimation analysis (Figure 3-6) shows that *A. marina* were originated during the Pliocene and divergent into three varieties in the Early Pleistocene (Figure 3-6). *Rhizophora mucronata* is older, evolved in the Late Miocene and diverged in the Pliocene. The divergence of the Red Sea lineages of *A. marina* and *R. mucronata* dates back to the Quaternary (0.0000031-1.4 Mya). Mean ages of species origin and diversification with 95% highest posterior density confidence interval HPD for the Farasan Islands mangrove species included in this study are listed in Table 4-6.

Table 4-6. Divergence ages of the Farasan Islands species included in this study calculated in BEAST

	Age (Mya)	
	Avicennia marina var. *marina*	*Rhizophora mucronata*
Species age (stem)	3.2011(95%HDP: 1.703-4.59)	6.94 (95%HDP: 0.12-15.77)
Species diversification (crown)	2.1(95%HDP: 1.02-3.3)	3.6 (95%HDP: 0.02-8.3)
Divergence of the Red Sea lineages	0.1(95%HDP: 0.0000031-0.569)	0.5 (95%HDP: 0.0027-1.4)
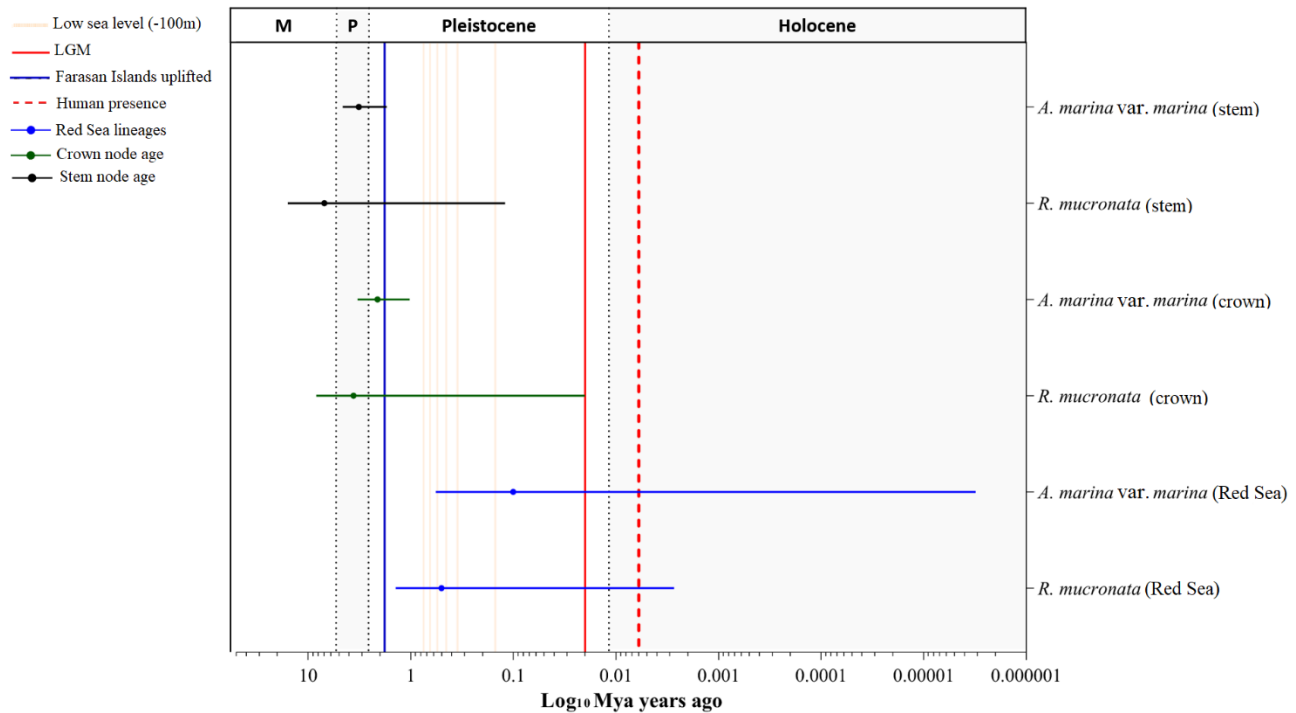
Figure 3-6. Diagram showing estimated lineage divergence times (mean and 95% HPD confidence intervals) for Farasan Islands mangrove species dated in this study and indicated in the MCC chronograms depicted in Appendix 3-3. Lowered sea-level (-100 m) data taken from Rohling et al. (2013) and Woodruff (2010). LGM= Last Glacial Maximum, M=Miocene, P=Pliocene.

3.4 Discussion

This study is the first to incorporate mangrove flora of the Red Sea into global phylogenies and to assess their affinities using DNA-sequence based phylogeny. The phylogenies of *A. marina* and *R. mucronata* based on combined cpDNA and ITS data indicate that the Red Sea mangrove forms a distinct clade closely related to the East African populations, but without statistical support (Figure 3-3, 3-5). However, the concatenated six nuclear regions strongly confirm the distinctiveness of the Red Sea lineages of *A. marina* but could not confirm the observed relatively close relationship with East Africa owing to the lack of sample from this region in the phylogeny of the *Avicennia* data set 2 (Figure 3-4). Sequencing multiple loci has been a successful strategy used to resolve phylogenetic relationships among numerous species (Li et al., 2016; Prum et al., 2015; Zou et al., 2008). Further analysis with additional DNA markers and samples needs to be carried out in order to resolve this uncertainty in the placement of the Red Sea *A. marina* and *R. mucronata*.

The close biogeographic relationship between the mangrove in the Red Sea and East Africa is plausible. The Red Sea mangroves have been biogeographically classified as part of the Indo-Pacific-East-African mangrove realm (Saenger, 2002). According to Duke et al. (2002), the current dispersal and gene exchange of East African mangrove communities likely followed the continent's northern coastline through the Middle East and India, across to Southeast Asia and down to Australia. Circulations of the Indian Ocean currents may explain this flow, which is driven by seasonally directed monsoons. In summer (May-September), the southwest monsoon causes the East African Current to flow northward along the African coast, water mass is then introduced into the Red Sea through the Gulf of Aden (Aiki et al., 2006). This current is synchronised with the maturation period of the water-dispersed propagules of *A. marina* and *R. mucronata* (Mandura et al., 1987; Duke, 1991). The affinity of Red Sea marine organisms with East Africa is also found in some endemic reef fish, such as sleeper rays, toadfishes, silversides, velvetfishes, cardinalfishes and dottybacks (DiBattista et al., 2016).

In terms of the intraspecific relationships of *A. marina*, neither combined cpDNA and ITS data nor concatenated multiple nuclear regions could distinguish between Farasan Islands and other populations in the Red Sea basin (Jazan and Egypt). This is due to the low level of DNA sequence divergence, consistent with the case for 18S gene in Sinai Peninsula (Sabri et al., 2018). The possible explanation for the observed low level of sequence divergence is that these populations were recently isolated, or they may still interact, although this would need much more in-depth population analysis to resolve the detail.

Avicennia marina varieties have been estimated to diverge more recently in the Early Pleistocene possibly during periods of lowered sea levels associated with the ice age (Duke et al., 1998; Li et al., 2016). While *R. mucronata* is older and evolved at around the middle Miocene and well-established along old southern shores of Tethys Sea from East Africa to India (Duke et al., 2002; Lo et al., 2014). The results of the divergence time estimation in current study are in line with the former findings (Figure 3-6) and show that the genetic divergence of the Red Sea mangrove lineages dates back to the Pleistocene. This finding may indicate a possible effect of Pleistocene sea-level changes on the genetic structure of these marginal populations. Several phylogeographical studies have shown that the genetic divergence of many mangrove species across the IWP region has been linked with periods of glaciation (Ge & Sun, 2001; Nettel & Dodd, 2007; Yang et al., 2017; Guo et al., 2018). During lower sea levels, mangrove populations experienced bottlenecks and even extinction due to the rapid decline in suitable habitats that accompanied climate change. This led to a considerable loss of genetic diversity, especially in populations at the range margins. During interglacial periods of raised sea levels, the founder effect that took place during recolonisation of shores by mangroves led to a further decrease in the genetic diversity of peripheral populations, such as those in the Red

Sea (Yang et al., 2017). Molecular evidence from Red Sea reef fauna shows that five of the seven species examined (*Acanthurus nigrofuscus, Cephalopholis argusand, Chaetodon auriga, Neoniphon samara* and *Pygoplites diacanthus*) were genetically differentiated from those in the West Indian Ocean, a change that probably took place in response to Pleistocene conditions (DiBattista et al., 2013). The potential effects of Quaternary climatic changes and sea level fluctuations on the genetic structure of the peripheral populations of *A. marina* and *R. mucronata* in the Red Sea remain unstudied.

Given that the Farasan Islands took their present configuration after the postglacial sea-level rise in the middle-Holocene c.0.006 Mya, current mangrove flora of the Farasan Islands seems to be very recent. The age interval estimated in this study for *A. marina* (0.0000031- 0.569 Mya) and *R. mucronata* (0.0027-1.4 Mya) from Farasan Islands overlapping with the results from dated molluscan shells of *Terebralia palustris,* characteristic molluscs of mangrove, from the Red Sea. Molluscan shells indicated that the flourish of mangrove in the Red Sea was dated to middle Holocene (Mayer & Beyin, 2009; Plaziat et al., 1995; Plaziat, 1995; Tosi, 1986; Vermeersch et al., 2005).

3.5 Summary

The Farasan Islands populations of *A. marina* and *R. mucronata* are the most well-preserved mangrove populations in their northern range of the Red Sea region. This phylogenetic investigation clearly distinguishes the Red Sea mangroves from other populations in the Indo West Pacific region and reveals that their closest genetic association is with East African populations. Furthermore, the diversification of the lineages was dated to the Pleistocene and may indicate that sea-level fluctuations and associated climate change during the glacial periods in the Red Sea region may have had an effect on the species' genetic structures. However, the specific relationships between mangrove populations in the Red Sea basin and the effect of Late Quaternary glacial-interglacial cycles on the genetic structure of Red Sea mangrove flora remain unstudied. The divergence time estimate supports the results of dated molluscan shells that the flourish of mangrove in the Red Sea was dated to middle Holocene after the postglacial sea-level rise.

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Appendices

Appendix 3-1a. Sequences downloaded from GenBank, part of previously published studies of genus *Avicennia*. All data belonging to Li et al. (2016) except accessions marked with * and ** are taken from Nettel et al. (2008) and Sahu et al. (2016) respectively. Outgroup taxa shaded by gray.

Taxon	Locality	GenBank accession *trnD-T*	GenBank accession *trnH*	GenBank accession ITS	GenBank accession 0259	GenBank accession 0347	GenBank accession c099	GenBank accession c121	GenBank accession c138
Avicennia abla Blume	Thailand	-	-	KX641594	KP026034	KP026052	KP026113	KT453713	KT453686
	Indonesia	EF540951*	EU352162*	EF540977*	-	-	-	-	-
Avicennia integra N. C. Duke	Australia	KT453646	KT453637	-	-	KX240515	KX240564	-	KX240590
Avicennia officinalis L.	Thailand	KT453649	KT453644	KX641597	KT453682	KP026057	KP026118	KT453714	KP026070
Avicennia rumphiana Hallier f.	Malaysia	KT453650	KT453643	KX641595	KP026040	KP026058	KP026119	KT453712	KP026071
Avicennia marina (Forssk.) Vierh. var. *australasica*	New Zealand	KT453651	KT453638	KX641591	KP026038	KP026056	KP026116	KT453711	-
Avicennia marina (Forssk.) Vierh. *var. eucalyptifolia*	Australia	KT453654	KT453641	KX641592	KP026036	KT453684	KP026115	-	KT453687
Avicennia marina (Forssk.) Vierh. *var. marina*	China	KT453648	KT453642	KX641593	KP026037	KP026055	KT453697	KT453710	KT453688
	Australia	EF540944*	EU352163*	EF540978*	-	-	-	-	-
	Madagascar	EF540943*	EU352164*	DQ46986*	-	-	-	-	-
	India	-	-	KJ784552**	-	-	-	-	-
	Kenya	KX812716	-	-	-	-	-	-	-
	Malysia	KX812717	-	-	-	-	-	-	-
Avicennia germinans (L.) Stearn	Mexico	-	-	KX641596	KP026035	KP026053	KP026114	KT453715	KP026067
	Panama	EF540954*	EU352182*	DQ469844*	-	-	-	-	-
Avicennia bicolor Standl.	Costa Rica	EF540988*	EF540948*	EF540988*	-	-	-	-	-
Avicennia schaueriana Stapf & Leechm. ex Moldenke	Brazil	EF540952*	EU352166*	DQ469862*	-	-	-	-	-
Thunbergia grandiflora (Rottl. ex Willd.) Roxb.	China	KT453647	KT453645	KX641599	-	-	-	-	-

Appendix 3-1b. Sequences downloaded from GenBank, part of previously published study of genus *Rhizophora* (Lo et al., 2014). Outgroup taxa shaded by gray.

Taxon	Locality	GenBank accession ITS	GenBank accession *trnH-rpl2*	GenBank accession *trnS-trnG*
Rhizophora apiculata Blume	Sri Lanka	HQ337917	HQ338004	HQ338060
	Federated States of Micronesia	KJ194254	KJ417116	KJ417024
Rhizophora mangle L.	Panama	HQ337954	HQ338012	HQ338068
	Hawaii	KJ194269	KJ417122	KJ417030
Rhizophora mucronata Lam.	Australia	KJ194235	KJ417077	KJ416984
	Kenya	HQ337949	HQ337974	HQ338030
	Kenya	HQ337948	HQ337975	HQ338031
	Federated States of Micronesia	HQ337945	HQ337968	HQ338024
	Japan	HQ337943	HQ337966	HQ338020
	Indonesia	KJ194224	KJ417086	KJ416994
	Malaysia	HQ337951	HQ337972	HQ338028
	Thailand	HQ337950	HQ337973	HQ338029
	Philippines	HQ337947	HQ337970	HQ338026
	Sri Lanka	HQ337946	HQ337969	HQ338025
Rhizophora racemosa G.Mey.	Panama	KJ194273	KJ417046	KJ417042
Rhizophora samoensis (Hochr.) Salvosa	Fiji	KJ194272	KJ417130	KJ417038
Rhizophora stylosa Griff.	Fiji	HQ337928	HQ337990	HQ338046
	Malaysia	HQ337935	HQ337985	HQ338041
Bruguiera gymnorrhiza (L.) Lam.	Thailand	HQ337961	HQ338017	-

Appendix 3-2. Voucher information of the samples sequenced for this study. Ext. code= DNA extraction code.

Species	Locality of collection	Collector name & No.	Year of collection	Herb. code	Ext. code	Sequenced region							
Genus *Avicennia*						*trnD-T*	*trnH*	ITS	0259	0347	c099	c121	c138
Avicennia marina (Forssk.) Vierh. var. *marina*	Farasan Alkabir Island	S. Alharbi & Al Qthanin 5	2016	RNG	Af5	MN885635	MN885639	MN883385	MN901111	MN891713	MN891717	MN891704	MN891707
	Sajid Island	S. Alharbi & Al Qthanin 32	2016	-	As1	MN885632	MN885637	MN883387	MN901109	MN891711	MN891715	MN891705	MN891708
	Zifaf Island	S. Alharbi & Al Qthanin 105	2016	-	Az3	MN885633	MN885638	x	MN901108	MN891710	MN891716	MN891702	MN891706
	Jazan City, Saudi Arabia	S. Alharbi 161	2016	RNG	Am1	MN885636	MN885640	MN883386	MN901109	MN891712	MN891714	MN891703	MN891709
	Al-Quatif, Saudi Arabia	John D. Dwyer 13957	1979	RNG	H18	x	x	x	x	x	x	x	x
	Kuwait	Abdullah MTA 195	2012	E	AK19h	x	x	x	x	x	x	x	x
	West Asia and Egypt	John Martin 118	1992	E	AEg11h	MN885634	MN885641	MN883384	x	x	x	x	x
	Eritrea	Ghebrehiwet M 1007	2002	EIT	AE10h	x	x	x	x	x	x	x	x
Genus *Rhizophora*						ITS		*trnH-rpl2*			*trnS-trnG*		
Rhizophora mucronata Lam	Farasan Alkabir Island, Farasan Group	S. Alharbi & Al Qthanin 38	2016	RNG	Rf6	MN883389		MN896990			x		
	Zifaf Island, Farasan Group	S. Alharbi & Al Qthanin 106	2016	-	Rz1	MN883390		MN896992			x		
	North Solain Island, Farasan Group, Saudi Arabia	I.S. Collenette 6835	unknown	K	RSu68h	MN883388		MN896991			x		
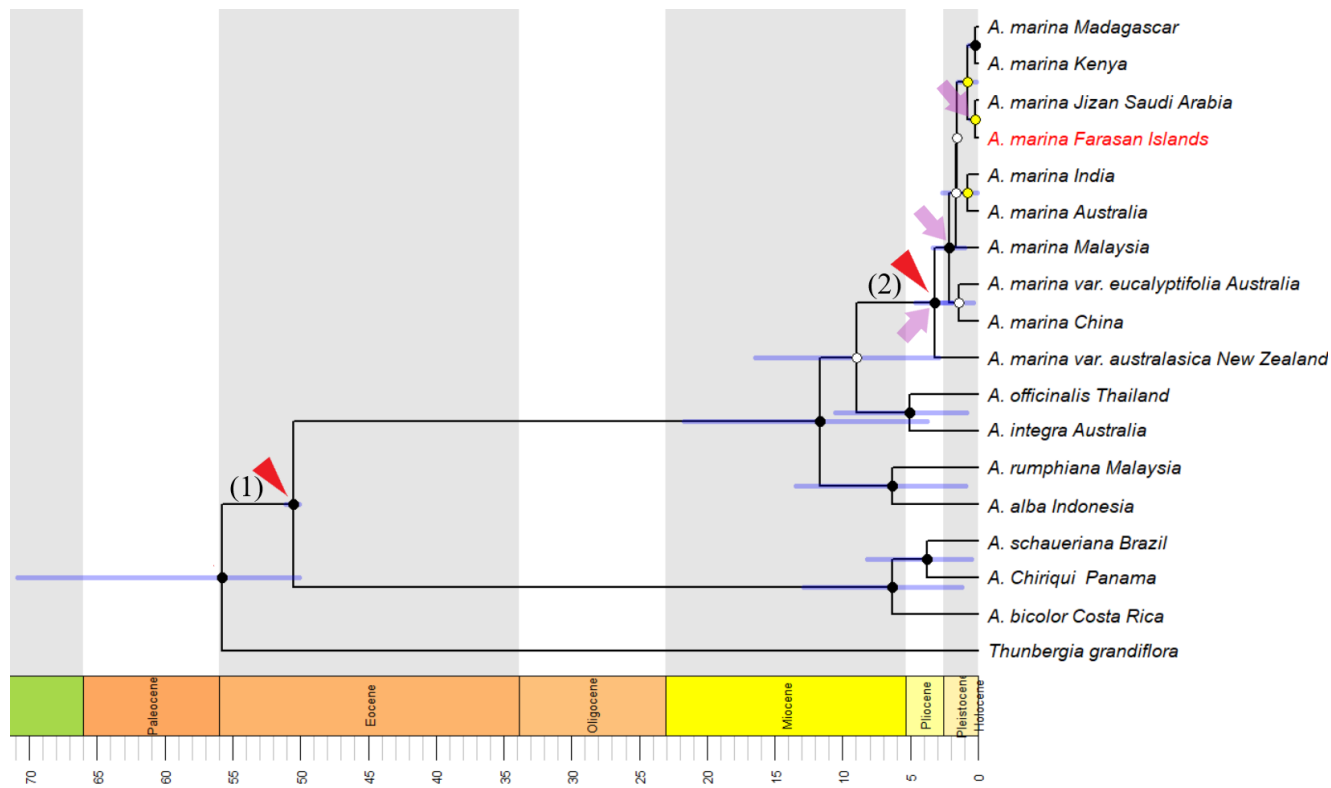
Appendix 3-3a. Maximum clade credibility chronogram of *Avicennia* based on ITS and cpDNA of all extant species. Node heights indicate mean ages with blue bars indicate the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 3-4; pink arrows indicate node ages summarised in Figure 3-6.
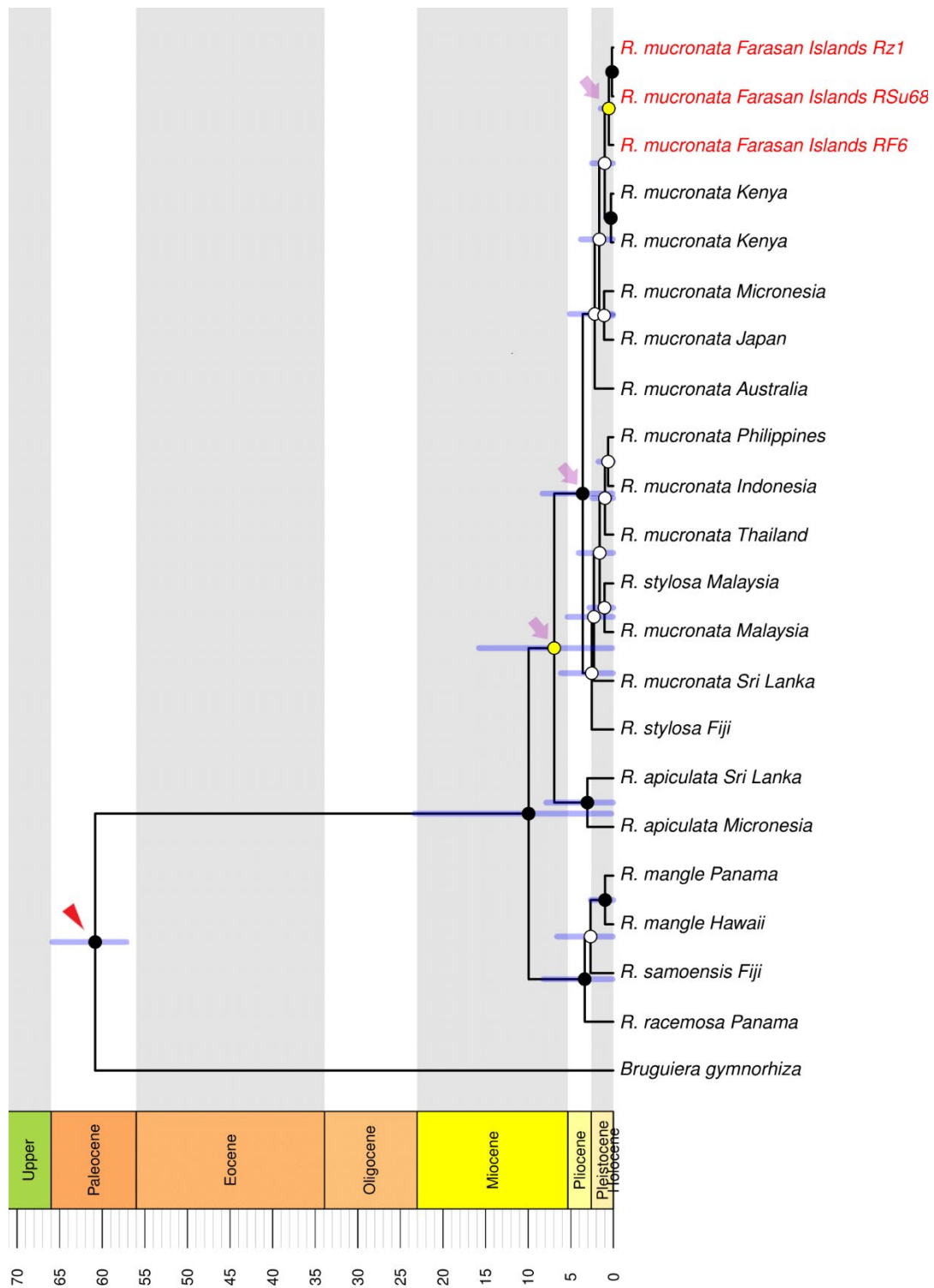
Appendix 3-3b. Maximum clade credibility chronogram of *Rhizophora* of chloroplast and nuclear regions. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows, pink arrows indicate node ages summarised in Figure 3-6.

CHAPTER 4: Phylogenetic affinities and divergence time of the Farasan Islands sandplain and salt marshes species of selected genera (*Cyperus* L., *Heliotropium* L., *Suaeda* Forssk. ex J.F.Gmel., and *Tetraena* Maxim.)

4.1 Introduction

The Farasan Archipelago has a total of 630 km of coastline. The shorelines of the larger islands are predominantly composed of a combination of salt marshes and sandy beaches, but mudflats and coralline limestone rock are also common, and sheltered coastal inlets contain silty mud that supports mangrove stands (Figure 4-1, 4-2, A & B) (Hall et al., 2010). The shorelines are low in species diversity and are dominated by halophytes that are tolerant to waterlogging and salt spray, such as *Cyperus conglomeratus, Arthrocnemum macrostachyum, Halopeplis perfoliata, Limonium axillare, Aeluropus lagopoides Suaeda fruticosa, Suaeda monoica, Tetraena coccinea* and *Tetraena simplex* (Al Mutairi et al., 2012). These taxa characterise the sand dunes and salt marshes of the shore zones of the Red Sea (El-Demerdash et al., 1994; Hassan & Al-Hemaid, 1996; Hegazy et al., 1998; Zahran, 2002; Alfarhan et al., 2005).

Salt marshes habitats have the capacity to act as CO_2 sinks, and protect the coast against erosion from increasing wave action (Duarte et al., 2013). The coastal ecosystem (and its dependent biota) of the Farasan Islands is of ecological importance because it serves as an important nesting site for migratory shorebirds, such as plovers and sooty gulls (Chaudhary, 2001b; AlRashidi et al., 2011). *Tetraena simplex* and *Suaeda monoica* form a major proportion of the Idmi gazelle's diet (Alwelaie et al., 1993; Wronski & Schulz-Kornas, 2015). This ecosystem is currently under serious threat from anthropogenic activities and infrastructure, such as port development and tourism (Figure 4-2, C & D). It is also vulnerable to the direct, large-scale impacts of climate change, primarily due to its sensitivity to rising sea levels with the heaviest impact predicted for salt marshes and sandy beaches (AlRashidi et al., 2012).

The evolutionary and biogeographical history of the Farasan flora in general and coastal vegetation in particular has been afforded little consideration. One hypothesis posited that the perennial halophytes in the Farasan Islands have a great affinity with the Arabian Peninsula (Hassan & Al-Hemaid, 1996). This hypothesis has been inferred from the distribution patterns of present taxa. Although comprehensive molecular phylogeny has been carried out for Saudi Arabian *Tetraena* (Alzahrani & Albokhari, 2017) no samples from the Farasan Islands were included. Therefore, the purpose of this chapter is to determine the age and biogeographic affinities of the Farasan Islands representatives of selected genera (*Cyperus* L., *Heliotropium* L., *Suaeda* Forssk. ex J.F.Gmel., and *Tetraena* Maxim.) that dominated in the coastal region by placing them in a global phylogenetic context and to infer molecular divergence age estimates. The study aims to test the null hypothesis that all the halophytes in the Farasan Islands derived from the Arabian Peninsula and to discover the timescale for the colonisation events.
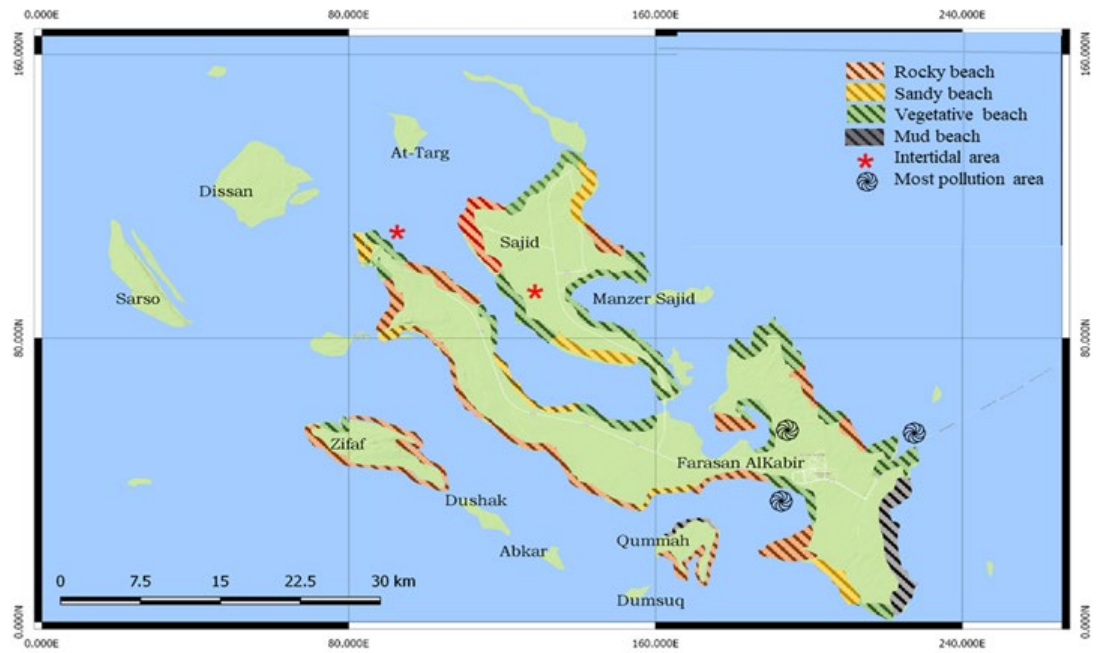
Figure 4-1. Map of the Farasan islands showing the natural composition of shorelines in the largest islands and the highly polluted coastal sites (reproduce from Al Qthanin (2019)).

Figure 4-2. Shorelines in the Farasan Islands: **(A)** salt marshes, **(B)** sandplains, **(C & D)** degradation in the salt marshes in Al-Ghadeer Beach, SW. Farasan Alkabir Island noticed between 2016-2017 due to the tourist's off-road traffic.

4.2 Materials and Methods

4.2.1 Study group and DNA region selection

Four genera were chosen as representatives of the Farasan Islands coastal vegetation (*Cyperus*, *Heliotropium*, *Suaeda* and *Tetraena*) based on the criteria summarised on chapter 1, section 1.5. Seventeen native species were recorded in the islands for these genera as follows: *Cyperus* (5 spp.), *Heliotropium* (4 spp.), *Suaeda* (3 spp.), *Tetraena* (5 spp.) (Alwelaie et al., 1993; El-Demerdash, 1996; Hassan & Al-Hemaid, 1996; Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Hall et al., 2010; Thomas et al., 2010; Al Mutairi et al., 2012a; Alzahrani & Albokhari, 2018). These genera in the Farasan Archipelago occur not only on sandplains and saltmarshes but also in cultivated areas (e.g. *Cyperus rotundus* and *Heliotropium longiflorum*) and some may be predominantly plants of cultivated land. Details including species names, habitat, geographical distribution, dispersal syndrome, flowering and fruiting period, and uses are listed in Table 4-1. Species global range maps are shown in Figure 4-3.

Table 4-1. List of all recorded native species of *Cyperus, Heliotropium, Suaeda* and *Tetraena* in the Farasan Islands, including information on the habitat, distribution range in the islands, dispersal syndromes, Flowering & fruiting and use. Species marked with an asterisk were sampled for phylogenetic study. F. D = distribution in the Farasan Islands, W. D = world distribution.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Cyperus bulbosus **Vahl***	Perennial small sedge[2]	Seasonally wet grasslands[1]	**F.D:** Farasan Alkabir Island **W.D:** W. E. & N. Africa, Arabia, Iran, Pakistan, India, E. Asia, Australia[2].	Achene[28]	Zoochorous[26]	August[4]	Tuber is edible[1]
Cyperus conglomeratus **Rottb.***	Perennial sedge[2]	Sandy soils, clay soils and rocky areas[1]	**F.D:** Widespread in Farasan Islands **W.D:** Africa, Arabia to India[1]	Achene[28]	Zoochorous[26]	January-April[4]	Medicinal herb[1]
Cyperus jeminicus **Rottb.**	Perennial, tufted herb[2]	Sandy soils in open areas[1]	**F.D:** Farasan Alkabir and Sajid Islands **W.D:** N. & NE. Africa, Arabia, Palestine[1,2]	Achene[28]	Zoochorous[26]	June-October[21]	No data available
Cyperus rubicundus **Vahl.**	Annual or perennial sedge[2]	Seasonally wet habitats, often on shallow soils[1]	**F.D:** Farasan Alkabir Island **W.D:** S. & NE Trop. Africa, Arabia, India[1]	Achene[28]	Zoochorous[26]	No data available	Medicinal plant[1]
Cyperus rotundus **L.* (new record for Farasan Islands)**	Perennial sedge[2]	Open or slightly shaded areas, often in cultivation areas[1]	**F.D:** Sajid Island **W.D:** Pantropical[1]	Nutlet[6]	Zoochorous[26]	April-October[4]	Medicinal plant[1]
Heliotropium longiflorum **Hochst. & Steud. ***	Perennial herb[8]	Alluvial soil[7], weed in agricultural farms in Saudi Arabia[24]	**F.D:** Farasan Alkabir and Sajid Islands **W.D:** NE. Trop. Africa, Arabia[8]	Nutlet[6]	Anemochorous[6]	All year round[25]	Medicinal herb[10,11]
Heliotropium pterocarpum **(DC.) Hochst. & Steud. ex Bunge**	Annual herb[8]	Sandy desert and at the edges of littoral salt marshes[9]	**F.D:** Farasan Islands **W.D:** N. & NE. Trop. Africa, Arabia[8]	Nutlet[6]	Anemochorous[12]	All year round[25]	Medicinal herb[9]
Heliotropium ramosissimum **(Lehm.) Sieb. ex DC.***	Annual herb[8]	Sand dunes of the coasts and inland, dry and sediment-rich high banks of rivers[6]	**F.D:** Farasan Alkabir and Sajid Islands **W.D:** N. & NE. Africa, Arabia, Iran, Afghanistan[8]	Nutlet[6]	Anemochorous[6]	All year round[7]	Medicinal herb[9]

Table 4-1. Continued.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Heliotropium zeylanicum (Burm.f.) Lam.	Annual herb[8]	Dry woodland, savanna, open localities, on sandy riverbanks and on edges of lakes and rivers[9]	**F.D:** Farasan Islands **W.D:** From India to Trop. & S. Africa[7]	Nutlet[6]	Unspecialized [13]	All year-round[25]	Medicinal herb[9]
Suaeda aegyptiaca (Hasselq.) Zoh.*	Annual or short-lived perennial undershrub[8]	Coastal and inland saline and common weed in agricultural lands in Saudi Arabia[14]	**F.D:** Qummah Island **W.D:** N. Africa, NE. Trop. Africa, SW. Asia, S. Australia[14]	Utricles[27]	Zoochorous[3]	February-August[15]	Medicinal herb[16]
Suaeda fruticosa Forssk. ex J.F.Gmel. (Inc. *S. vera* Forssk. ex J.F.Gmel. and *S. vermiculata* Forssk. ex J.F.Gmel.)*	Perennial undershrub[8]	Salty alluvial flats with clayey or sandy soils subjected to episodic waterlogging, on drier sites and coastal belts[4]	**F.D:** Widespread in Farasan Islands **W.D:** W. N. E. Africa, Arabia, Palestine, from Iran to India[17]	Utricles[27]	Zoochorous[3]	September-May[15]	No data available
Suaeda monoica Forssk. ex J.F.Gmel.	Shrub or small tree[8]	Coastal and inland blocked saline wadis[14]	**F.D:** Farasan Alkabir, Sajid and Dawshak Islands **W.D:** The Cape Verde Islands, N. & NE. Trop. Africa, India[17]	Utricles[27]	Zoochorous[3]	April-October[15]	medicinal herb[16]
Tetraena alba (L.f.) Beier & Thulin var. *alba*ature*	Perennial undershrub[8]	Salt marshy habitats, coastal and inland saline sandy soils, dunes sheets, and in saline depressions[18]	**F.D:** Dawshak Island **W.D:** Red Sea coasts, Mediterranean, NE. Trop. Africa [18, 19], S. Europe (S. Spain, Crete), Cyprus; Turkey[20].	Mericarp[22]	Anemochorous Zoochorous [22,23]	February-June[18]	Medicinal herb[16]
Tetraena propinqua (Decne.) Ghaz. & Osborne (Syn. *Tetraena boulosii* (A.I.Hosny) M.Hall	Small perennial shrub[18]	Sandy and gravel desert, sandy depressions, shallow runnels[20]	**F.D:** Farasan Alkabir Island **W.D:** Egypt (Sinai), Jordan, Syria, Palestine, Arabia, Iraq, S. Iran, Afghanistan, Pakistan[20].	Mericarp[22]	Anemochorous Zoochorous[22,23]	Mainly April-June and September-October, occasionally July and August.	No data available

Table 4-1. Continued.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Tetraena coccinea (L) Beier & Thulin*	Perennial shrublet[8]	Sandy and gravel desert, sandy depressions, sandy shores and low coastal dunes[20]	**F.D:** Widespread in Farasan Islands **W.D:** Red Sea coasts, S. Arabia, Iran, Palestine[20]	Mericarp[22]	Anemochorous Zoochorous[22,23]	February-June[18]	Medicinal herb[16]
Tetraena hamiensis (Schwein f.) Beier & Thulin var. *mandavillei* (Hadidi ex Beier & Thulin) Alzahrnai & Albokhari	Perennial shrublet[8]	Red sands, gravels or saline areas[18]	**F.D:** Farasan Alkabir Island **W.D:** Arabia[18]	Mericarp[22]	Anemochorous Zoochorous[22,23]	February-June and September-November[18].	No data available
Tetraena simplex (L.) Beier & Thulin*	Annual herb[18]	Sandy soils[18]	**F.D:** Widespread in Farasan Islands **W.D:** Arabia, Africa, Jordan, Palestine, Iran, Pakistan, India[18]	Mericarp[22]	Anemochorous Zoochorous[22,23]	February-June and September-November[18]	Medicinal herb[16]

1= (Simpson & Inglis, 2001), 2 = (Chaudhary, 2001), 3 = (Green et. al., 2002), 4 = (Tropicos. org. Missouri Botanical Garden, 2011), 5 = (Alwelaie et al., 1993), 6 = (Förther, 1998), 7= (Akhani, 2007), 8 = (Alfarhan et al., 2005), 9 = (Schmelzer & Gurib-Fakim, 2008), 10 = (Ghazanfar, 1994), 11 = (Abdoul-Latif et al., 2016), 12 = (Diane et al., 2016), 13 = (Royal Botanic Gardens Kew., 2019), 14 = (Chaudhary, 1999), 15 = (Zohary, 1966), 16 = (Basahi, 1999), 17 = (Boulos, 1991), 18 = (Alzahrani & Albokhari, 2018), 19 = (Euro+Med, 2006), 20 = (Ghazanfar & Osborne, 2015), 21 = (Zohary, 1986), 22 = (Van Zyl, 2000), 23 = (Sheahan, 2007), 24 = (Thomas, 2011), 25 = Samples examined through The Royal Botanic Garden Edinburgh (2018), 26 = (Kubitzki et al., 1998), 27 = (Khan et al., 2014), 28 = (Mohlenbrock, 2001).
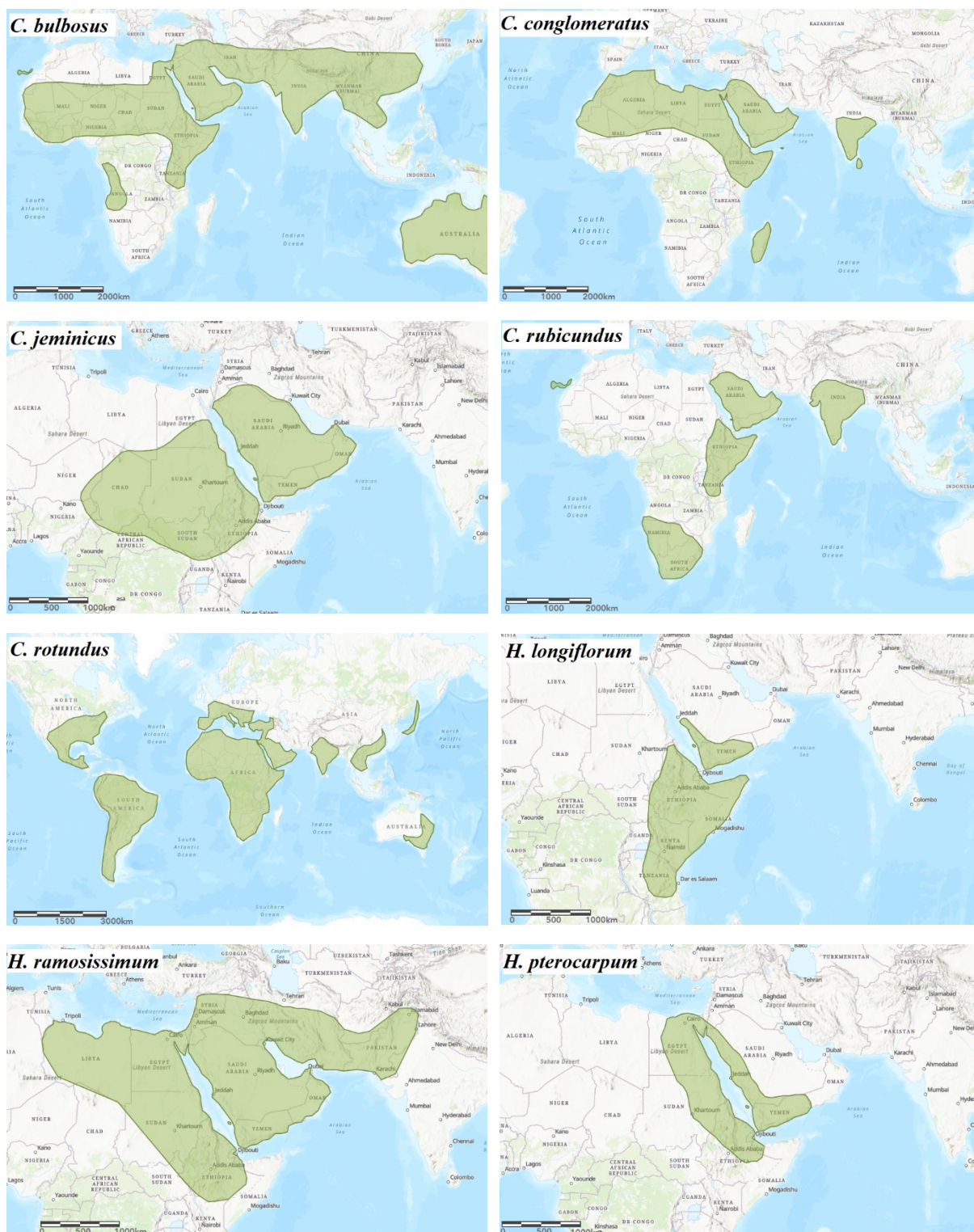
Figure 4-3. Distribution maps of the study group species based on data taken from Alfarhan et al. (2005); Akhani (2007); African Plant Database (version 3.4.0) (2012); Alzahrani and Albokhari (2018); Boulos (1991); Euro+Med (2006); Ghazanfar and Osborne (2015); Simpson et al. (2011), created using ArcGIS Online (Esri, "Topography"). *C = Cyperus, H = Heliotropium, S = Suaeda, T = Tetraena.*

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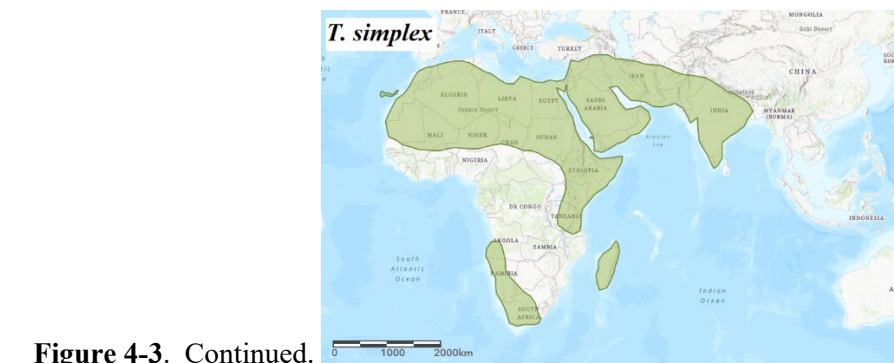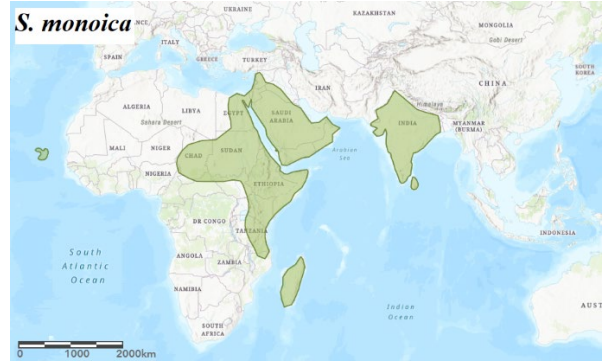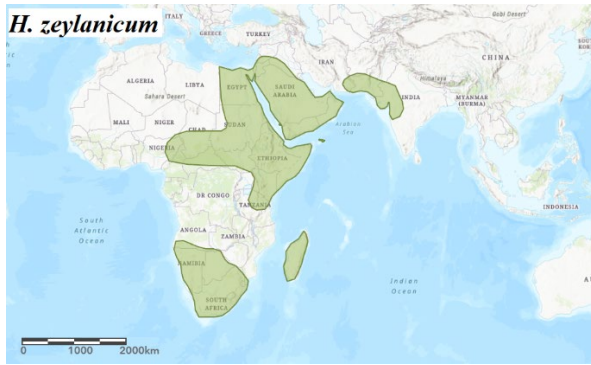
Figure 4-3. Continued.

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Brief descriptions of the four selected genera and the available molecular sequence data with the highest taxonomic coverage and relevant source regions are given below.

Cyperus (Cyperaceae) is a cosmopolitan sedge genus (Mabberley, 2017) and one of the most ecologically important angiosperm genera in tropical wetlands (Larridon et al., 2014). It comprises c.950 species after a broad circumscription based on recent molecular studies (Simpson et al., 2007; Muasya et al., 2009; Larridon et al., 2011; Larridon et al., 2013). All *Cyperus* species present in Saudi Arabia belong to *Cyperus* subg. *Cyperus,* which uses C_4 photosynthetic pathway and has a centre of diversity in East Africa (Larridon et al., 2013). Around 100 species, mostly from Africa, were sequenced for nuclear ribosomal DNA ETS1f and two non-coding plastid DNA the *rpl32-trnL* intergenic spacer of the small single-copy region of the plastid genome and the *trnH-psbA* intergenic spacer (Larridon et al., 2013).

Heliotropium (Heliotropiaceae) is cosmopolitan (Good, 1974) and the most diverse genus of the family with around 300 species (Förther, 1998), distributed in tropical and temperate regions of both hemispheres (Al-Turki et al., 2001). Fifty out of 300 species were the subject of phylogenetic analysis using nuclear ITS1 and chloroplast *trnL* intron (Hilger & Diane, 2003). Arabia and Africa were poorly represented in this investigation with only 13 African species included.

Suaeda (Amaranthaceae) is a genus of halophytic plants that comprises c.80–100 species with a worldwide distribution (Brandt et al., 2015). It has its centre of origin from southwest Asia to central Asia, and usually found on saline soils and saltmarshes in arid to semiarid regions (Sage et al., 2018). All *Suaeda* species in the Farasan Islands use the C_4 photosynthetic pathway (Schutze et al., 2003). Sixty eight of 80 species were sequenced for ITS, the chloroplast *atpB-rbcL* spacer and the *psbB-psbH* spacer (Schütze, 2008). Two species from Africa and no species from Arabia were sampled.

Tetraena (Zygophyllaceae) comprises c.40 species distributed in arid and semi-arid regions from the Canary Islands to South Africa and China (Sheahan & Chase, 2000; Beier et al., 2003). Molecular investigations based on chloroplast data of *rbcL* and non-coding *trn*L-F were carried out in 21species from Africa (Bellstedt et al., 2008) and seven species from Saudi Arabia (Alzahrani & Albokhari, 2017).

A summary of DNA regions and relevant publications is shown in Table 4-2. Four hundred and fifty-six accessions, represent ingroup and outgroup, were downloaded from GenBank: *Cyperus* (134); *Heliotropium* (80); *Suaeda* (156); *Tetraena* (86). Details of the GenBank accessions shown in Appendix 4-1.

Table 4-2. Summary of selected DNA regions for each genus

Genus	Sequence region	Publication
Cyperus	ETS1f, *rpl32-trnL* and *trnH-psbA*	Larridon et al. (2013)
Heliotropium	ITS$_1$ and *trnL* $_{LUAA}$ intron	Hilger & Diane (2003)
Suaeda	ITS, *atpB-rbcL* and *psbB-psbH*	Schütze et al. (2003); Schütze (2008)
Tetaena	*rbcL* and *trnL-F*	Bellstedt et al. (2012) and Alzahrani and Albokhari, (2017)

4.2.2 Taxon Sampling

Ten out of the seventeen study group species recorded in the archipelago were sampled in the field and obtained from E, including *Cyperus* (3 spp.), *Heliotropium* (2 spp.), *Suaeda* (2 spp.) and *Tetraena* (3 spp.) (outlined in Chapter 2). To supplement the available molecular data of the chosen genera that lack complete geographical coverage (especially from the adjacent mainland to the Farasan Islands), 58 samples were obtained either from the field or from local and international herbaria whenever possible. Those herbaria were KSU, E, RNG, EIT; voucher details are listed in Appendix 4-2.

4.2.3 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 350 mg silica dried leaves and herbarium dried leaves using a cetyltrimethylammonium bromide (CTAB) extraction protocol based on Doyle & Doyle (1987). For the herbarium specimens, the extraction protocol has been modified to include one-week isopropanol precipitation. For some samples, DNeasy QIAGEN extraction kit (QIAGEN, Hilden, Germany) was used following the manufacturer's instructions. Plant tissues were ground two or more times in a 2 ml Eppendorf tube with a small amount of sand and two tungsten beads (QIAGEN 69997) using the Qiagen TissueLyser II (QIAGEN) at 30 Hz for 45 seconds. DNA extractions were stained with GelRed™ (Biotium, Fremont, CA, USA) and visualised under UV light using T:Genius gel documentation (Synoptics Ltd, Cambridge, UK) following gel electrophoresis on a 0.7% agarose gel (Fisher Scientific Midi Submarine Gel) in 1x TAE buffer PH 8.0; photographs were taken to record the extractions. DNA size and concentration were determined using HyperLadder™ 1kb (Bioline Reagents Ltd., London, UK) for marking. Concentration and quality were also determined using spectrophotometry (NanoDrop™ Lite, Thermo Fisher Scientific Inc., Waltham, MA, USA). Fifty-microliter (50

µl) aliquots of 2–10 ng/µl were prepared for polymerase chain reaction (PCR) amplification and stored at -20 °C until required.

PCR was done using the Applied Biosystems Thermal Cycler SimpliAmp™ (Thermo Fisher Scientific Inc.), and Gradient PCRs were performed for some samples in order to gather optimum annealing temperatures using the Eppendorf Mastercycler® gradient (Eppendorf, Hamburg, Germany). Degraded herbarium materials amplified in two segments using internal primers. Table 4-3 provides details of primer sequences, PCR reactions and cycling conditions for each marker for *Cyperus, Heliotropium*, *Suaeda* and *Tetraena.* Ten percent (10%) DMSO (dimethyl sulfoxide; Sigma-Aldrich, Schnelldorf, Germany) was added in nrDNA regions reactions and, 0.5 µl 100 mg/ml BSA (Bovine serum albumin, Sigma-Aldrich) in cpDNA regions to enhance the PCR reaction.

The PCR products were separated on 1% agarose gels in 1x TAE buffer stained with GelRed™ and visualised under UV light. Approximate size and concentration of the PCR products were determined by comparison with HyperLadder™ 1kb. PCR products were purified and sequenced by Eurofins Genomics (Cologne, Germany), each region was sequenced bi-directional using the same primers as the amplification primers.

4.2.4 Sequence editing and alignment

Outlined in Chapter 3 section 3.2.4. The newly generated sequences were deposited in the GenBank, the accession numbers shown in Appendix 4-2.

4.2.5 Phylogenetic analysis and Bayesian divergence time estimation.

Outlined in in Chapter 3 section 3.2.5.

4.2.6 Fossil constraints and secondary calibrations

To make calibration of several nodes possible in the dated phylogeny analysis, a broad outgroup was selected for the given genera. Varieties of fossil and secondary calibration points were used in the dating analysis of each genus. Details of primary and secondary constrained nodes, fossil name, organ, offsets and priors for all genera are listed in Table 4-4.

Table 4-3 List of Polymerase Chain Reaction (PCR) Primers, Master Mix recipe and cycling conditions in. *Suaeda*, *Tetraena*, *Cyperus* and *Heliotropium.*

Genus	Region ID	Primer sequences 5'-3'	Master Mix recipe	Cycling conditions
Cyperus	*rpl32-trnL*	rpl32-F: AGTTCCAAAAAAACGTACTTC trnL(UAG): TGCTTCCTAAGAGCAGCGT (Shaw et al., 2007)	**25 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 12.5 µl 2x BioMix Red 0.25 µl of each primer (10 mM) 10.5 µl Milli-Q water 0.5 µl BSA Modified from Larridon et al. (2011)	80°C for 5 min; 30 cycles of 95°C for 1 min, 50°C for 1 min, and 65 °C for 4 min; final extension 65°C for 5 min. (Shaw et al., 2007)
	trnH-psbA	Pe-trnH: ATTCACAATCCACTGCCTTGAT Pe-psbA: AATGCACACAACTTCCCTCTA (Shaw et al., 2005) and psbAF: TWATGCAYGAACGTAATGCTC psbA r: GCGCATGGTGGATTCACAATCC (Sang et al., 1997)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 10 µl 2x BioMix Red 0.2 µl of each primer (10 mM) 8.1 µl Milli-Q water 0.5 µl BSA Modified from Larridon et al. (2011)	80°C for 5 min ; 35 cycles of 94°C for 30 sec, 50-56°C for 30 sec, and 72°C for 1 min; final extension 72°C for 10 min. (Shaw *et al.,* 2005)
	ETS1f	ETS-F: CTGTGGCGTCGCATGAGTTG 18S-R: AGACAAGCATATGACTACTGG CAGG (Starr et al., 2003)	**50 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 25 µl 2x BioMix Red 2.5 µl of each primer (10 mM) 18.5 µl Milli-Q water 0.5 µl BSA Modified from Larridon et al. (2011)	95°C for 3 min; 35 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min 20 sec; final extension 72°C for 10 min. (Starr et al.,2003)
Heliotropium	*trnL* intron	C: CGAAATCGGTAGACGCTACG D: GGGGATAGAGGGACTTGAAC (Taberlet et al. 1991)	**20 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 12.5 µl 2x BioMix Red 2.5 µl of each primer (10 mM) 5 µl Milli-Q water 0.5 µl BSA	95°C for 4 min; 35 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 2min; final extension 72°C for 7 min.
	ITS$_1$	P1: TTCAACGAGGAATTCCTAGT P2: TACGTTCTTCATCGATGCGA (Diane et al., 2002) ITS-A: GAAGGAGAAGTCGTAACAAGG ITS-C: GCAATTCACACCAAGTATCGC (Blattner, 1999)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 10 µl 2x BioMix Red 0.6 µl of each primer (10 mM) 5.3 µl Milli-Q water 0.5 µl BSA 2 µl DMSO	93°C for 4 min; 30 cycles of 93°C for 1 min, 50-55°C for 90 sec, and 72°C for 2min; final extension 72°C for 8 min.
Suaeda	ITS	ITS-A: GAAGGAGAAGTCGTAACAAGG ITS-B: CTTTTCCTCCGCTTATTGATATG ITS-C: GCAATTCACACCAAGTATCGC ITS-D: CTCTCGGCAACGGATATCTCG (Blattner, 1999) In one part: A+B In two parts: A+C/D+B	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 10 µl 2x BioMix Red 0.5 µl of each primer (10 mM) 7µl Milli-Q water 2 µl DMSO Modified from Schutze et al. (2003)	94°C for 3 min; 30 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 90 sec; final extension 72°C for 10 min. (Schutze et al., 2003)
	atpB-rbcL	F: GAAGTAGTAGGATTGATTCTC R: CAACACTTGCTTAGTCTCTG (Xu et al., 2000)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 10 µl 2x BioMix Red 0.4 µl of each primer (10 mM) 7.7µl Milli-Q water 0.5 µl BSA Modified from Schutze et al. (2003)	94°C for 3 min; 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec; final extension 72°C for 10 min. (Schutze et al., 2003)
	*psb*B-*psb*H	F: AGATGTTTTTGCTGGTATTGA R: TTCAACAGTTTGTGTAGCCA (Xu et al., 2000)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 10 µl 2x BioMix Red 0.2 µl of each primer (10 mM) 8.1 µl Milli-Q water 0.5 µl BSA Modified from Schutze et al. (2003)	94°C for 3 min; 30 cycles of 94°C for 30 sec, 53°C for 30 sec, and 72°C for 90 sec; final extension 72°C for 10 min. (Schutze et al., 2003)
Tetraena	*trnL-F*	C: CGAAATCGGTAGACGCTACG F: ATTTGAACTGGTGACACGAG internal primers D: GGGGATAGAGGGACTTGAAC E: GGTTCAAGTCCCTCTATCCC C+D/F+E (Taberlet et al., 1991)	**25 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 12.5 µl 2x BioMix Red 2µl of each primer (10 mM) 6 µl Milli-Q water 0.5 µl BSA Modified from Bellstedt et al. (2008)	94°C for 5 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 90 sec; final extension 72°C for 6 min. (Bellstedt et al., 2008)
	rbcL	1F: ATGTCACCACAAACAGAAAC 1460R:CCTTTTAGTAAAAGATTGGGCCGAG internal primers 636 F: GCGTTGGAGAGATCGTTTCT 724R: TCGCATGTACCTGCAGTAGC 636F+1460R/ 724R+1F (Savolainen et al., 2000)	**25 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 12.5 µl 2x BioMix Red 2µl of each primer (10 mM) 6 µl Milli-Q water 0.5 µl BSA Modified from Bellstedt et al. (2008)	94°C for 5 min; 30 cycles of 94°C for 30 sec, 49-52°C for 50 sec, and 72°C for 1min; final extension 72°C for 6 min. (Bellstedt et al., 2008)

Table 4-4a. List of fossil priors utilized in BEAST analysis. (Mya= millions of years ago).

Constrained node	Fossil name (Organ)	Age (Mya)	Source	Prior distribution	Offset (Mya)	Mean	Log (SD)
Cyperus							
1. *Cyperus* crown	*Cyperus* L. (fruit)	Late Oligocene (28.4 - 23.03)	Smith et al. (2010)	Lognormal	23.03	1.5	1.25
2. *Scirpus* crown (outgroup)	*Scirpus* L. (fruit)	Middle Oligocene (28.4)	Smith et al. (2010)	Lognormal	28.4	1.5	1.0
3. *Carex* crown (outgroup)	*Carex colwellensis* M.Chandler (fruit)	Late Eocene (38-33.9)	Jiménez-Mejías et al. (2016)	Lognormal	33.9	1.5	0.85
Heliotropium 1. *Heliotropium* I crown	*Turnefortia* L. (pollen grains)	Early Oligocene (c.33.9-28.1)	Graham and Jarzen (1969)	Lognormal	28.1	1.1	1.0
2. *Ehretia* crown (outgroup)	*Ehretia* P.Browne (fruit)	Early Eocene (56–47.8)	Chandler, 1961, 1962, 1964 , cited in Luebert et al. (2017), p.160	Lognormal	47.8	1.4	1.0
Suaeda 1. Salicornioideae crown (outgroup)	*Salicornites massalongoi* Principi (stem system)	The Oligocene (35.4-23.3)	Collinson et al. (1993)	Lognormal	23.3	1.0	1.25
2. Chenopodioideae (root)	*Chenopodiopollis multiplex* (pollen grains)	The Paleocene (65-56.5)	Nichols and Traverse (1971)	Lognormal	56.5	1.0	1.25

Table 4-4b. List of secondary priors utilized in BEAST analysis. (Mya= millions of years ago).

Constrained node	Age (Mya)	Source	Prior distribution	Mean	Log (SD)
Cyperus	Late Cretaceous (c.85)		Normal	85	5.25
4. Cyperaceae (root)		Léveillé-Bourret et al. (2018); Escudero and Hipp (2013); Spalink et al. (2016)			
Heliotropium					
3. Heliotropiaceae crown	Early Eocene (c.54.01)	Luebert et al. (2017); Luebert & Wen (2008)	Normal	54.01	3.93
4. Boraginales II (root)	Late Cretaceous (c.72.11)	Luebert et al. (2017)	Normal	72.11	4. 35
Suaeda					
3. *Suaeda* crown	The Oligocene (c.25)	Kadereit et al. (2017)	Normal	25	4.0
Tetraena					
1. Zygophylloideae	Late Eocene (c.37.57)	Wu et al. (2015)	Normal	37.57	4.6
2. Zygophyllaceae (root)	Middle Paleocene (c.60.88)	Magallón et al. (2015)	Normal	60.88	14.06

4.3 Results

4.3.1 Phylogenetic analyses

Sequences were successfully obtained for 80% of all samples (Appendix 4-2), descriptive statistics for plastid and nucleotide partitions for all genera including amplicon length, alignment length, number of taxa, number of variable sites and the best-fit model of nucleotide substitution are given in Table 4-5. For any given genus DNA partitions were combined after the ILD test indicated no significant difference between the datasets (P>0.05). In all genera, the combined data BI tree provided better resolution and well-supported clades compared to separate sequence analyses. Thus, combined gene regions phylogenies are carried towards the results and discussion.

Table 4-5. Descriptive statistics of nuclear and chloroplast datasets of *Cyperus, Heliotropium, Suaeda* and *Tetraena.* CP= Chloroplast region, NU= nuclear region.

Genus	Sequence region	Amplicon length	Total aligned length (bp)	No. of taxa	Variable sites	Best-fit model of nucleotide substitution
Cyperus	*rpl32-trnL* (CP)	651	1278	45	317(24.8%)	GTR+ G
	trnH-psbA (CP)	636	1209	55	315(26.0%)	GTR+ G
	ETS1f (NU)	532	667	57	500(74.9%)	HKY+G
	CP + NU	-	3154	61	1132(35.8%)	GTR+I+G
Heliotropium	*trnL* intron (CP)	480	543	72	100 (18.4%)	HKY+G
	ITS$_1$ (NU)	430	288	68	200(66.8%)	HKY+G
	CP + NU	-	831	73	300 (36.1%)	GTR+I+G
Suaeda	*atpB-rbcL*	636	975	57	227 (23.2%)	GTR+I
	psbB-psbH	699	674	57	129 (19.1%)	GTR+I+G
	ITS	655	683	63	322(47%)	GTR+I+G
	CP + NU	-	2332	66	678 (29%)	GTR+I+G
Tetraena	*trnL-F*	650	997	60	330 (33%)	GTR+I+G
	rbcL	1521	1368	47	254(18.6%)	GTR+I+G
	Combined data	-	2365	60	584(51.6%)	GTR+G

Cyperus: The Farasan Islands *Cyperus* species were nested among C4 *Cyperus* clade which form a monophyletic group with very short branch lengths in the deepest node (Figure 4-4). *Cyperus bulbosus* individuals form well supported clade (PP=0.99), including samples from the Farasan Archipelago, Africa and Arabia. Similarly, *C. conglomeratus* populations from Farasan with northeast Africa and Arabian samples form a monophyletic group (PP=1.00), within this clade materials from Egypt and Jazan were closely related (PP=1.00). *Cyperus rotundus* from the islands and Oman formed a well-supported clade (PP=1.00), sister to clade comprises China and USA populations (PP=1.00).

Heliotropium: *Heliotropium* species from the Farasan Islands were nested among *Heliotropium* II clade that included the Old World *Heliotropium* species (Figure 4-5). *Heliotropium ramosissimum* from the Farasan Archipelago was nested within a large clade formed by species of section *Pterotropium* with a low supported backbone (Inc. *H. pterocarpum, H. bacciferum, H. dentatum, H. fartakense, H. riebeckii, H. ramosissimum H. asperrimum* and *H. erosum*). Species within sect. *Pterotropium* clade characterized by short branch lengths with limited taxonomic and biogeographic structure. Farasan *H. ramosissimum* individuals show close link with Arabia, sample (Hf122) clustered with *H. ramosissimum* from Saudi Arabia in a well-supported clade (PP=0.94). The Sarso Island sample (HSa92h) formed moderately supported clade (PP=0.6) with *H. dentatum* and *H. riebeckii* from Yemen and *H. ramosissimum* from the Arabian Gulf. The rejection of the identification of Sarso Island sample, which was obtained from E, as *H. pterocarpum* (see Chapter 2) was supported by this phylogeny. Conversely, *H. longiflorum* was of African affinity, which formed a strongly supported clade (PP=0.96) with Somalian accession.

Suaeda: Farasan Islands species were nested within Fruticosa subclade, a very diverse and species-rich monophyletic group using the C4 photosynthetic pathway belong to sect. *Salsina* (Freitag & Lomonosova, 2017; Schütze, 2008) (Figure 4-6). Among this clade, species grouped based on their geographical distribution rather than their taxonomy. *Suaeda aegyptiaca* from the Farasan Islands was distinguished from other populations in the Arabian mainland and formed strongly supported clade (PP=0.97) with the Eritrean accession. This clade shares a most recent common ancestor with an unidentified sample from Socotra (PP=0.52). *Suaeda aegyptiaca* from the middle part of the Arabian Peninsula and Jordan clustered in a moderately supported clade (PP=0.63) sister to the Red Sea/ Socotra clade. Similarly, *S. fruticosa* from Farasan Islands were distinct from other *S. fruticosa* populations on the mainland (Jordan,

Pakistan). Farasan samples and *S. paulayana* from Socotra form a highly supported clade (PP=0.97) sister to *S. micromeris* from Somalia (PP=1.00).

***Tetraena*:** This analysis strongly supported the monophyly of genus *Tetraena* (=*Zygophyllum* subgenus *Agrophyllum*) suggested by Beier et al. (2003) (Figure 4-7). The biogeographic relationships of Farasan Islands *Tetraena* species was not fully resolved. *Tetraena alba* was sister to clade consisting of *T. alba* and *T. propinqua* from Arabian Peninsula and *T. alba* and *T. cornuta* from the Mediterranean. Similarly, *T. coccinea* populations formed well supported clade, included samples from the Farasan Islands, Eretria and Saudi Arabia. Due to the lack of sequence variation, the intra-specific variability among populations not resolved. This supports our re-determine of *T. boulosii* (T8h) obtained from E as *T. coccinea* (see Chapter 2). *Tetraena simplex* formed a highly supported clade (PP=1.00); however, the intra-specific relationships unresolved. Thus, the Farasan Islands *T. simplex* affinity unclear.
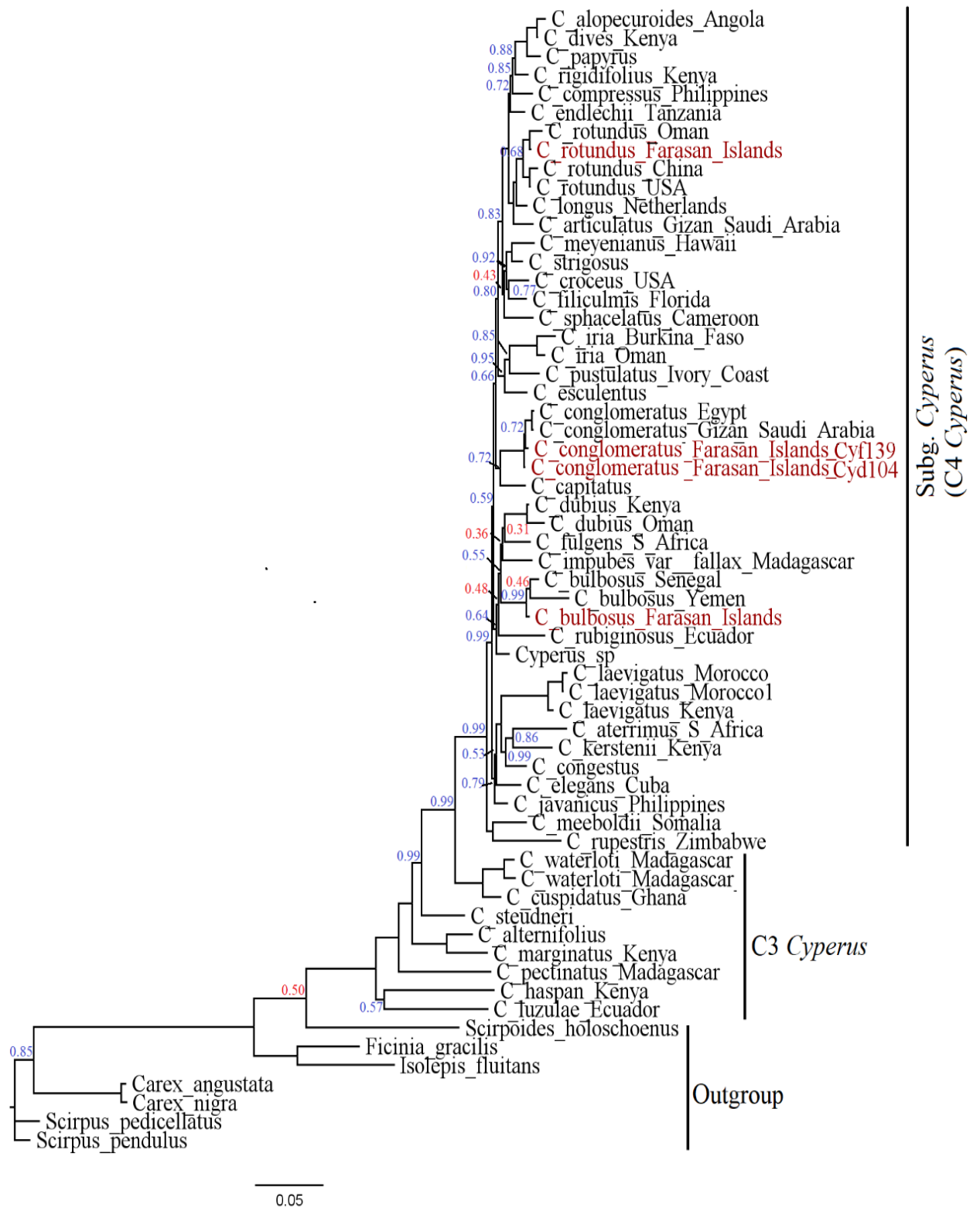
Figure 4-4 Combinable component consensus tree of *Cyperus* based on combined nuclear and chloroplast data. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.
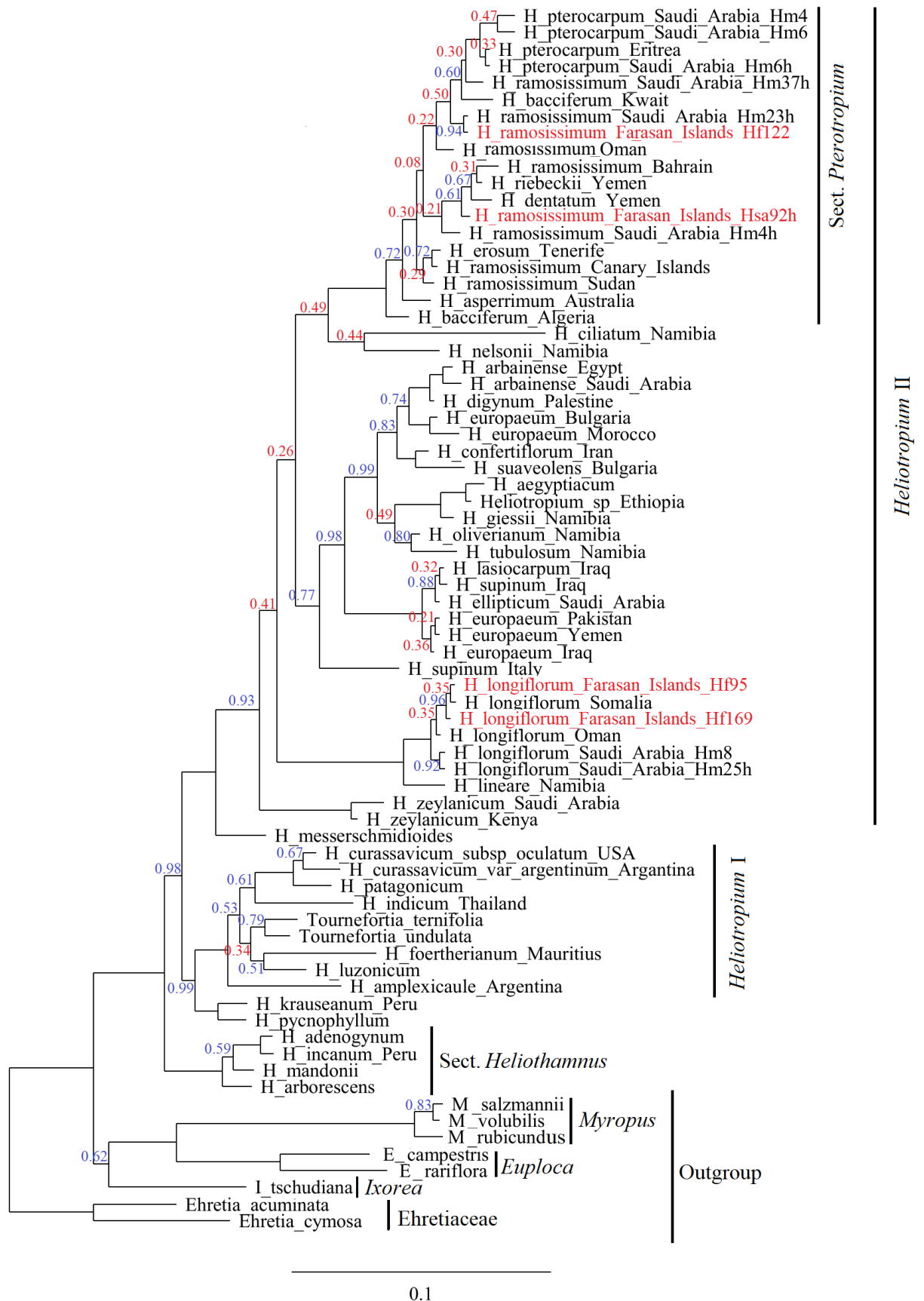
Figure 4-5. Combinable component consensus tree of *Heliotropium* based on combined chloroplast *trnL* and nuclear ITS$_1$ data. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows of substitutions per site.
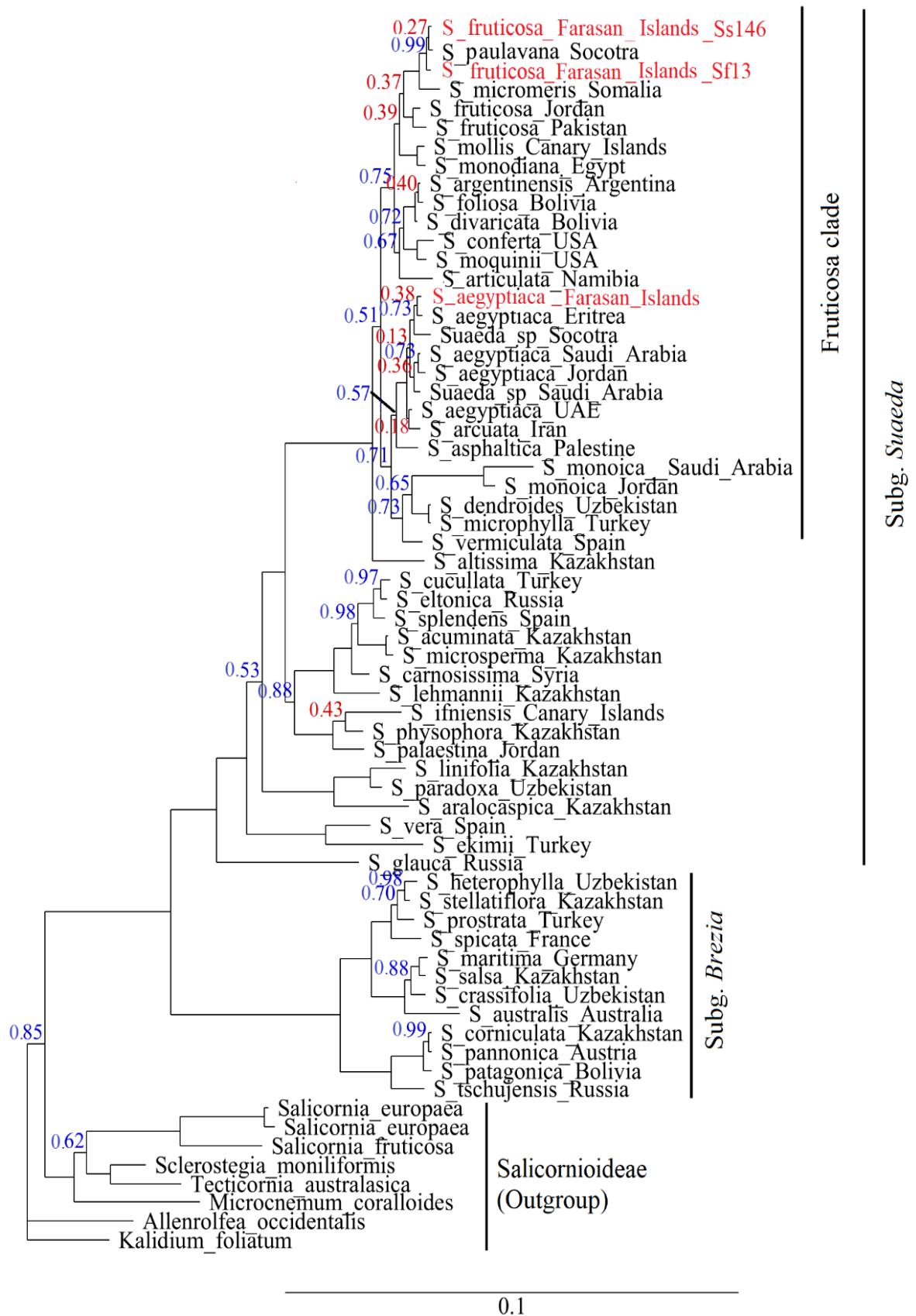
Figure 4-6. Combinable component consensus tree of *Suaeda* based on combined chloroplast and nuclear data. Posterior probabilities less than one are shown near nodes: blue > 0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.
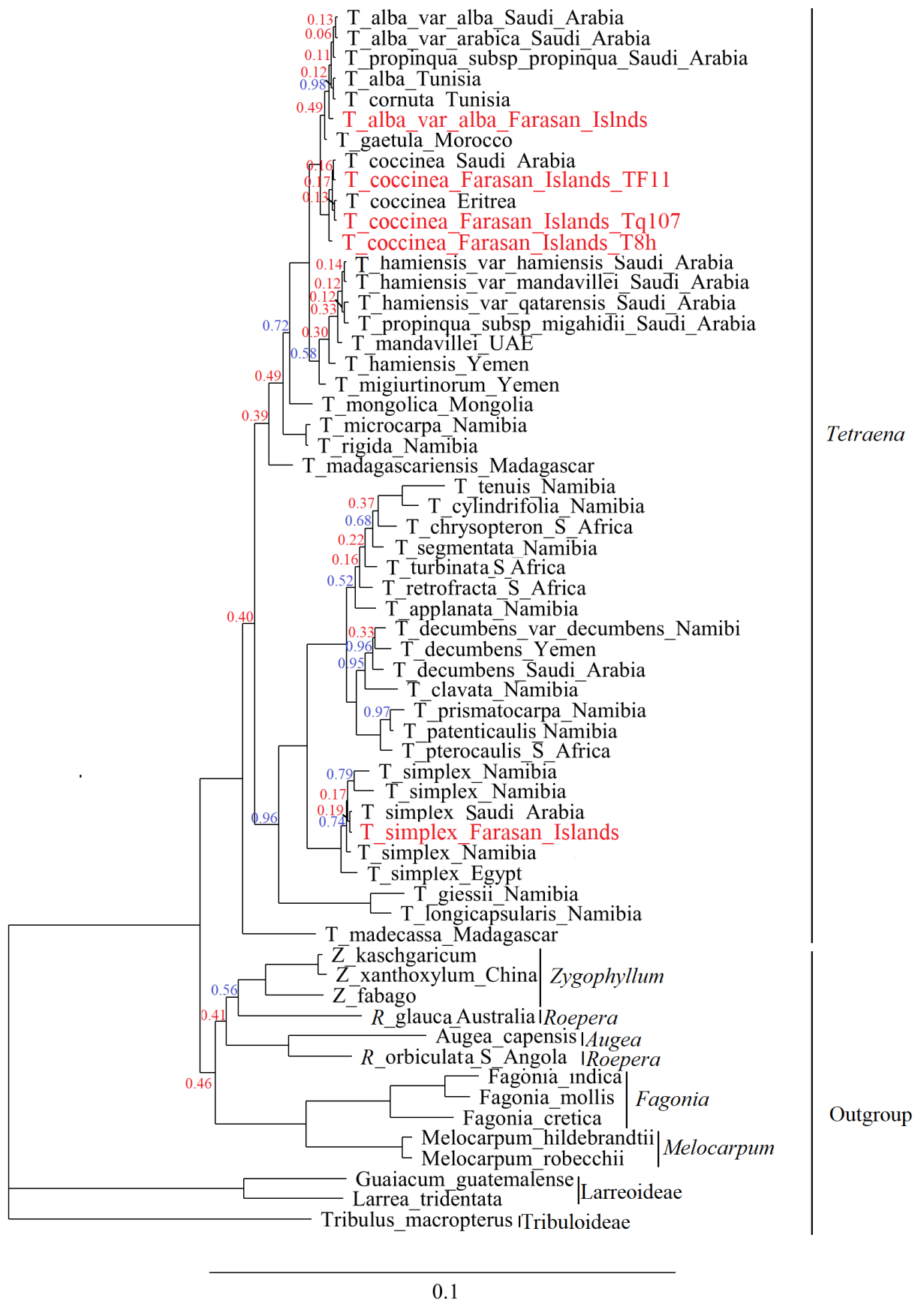
Figure 4-7. Combinable component consensus tree of *Tetraena* based on combined chloroplast data. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.

4.3.2 Divergence time estimates

The dated trees produced in the BEAST analysis (Appendix 4-3) for all genera are topologically the same as that produced by MrBayes for all nodes with a posterior probability > 50%, while clades weakly supported (<0.50) sometimes differed in their topological ordering. All the species originated from the Middle Miocene onward. The Farasan lineages then diversified during the late Pliocene and Pleistocene. When the crown nodes of the clades in which the Farasan Islands lineages were not fully supported, as was the case for *C. bulbosus, T. alba, T. coccinea, T. simplex* and *S. fruticosa*, the age of the next supported node was taken into consideration. Therefore, no reliable colonisation ages are available for these species, and they may have been established in the islands at any time during the Pleistocene. Mean ages with 95% HPD confidence intervals for the Farasan Islands species included in this study are listed in Table 4-6.

Table 4-6. Divergence ages of the Farasan Islands species included in this study calculated in BEAST.

Taxon	Species Age (Mya)	Divergence of Farasan lineages (Mya)
Cyperus bulbosus	11.5 (95%HDP: 5.2 – 19.7)	4.8 (95%HDP: 1.1 – 9.4)
Cyperus conglomeratus	10.8 (95%HDP: 3.8 – 18.9)	2.9 (95%HDP: 0.6 – 5.8)
Cyperus rotundus	6.4 (95%HDP: 2.5 – 11.3)	1.7 (95%HDP: 0.17 – 3.8)
Heliotropium ramosissimum	23.26 (95%HDP: 7.8 – 21.1)	0.8 (95%HDP: 0.00011 – 2.7)
Heliotropium longiflorum	9.8 (95%HDP: 3.5 – 17.6)	2.2 (95%HDP: 0.4 – 4.7)
Suaeda fruticosa	8.2 (95%HDP: 5.5 – 11.18)	0.8 (95%HDP: 0.22 – 1.69)
Suaeda aegyptiaca	4.29 (95%HDP: 2.0 – 6.7)	0.33 (95%HDP: 0.0000052 – 0.97)
Tetraena alba **var.** *alba*	2.03 (95%HDP: 0.7 – 3.6)	0.8 (95%HDP: 0.15 - 1.6)
Tetraena coccinea	2.03 (95%HDP: 0.7 – 3.6)	0.6 (95%HDP: 0.08 – 1.5)
Tetraena simplex	12.8 (95%HDP: 7.01 – 18.8)	1.05 (95%HDP: 0.05 – 2.4)

A
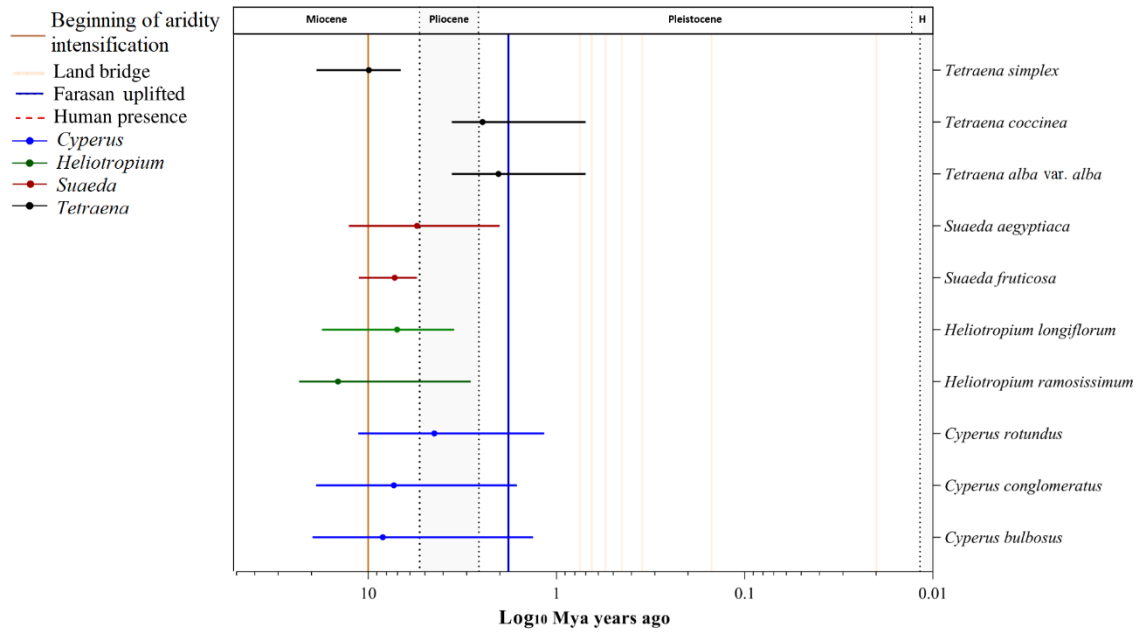
B
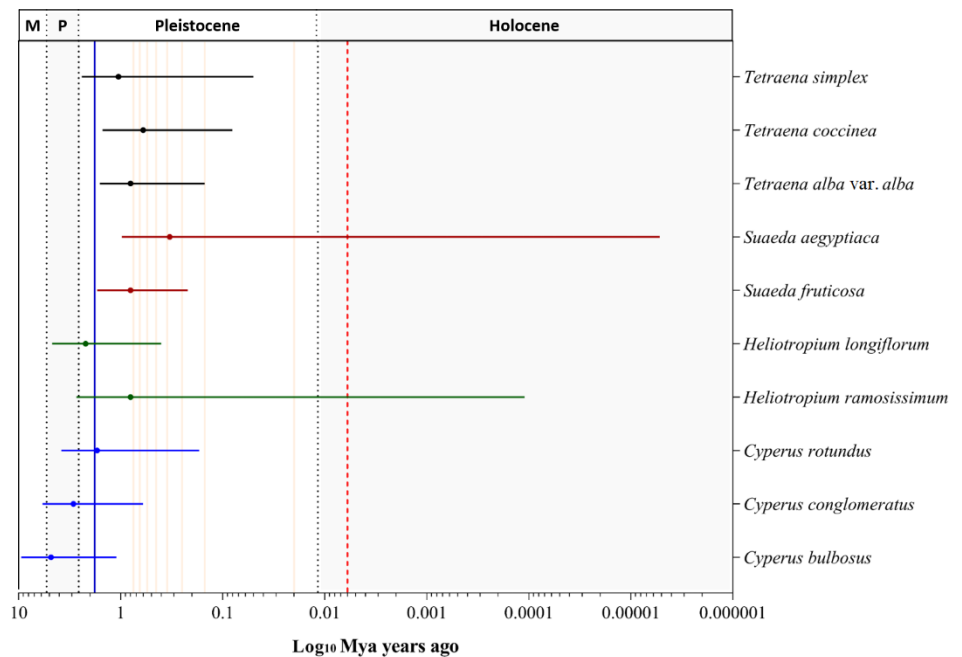
Figure 4-8. Diagram showing estimated lineage divergence times (mean and 95% HPD confidence intervals) for Farasan Islands coastal species dated in this study and indicated in the MCC chronograms depicted in Appendix 4-3. **(A)** Species origin, **(B)** divergence of Farasan Islands lineages. The age of land bridge formation is based on lowered sea level (-100 m) data from Rohling et al. (2013) and Woodruff (2010). H=Holocene, M=Miocene, P=Pliocene.

4.4 Discussion

This study is the first to incorporate the Farasan Archipelago coastal species into worldwide phylogenies and to infer molecular divergence age estimates for ten species belonging to four genera: *Cyperus, Heliotropium, Suaeda* and *Tetraena.*

All examined species are halophytic plants using the C_4 photosynthesis pathway, with the exception of *T. alba* and *T. coccinea*, which use the C_3 photosynthetic pathway (Lauterbach et al., 2016). The harsh conditions, characterised by high levels of aridity, salinity, temperature and irradiance with limited water availability, prevailing in the Farasan Islands (El-Demerdash, 1996; Ibrahim, 2008) make sand dunes and salt marshes the preferred habitats for C_4 plants to dominate. It has been estimated that the origin and convergent evolution of the C_4 pathway in several plant families coincided with the Miocene expansion of grassland ecosystems, accompanying increasing terrestrial aridity, and declining CO_2 concentrations (Sage et al., 2018). Salinity stress was also one of the major factors that promoted the evolution of C_4 pathway (Kadereit et al., 2012). The results of the present investigation are in line with those of previous studies, whereby the evolution of the examined species is concentrated in the transitional period between the Miocene and Pliocene (Figure 4-8, A). The expansion of the species in the Red Sea and Arabian Sea regions dates back to the Pliocene and Pleistocene, falling within the period during which the region experienced more arid conditions (Anderson et al., 2013) that increased the total area of suitable habitat to which arid-adapted plants could migrate. Phylogenetic analyses of the Zygophylloideae show evidence of migrations of *T. simplex* during the periodic aridification in the Pliocene/Pleistocene from southern to northern Africa (Bellstedt et al., 2012). The uplift of the Farasan Islands at the Pleistocene (Dabbagh et al., 1984) may have offered new habitats for these migrant species to colonise.

Hassan and Al-Hemaid (1996) hypothesised that the coastal flora of the Farasan Islands has a close link with the Arabian mainland. The phylogenetic evidence in this study has not refuted the hypothesis, but neither has it fully supported. Clear phylogenetic link with Arabia has been shown solely in one species, *H. ramosissimum* (10% of the study group species), whereas the other 80% (*C. bulbosus, C. conglomeratus, C. rotundus, S. fruticosa, S. aegyptiaca, T. alba, T. coccinea* and *T. simplex*) have unresolved relationships due to either the lack of sequence variation between individual accessions or data limitation. However, the colonisation events of these species appear to coincide with recurring land bridges with Arabia, suggesting their probable origin. The presence of the Arabian floristic elements of *T. propinqua* and *T. hamiensis* var. *mandavillei* (Figure 4-2) in the Farasan Islands provides further evidence of

Arabia's strong influence on the islands' flora (not included in the phylogeny, as no samples were available). Links with Africa were evident in one species, *H. longiflorum*. Thus, the present data support the strong influence of the nearby mainland on Farasan flora, as hypothesised by Thomas et al. (2010).

Seed dispersal is a key process in plants' colonisation of islands (Heleno & Vargas, 2015). The dispersal and establishment of the species along the coastal regions of the Farasan Islands may be due to birds, wind action, or anthropogenic factors, as none of the tested species possesses any buoyancy or special adaptation for floating, and they usually sink in water (Ridley, 1930). Migratory birds that nest in the wetlands of the Farasan Islands and frequent the salt marshes may play a significant role in coastal flora exchange. The dispersal of approximately 80% of species of the study group may have been mediated by birds externally or internally. Mud adhering to birds' feet was an important factor in the islands' flora formation, as has been suggested, for example, for 21.0% and 13.7% of the Juan Fernandez and Galápagos Islands colonists, respectively (Carlquist, 1996). Small *Cyperus* achenes and *Suaeda* seeds (Ridley, 1930; Carlquist, 1996) could benefit from external dispersal via mud. *Tetraene* seeds' coats become mucilaginous after dehydration, which may facilitate their external attachment to birds as well (Van Zyl, 2000; Sheahan, 2007). Shorebirds are also important consumers of seeds (Green et al., 2002). Birds have been observed to consume *Suaeda* seeds (Green et al., 2002) and *Cyperus* achenes (Kubitzki et al., 1998).

Adaptations for wind dispersal are found in the nutlets of *H. ramosissimum* and *H. longiflorum* (Förther, 1998; El Naggar et al., 2015) and in the *Tetraena* mericarps (Van Zyl, 2000; Sheahan, 2007). *Tetraena simplex* disperses largely in the manner of a tumbleweed, whereby the entire plant breaks off easily and rolls along the plains (Bellstedt et al., 2008).

The possibility of anthropogenic introduction of the species is strong because of their medicinal properties or growth as a weed in cultivated area. *Heliotropium ramosissimum* (Schmelzer & Gurib-Fakim, 2008) and *S. aegyptiaca* (Basahi, 1999) are medicinally used in Arabia and Africa, their probable colonisation events coincided with human existence in the islands. *Suaeda aegyptiaca* also grows as a weed in agricultural areas (Chaudhary, 1999). Thus, no single mode of dispersal can account for the entire coastal flora.

4.5 Summary

Phylogenetic affinities and divergence times were estimated for ten species belonging to *Cyperus, Heliotropium, Suaeda* and *Tetraena* occupying the salt marshes and sandplains in the Farasan Islands. All these species are halophytes using the C_4 photosynthesis pathway, with the exception of *T. alba* and, *T. coccinea*. The evolution of these species dates back to the Middle Miocene onward, and is associated with increased terrestrial aridity in continents' interiors and declining CO_2 concentration. The uplift of the Farasan Islands in the Pleistocene during the expansion of aridity in the area may have offered appropriate habitats for these species in the Red Sea to colonise, as is evident from the molecular dating.

It has been hypothesised that the coastal flora of the Farasan Islands has a close link with the Arabian mainland (Hassan & Al-Hemaid, 1996). The phylogenetic evidence has not refuted this hypothesis, but neither has not fully supported it, due to either the lack of sequence variation or data limitation in most of the species. However, the Arabian affinities are evident in other aspects, including the presence of Arabian floristic elements and the coincidence of colonisation events time with the recurring land bridges with Arabia. Birds, winds and man are possible dispersal vectors that contributed to the formation of littoral flora and maintained the islands' populations.

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Appendices

Appendix 4-1a. Sequences downloaded from GenBank, part of previously published studies of genus *Cyperus*. All data belonging to Larridon et al. (2013) except accessions marked with *, ** and*** are taken from Reid et al. (2017), Benítez-Benítez et al. (2018) and Léveillé-Bourret et al. (2018) respectively. Outgroup taxa shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession ETS1f	GenBank accession *trnH-psbA*	GenBank accession *rpl32-trnL*
Cyperus alopecuroides Rottb.	Hess 52/1581 (GENT)	Angola	HE993959	HE993898	HE993687
Cyperus alternifolius L.	Goetghebeur 11516 (GENT)	BG Ghent	HQ705948	HQ705818	HQ705878
Cyperus aterrimus Hochst.ex Steud.	Muasya & Ramdhani 2722 (BOL)	South Africa	HE993960	HE993899	HE993688
Cyperus bulbosus Vahl	Laegaard et al. 17024(GENT)	Senegal	HE993961	HE993900	HE993689
Cyperus capitatus Vand.	Goetghebeur 10744 (GENT)	BG Ghent	HE993962	HE993901	HE993690
Cyperus compressus L.	Reynders & Sabulao 15(GENT)	Philippines	HE993963	HE993902	HE993691
Cyperus congestus Vahl	Goetghebeur 11988 (GENT)	BG Ghent	HE993964	HE993903	HE993692
Cyperus croceus Vahl	Rostad s.n. GENT	USA	HE993965	HE993904	-
Cyperus cuspidatus Kunth	Jongkind & Nieuwhuis 2847 (GENT)	Ghana	HQ705954	HQ705823	HQ705884
Cyperus dives Delile	Muasya et al. 2529 (EA)	Kenya	HE993966	HE993905	HE993693
Cyperus dubius Rottb.	Muasya & Muthama 1251 (EA)	Kenya	HE993967	-	HE993694
Cyperus elegans L.	Goetghebeur 5601 (GENT)	Cuba	HQ705959	HQ705827	HQ705889
Cyperus endlichii Kük.	Muasya & Knox 954 (EA)	Tanzania	HE993968	-	-
Cyperus esculentus L.	Goetghebeur 11303 (GENT)	BG Nantes, BG Ghent	HQ705960	HQ705828	HQ705890
Cyperus filiculmis Vahl	Carter 4355 (GENT)	Florida	HE993969	HE993906	HE993695
Cyperus fulgens C.B.Clarke	Goetghebeur 4329 (GENT)	South Africa	HE993970	HE993907	HE993696
Cyperus haspan L.	Muasya & Muthama 1269(EA)	Kenya	HQ705927	HQ705803	HQ705803
Cyperus impubes Steud. var. *fallax* (Cherm.) Kük.	Dhondt 9 (GENT)	Madagascar	HE993971	-	HE993697
Cyperus iria L.	Desmet 77/13(GENT)	Burkina Faso	HE993972	HE993908	HE993698
Cyperus javanicus Houtt.	Reynders & Sabulao 60 (GENT)	Philippines	HE993973	-	HE993699
Cyperus kerstenii Boeck.	Muasya 984 (EA, K;Muasya et al., 2002)	Kenya	HQ705961	HQ705829	HQ705891
Cyperus laevigatus L.	Goetghebeur 10201 (GENT)	Morocco, BG Ghent	HE993975	HE993910	-
Cyperus laevigatus L.	Larridon et al. 2009-0033 (GENT)	Kenya	HE993974	HE993909	HE993700
Cyperus laevigatus L.	Goetghebeur 10202 (GENT)	Morocco, BG Ghent	HE993976	HE993911	HE993701
Cyperus longus L.	Farjon 217 (GENT)	Netherlands	HE993977	HE993912	HE993702
Cyperus luzulae (L.) Retz.	Van den Eynden 213 (GENT)	Ecuador	HQ705910	-	HQ705846
Cyperus marginatus Thunb.	Larridon et al. 2009-0076 (GENT)	Kenya	HQ705949	HQ705819	HQ705879
Cyperus meeboldii Kük.	Kilian & Lobin 6848 (GENT)	Somalia	HE993978	HE993913	HE993703
Cyperus meyenianus Kunth	Fosberg 47227 (GENT)	Hawaii	HE993979	HE993914	HE993704
Cyperus papyrus L.	Goetghebeur 5866 (GENT)	BG Ghent	HQ705962	HQ705830	HQ705892
Cyperus pectinatus Vahl	Larridon et al. 2010-0265 (GENT)	Madagascar	HQ705936	HQ705810	HQ705869
Cyperus pustulatus Vahl	Porembski 624 (GENT)	Ivory Coast	HE993980	HE993915	HE993705

Appendix 4-1a. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession ETS1f	GenBank accession *trnH-psbA*	GenBank accession *rpl32-trnL*
Cyperus rigidifolius Steud.	Samain 2005-001 (GENT)	Kenya	HE993981	HE993916	HE993706
Cyperus rotundus L.	Shaw 890 (K)	Hong Kong (China)	HQ705963	HQ705831	HQ705893
Cyperus rotundus L.	Reid 8050	USA	-	KX405809*	KX405701*
Cyperus rubiginosus Hook.f.	Unknown s.n. (GENT)	Ecuador	HE993982	HE993917	HE993707
Cyperus rupestris Kunth	Laegaard 15909 (GENT)	Zimbabwe	HE993983	HE993918	HE993708
Cyperus sp.	Goetghebeur 5965 (GENT)	BG Ghent	HE993985	HE993920	HE993710
Cyperus sphacelatus Rottb.	Goetghebeur 4908 (GENT)	Cameroon	HE993984	HE993919	HE993709
Cyperus spiralis Larridon	Muasya & Muthama 1247 (EA)	Kenya	HQ705953	HQ705822	HQ705883
Cyperus strigosus L.	BG 20051035G (GENT)	BG Poznan, BG Ghent	HE993986	HE993921	HE993711
Cyperus waterloti Cherm.	Larridon et al. 2010-0010 (GENT)	Madagascar	HQ705955	HQ705824	HQ705885
Cyperus waterloti Cherm.	Larridon et al. 2010-0043 (GENT)	Madagascar	HQ705956	HQ705825	HQ705886
Carex angustata Boott	-	-	GQ223478**	-	-
Carex nigra Reich.	S. Martín-Bravo et al. (UPOS)	Sweden	MF945740**	JN627681**	-
Ficinia gracilis Schrad.	Muasya 2713 (BOL)	South Africa	HQ705902	HQ705784	HQ705839
Isolepis fluitans (L.) R.Br.	Muasya & Knox 3195 (EA)	Kenya	HQ705901	HQ705783	HQ705838
Scirpoides holoschoenus (L.) Soják	Goetghebeur 11520 (GENT)	BG Porto, BG Ghent	HQ705900	HQ705782	HQ705837
Scirpus pendulus Muhl.	Cruise 1388	-	MF669145***	-	-
Scirpus pedicellatus Fernald.	Houle76-1185	-	MF669126***	-	-

Appendix 4-1b. Sequences downloaded from GenBank, part of previously published studies of genus *Heliotropium*. All data belonging to (Hilger and Diane, 2003) except accessions marked with * and ** are taken from Luebert and Wen (2008) and Gottschling et al. (2014) respectively. Outgroup taxa shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS1	GenBank accession *trnL*
Heliotropium adenogynum I.M.Johnst.	Cano 10058 (M)	Peru	AY377792	AY376172
Heliotropium aegyptiacum Lehm.	Schultka 1995/5 (BSB)	Kenya	AF396918	AY376173
Heliotropium amplexicaule Vahl.	-	Argentina	AY176076*	AY376174*
Heliotropium arbainense Fresen.	Förther 4049 (BSB)	Egypt	AF396916	AY376176
Heliotropium arborescens L.	commercial cultivated plant (BSB)	-	AF396896	AY376177
Heliotropium asperrimum R.Br.	Craven 9671 (CANB)	Australia	AF402586	AY376178
Heliotropium bacciferum Forssk.	Podlech 35182 (M)	Algeria	AY377793	AY376179
Heliotropium ciliatum Kaplan.	Hilger Nam_93/10 (BSB)	Namibia	AY377795	AY376183
Heliotropium confertiflorum Boiss. & Noe	Akhani & Lari 5591 (KAS)	Iran	AY377798	AY376184
Heliotropium curassavicum L. var. *argentinum* M.Johnst.	Hilger Arg_95/82 (BSB)	Argentina	AF396898	AY376185

Appendix 4-1b. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS$_1$	GenBank accession *trnL*
Heliotropium curassavicum L. subsp. *oculatum* (A.Heller) Thorne	Hilger USA_94/21 (BSB)	USA	AF396897	AY376186
Heliotropium digynum (Forssk.) Asch. ex C.Chr.	Hilger Israel_94/23 (BSB)	Palestine	AF396915	AY376188
Heliotropium erosum Lehm.	Zippel 2000/69 (BSB)	Spain: Tenerife	AY377801	AY376189
Heliotropium europaeum L.	Hilger Bg_97/6 (BSB)	Bulgaria	AF402587	AY376193
Heliotropium foertherianum Diane & Hilger	Tillich 3555, cult. Botanical Garden of München-Nymphenburg, Germany	Mauritius	AF396900	AY376222
Heliotropium giessii Friedr.-Holzh.	Hilger Nam_93/3 (BSB)	Namibia	AF396917	AY376194
Heliotropium incanum Ruiz & Pav.	Weigend 2000/162 (M)	Peru	AY176077	AY376192
Heliotropium krauseanum Fedde	Weigend & Förther 97/727 (M)	Peru	AF396909	AY376195
Heliotropium lineare (A.DC.) Gürke	Hilger Arg_95/15 (BSB)	Namibia	AY37780	AY376196
Heliotropium luzonicum (I. M. Johnst.) Craven	Liede 3302 (BSB)	Philippines	AF396899	AY376228
Heliotropium mandonii I.M.Johnst.	Weigend, cult. Botanical Garden of München-Nymphenburg, Germany 1.9.1997 (BSB)	Ecuador	AF396895	AY376197
Heliotropium messerschmidioides Kuntze	-	-	AY377791*	AY376171*
Heliotropium nelsonii C.H.Wright	Hilger Nam_93/6 (BSB)	Namibia	AY377806	AY376200
Heliotropium oliverianum Schinz	Hilger Nam_93/16 (BSB)	Namibia	AF396913	AY376202
Heliotropium patagonicum (Speg.) I.M.Johnst.	Weigend et al. 5940 (BSB)	Argentina	AY377809	AY376205
Heliotropium pycnophyllum Phil.	Dillon & Dillon 6041 (M)	Chile	AY377812	AY376208
Heliotropium suaveolens M.Bieb.	Hilger Bg_97/5 (BSB)	Bulgaria	AF396911	AY376210
Heliotropium supinum L.	Hilger s.n. anno 1985 (BSB)	Italy	AF396919	AY376211
Heliotropium tubulosum DC.	Hilger Nam_93/18 (BSB)	Namibia	AY377817	AY376214
Heliotropium zeylanicum (Burm.f.) Lam.	Hilger Kenya_94/4 (BSB)	Kenya	AY377819	AY376216
Ixorhea tschudiana Fenzl	cult. Botanical Garden of München-Nymphenburg, Germany (BSB)	Argentina	AF396880	AY376218
Myriopus rubicundus (Salzm. ex DC.) Luebert (syn. *Tournefortia rubicunda* Salzm. ex DC)	-	-	EF688852*	EF688799*
Myriopus salzmannii (DC.) Diane & Hilger	Nee & Wen 53848 (US)	-	EF688853*	EF688800*
Myriopus volubilis (L.) Small	Hilger Mex_1980/6 (BSB)	Mexico	AF396882	AY376233
Tournefortia ternifolia Kunth	Weigend & Förther 1997/880 (BSB)	Peru	AY377831	AY376232
Tournefortia undulata Benth.	Weigend et al. 2001/25 (BSB)	Peru	AY377830	AY376231
Euploca rariflora (Stocks) Diane & Hilger	Hilger Nam_93/23 (BSB)	Namibia	AF396889	AY376209
Euploca campestris (Craven) Diane & Hilger	Nee & Wen 53873 (US)	-	EF688856*	EF688803*
Ehretia cymosa Thonn.	de Wilde 4230 (B)	Ethiopia	AF385790**	EU600012**
Ehretia acuminata R.Br.	cult. Botanical Garden of Adelaide (BSB)	Australia	AF385798	AY376167

Appendix 4-1c. Sequences downloaded from GenBank, part of previously published studies of genus *Suaeda*. All data belonging to Schütze (2008). Names in [] is the new name based on Kapralov et al. (2006). Outgroup taxa shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession *atp*B-*rbc*L	GenBank accession *psb*B-*psb*H	GenBank accession ITS
Alexandra lehmannii Bunge[=*Suaeda lehmannii* (Bunge) Kapralov, Akhani &E.H.Roalson]	Diomina 5171a (KAS)	E Kazakhstan	FJ449756	DQ499432	FJ449821
Borszczowia aralocaspica Bunge [=*Suaeda aralocaspica* (Bunge) Freitag & Schü tze]	Ogar 10.2000 (KAS)	E Kazakhstan	AY181807	AY181934	AY181872
Suaeda acuminata (C. A. Mey.) Moq.	Lomonosova 053a_ (NS,KAS)	Kazakhstan	FJ449757	AY181912	AY181848
Suaeda aegyptiaca (Hasselq.) Zohary	Freitag 30.120 (KAS)	E Jordan	AY181788	AY181917	AY181853
Suaeda altissima Pall.	Freitag 28.150 (KAS)	N Kazakhstan	AY181785	AY181914	AY181850
Suaeda arcuata Bunge	Lö ffler 1/2001 (W)	Iran	AY181789	AY181918	AY181854
Suaeda argentinensis Soriano	Fortunato 4303 (NY)	Argentina	-	-	FJ449786
Suaeda articulata Aellen	Okaukungo 23.04.68 (W)	Namibia	AY181795	AY181924	AY181860
Suaeda asphaltica Boiss.	Danin 2000 (HUJ)	Israel Palestine	AY181786	AY181915	AY181851
Suaeda australis Moq.	Schmalz 55 (MJG)	Australia	AY181766	AY181891	AY181826
Suaeda carnosissima Post	Freitag 31.159 (KAS)	SE Syria	AY181783	AY181910	AY181846
Suaeda conferta (Small) I. M. Johnst.	Schenk 06/10/1994 RSA)	USA	-	-	FJ449789
Suaeda corniculata group	Lomonosova 71a (NS, KAS)	Kazakhstan	AY181779	AY181904	AY181840
Suaeda crassifolia Pall.	Freitag 30.134 (KAS)	W Uzbekistan	AY181760	AY181885	AY181820
Suaeda cucullata Aellen	Freitag 28.729 (KAS)	Turkey	-	AY181909	AY181845
Suaeda dendroides (C.A. Mey.) Moq.	Freitag 30.127 (KAS)	Uzbekistan	AY181791	AY181920	AY181856
Suaeda divaricata Moq.	Torrico-Peca 101 (LPB, KAS)	Bolivia	AY181797	AY181926	AY181863
Suaeda eltonica Iljin	Freitag 28.242 (KAS)	Russia	AY181784	AY181911	AY181847
Suaeda foliosa Moq.	R. de Michel 2982 (LPB, KAS)	Bolivia	AY181796	AY181925	AY181862
Suaeda fruticosa Forssk. ex J.F.Gmel.	Freitag 31.138 (KAS)	Jordan	AY181793	AY181922	AY181858
Suaeda fruticosa Forssk. ex J.F.Gmel.	Freitag 21.500 (KAS)	Pakistan	AY181792	AY181921	AY181857
Suaeda glauca Bunge	Nechayev & Pavlova 04/10/2003 (KAS)	Russia	FJ449761	FJ449835	FJ449825
Suaeda heterophylla group	Freitag 30.132 (KAS)	Uzbekistan	AY181774	AY181899	AY181835
Suaeda ifniensis Caball.	Reys-Betancort (TFC 41074, KAS)	Canary Islands	AY181800	AY181928	AY181866
Suaeda linifolia Pall.	Freitag 28.092 (KAS)	Kazakhstan	AY181805	AY181932	AY181870
Suaeda maritima (L.) Dumort.	Schü tze 10.09.01(KAS)	Germany	AY181758	AY181883	AY181818
Suaeda micromeris Brenan	4007 (Herb. Mogadishu) (MOG)	Somalia	-	-	FJ449798
Suaeda microphylla Pall.	Freitag 28.686 (KAS)	Turkey	AY181790	AY181919	AY181855
Suaeda microsperma (C.A. Mey.) Fenzl	Lomonosova 45a (NS, KAS)	Kazakhstan	-	AY181913	AY181849
Suaeda mollis Delile	TFC 41066 (TFC, KAS)	Canary Islands	-	-	FJ449799
Suaeda monodiana Maire	Bornkamm 28.09.86 (B, KAS)	Egypt	-	-	AY181861
Suaeda monoica Forssk. ex J.F.Gmel.	Le´onard 7466 (BR, KAS)	Jordan	AY181794	AY181923	AY181859
Suaeda moquinii (Torr.) Greene	Ickert-Bond 1122 (ASU, KAS)	USA	AY181798	-	AY181864
Suaeda palaestina Eig. & Zohary	Freitag 30.165 (KAS)	Jordan	AY181799	AY181927	AY181865
Suaeda pannonica (Beck) Graebn.	Freitag 27.156 (KAS)	Austria	AY181778	AY181903	AY181839

Taxon	Voucher (herbarium)	Locality	GenBank accession	GenBank accession	GenBank accession
			*atp*B-*rbc*L	*psb*B-*psb*H	ITS
Suaeda paradoxa Bunge	Freitag 30.128 (KAS)	Uzbekistan	AY181806	AY181933	AY181871
Suaeda patagonica Speg.	R. de Michel 2862 (LPB, KAS)	Bolivia	AY181782	AY181907	AY181843
Suaeda paulayana Vierh.	Kilian YP3782 (KAS)	Socotra, Yemen	FJ449767	FJ449834	FJ449823
Suaeda physophora Pall.	Freitag 28.041 (KAS)	W Kazakhstan	AY181802	-	-
Suaeda prostrata group	Freitag 28.793 (KAS)	Turkey	AY181769	AY181894	AY181822
Suaeda salsa (L.) Pall.	Freitag 28.053 (KAS)	W Kazakhstan	AY181762	AY181887	AY181822
Suaeda sp. Sokotra	Kilian YP3644 (KAS)	Socotra, Yemen	FJ449775	-	FJ449822
Suaeda ''ekimii''	Freitag 10.2002 (KAS)	Turkey	AY181804	AY181931	AY181869
Suaeda spicata (Willd.) Moq.	Schü tze ER361 (KAS)	S France	AY181767	AY181892	AY181828
Suaeda splendens (Pourr.) Gren. & Godr.	Freitag 27.205a (KAS)	Spain	-	AY181908	AY181844
Suaeda stellatiflora G.L.Chu	Lomonosova 67b (NS, KAS)	E Kazakhstan	AY181771	AY181896	AY181832
Suaeda tschujensis Lomonosova & Freitag	Lomonosova 82 (NS, KAS)	Russia	AY181777	AY181902	AY181838
Suaeda vera Forssk. ex J.F. Gmel.	Schü tze ER311 (KAS)	NE Spain	AY181803	AY181930	AY181868
Suaeda vermiculata Forssk.ex J.F. Gmelin	Hensen 03.04.01 (KAS)	SE Spain	AY181787	AY181916	AY181852
Allenrolfea occidentalis (S. Watson) Kuntze	Piep. & Long 120(UTG)	USA	AY181810	AY181937	AY181875
Kalidium foliatum (Pall.) Moq	Freitag 28.141 1141	NW Kazakhstan	AY181809	AY181936	AY181874
Microcnemum coralloides (Loscos & Pardo) Buen	Vural 7558 (GAZI, KAS)	Turkey	AY181811	AY181938	AY181876
Salicornia europaea L.	Schü tze 07.09.01(KAS)	Germany	AY181814	AY181941	AY181879
Salicornia europaea L.	Schü tze ER313 (KAS)	Spain	AY181815	AY181942	AY181880
Salicornia fruticosa L.	Freitag 27.202 (KAS)	SW Spain	AY181816	AY181943	AY181881
Sclerostegia moniliformis Paul G. Wilson	Schmalz 184 (MJG)	Australia	AY181813	AY181940	AY181878
Tecticornia australasica (Moq.) Paul G. Wilson	Jacobs 8685 (NSW)	Australia	AY181812	AY181939	AY181877

Appendix 4-1d. Sequences downloaded from GenBank, part of previously published studies of genus *Tetraena* (Alzahrani and Albokhari, 2017; Beier et al., 2003; Bellstedt et al., 2008; Sheahan and Chase, 2000; Wu et al., 2015). Synonyms between brackets based on Lauterbach et al. (2016). Outgroup taxa shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession	GenBank accession
			*rbc*L	*trn*L-F
Tetraena alba (L.f.) Beier & Thulin var. *alba*	Alzahrani & Albokhari 139 (KAUH)	Umluj, Saudi Arabia	MG664309	MG664340
Tetraena alba (L.f.) Beier & Thulin var. *arabica* Alzahrani & Albokhari	Alzahrani & Albokhari 138 (KAUH)	Umluj, Saudi Arabia	MG664310	MG664341
Tetraena applanata (Van Zyl) Beier & Thulin	Bellstedt 870 (STE)	Namibia	EF655988	EF656012
Tetraena chrysopteron (Retief) Beier & Thulin	Marais 427 (STE)	S Africa	-	EF656013
Tetraena clavata (Schltr. & Diels) Beier & Thulin	Bellstedt 878	Namibia	EF655986	EF656010
Tetraena coccinea (L) Beier & Thulin	Alzahrani & Albokhari 128 (KAUH)	Western region, Saudi Arabia	MG664305	MG664336
	Ryding 1347 (K)	Eritrea	AJ133863	AJ387965
Tetraena cylindrifolia (Schinz) Beier & Thulin	Craven 3800 (WIND)	Namibia	AJ133864	AJ387966
Tetraena decumbens (Delile) Beier & Thulin	Thulin et al. 7981 (UPS)	Yemen	AJ133865	AJ387967
Tetraena decumbens (Delile) Beier & Thulin var. *decumbens*	van Zyl 4588 (STE)	Namibia	EF655991	EF 656011
Tetraena decumbens (Delile) Beier & Thulin	Alzahrani & Albokhari 142 (KAUH)	Umluj, Saudi Arabia	MG664307	MG664338

Appendix 4-1d. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession *rbcL*	GenBank accession *trnL-F*
Tetraena giessii (Merxm. & A. Schreib.) Beier & Thulin	Bellstedt 874 (STE)	Namibia	EF655980	EF 656000
Tetraena hamiensis (Schweinf.) Beier & Thulin	Thulin et al. 9840 (UPS)	Yemen	-	AY300783
Tetraena hamiensis (Schweinf.) Beier & Thulin var. *hamiensis*	Alzahrani D24 (KAUH)	Al Ahsa, Saudi Arabia	MG664292	MG664323
Tetraena hamiensis (Schweinf.) Beier & Thulin var. *qatarensis* (Hadidi ex Beier & Thulin) Alzahrani & Albokhari	Alzahrani D16 (KAUH)	Al Ahsa, Saudi Arabia	MG664295	MG664326
Tetraena hamiensis (Schweinf.) Beier & Thulin var. *mandavillei* (Hadidi ex Beier &Thulin) Alzahrnai & Albokhari	Alzahrani D13 (KAUH)	Khurais, Saudi Arabia	MG664299	MG664330
Tetraena longicapsularis (Schinz) Beier & Thulin	Bellstedt 879 (STE)	Namibia	EF655981	EF 656001
Tetraena madagascariensis (Baill.) Beier &Thulin	Keating Miller 2236 (K)	Madagascar	-	AY300784
Tetraena madecassa (H. Perrier) Beier & Thulin	Lorence s.n. (K)	Madagascar	-	AY300785
Tetraena microcarpa (Licht. ex Cham.) Beier & Thulin	van Zyl 4591 (STE)	Namibia	EF655983	EF656002
Tetraena migiurtinorum (Chiov.) Beier & Thulin	Thulin et al. 9553 (UPS)	Yemen	-	AY300786
Tetraena mongolica Maxim.	Sheahan 1994 (K)	Mongolia	Y15027	AJ387959
Tetraena patenticaulis Van Zyl ined. (syn. *Zygophyllum patenticaule* Van Zyl)	Bellstedt 868 (STE)	Namibia	EF655989	EF656008
Tetraena prismatocarpa (Sond.) Beier & Thulin	Bellstedt 860 (STE)	Namibia	EF655990	EF656009
Tetraena propinqua (Decne.) Ghaz. & Osborne subsp. *propinqua.*	Alzahrani & Albokhari 109 (KAUH)	Shuaibah	MG664288	MG664319
*Tetraena propinqua (*Decne.) Ghaz. & Osborne subsp. *migahidii* (Hadidi ex Beier & Thulin) Alzahrani & Albokhari	Alzahrani & Albokhari 145 (KAUH)	Hail, Saudi Arabia	MG664287	MG664318
Tetraena pterocaulis (Van Zyl) Beier & Thulin	Mucina 270806/25 (STE)	N Cape	-	EF656007
Tetraena retrofracta (Thunb.) Beier & Thulin	Marais 430 (STE)	S Africa	-	EF656014
Tetraena rigida (Schinz) Beier & Thulin	van Zyl 4590 (STE)	Namibia	EF655982	EF656003
Tetraena simplex (L.) Beier & Thulin	Chase 806 (K)	Egypt	Y15031	AJ387974
	Bellstedt 854 (STE)	Namibia	EF655984	EF656004
	Alzahrani & Albokhari 146	Hail, Saudi Arabia	MG664280	MG664311
Tetraena simplex (L.) Beier & Thulin (syn. *Zygophyllum inflatum* Van Zyl)	HK 1490 (WIND)	Namibia	-	EF656005
Tetraena simplex (L.) Beier & Thulin (syn. *Zygophyllum spongiosum* Van Zyl)	HK 1573 (WIND)	Namibia	EF655985	EF656006
Tetraena segmentata (Van Zyl) ined. (syn. *Zygophyllum segmentatum* Van Zyl)	Bellstedt 861 (STE)	Namibia	EF655987	EF 656015
Tetraena tenuis (Glover) Beier & Thulin	van Zyl 4593 (STE)	Namibia	-	EF656017
Tetraena turbinata Van Zyl ined. (syn. *Zygophyllum turbinatum* Van Zyl)	Bellstedt 799 (STE)	S Africa	-	EF656016
Melocarpum robecchii (Engl.) Beier & Thulin	van Zyl 3894 (STE)	Horn of Africa	AJ133870	AJ387972
Melocarpum hildebrandtii (Engl.) Beier & Thulin	Thulin et al. 9012 (UPS)	Horn of Africa	AJ133868	AJ387971
Fagonia cretica L.	Chase 3432 (K)	North Africa	AJ133855	AJ387942
Fagonia indica Burm. f.	Collenette 10/93 (K)	Horn of Africa	Y15018	AJ387943
Fagonia mollis Delile	Townsend 86/12 (K)		-	AY641601
Augea capensis Thunb.	Bellstedt 934 (STE)	W Cape	EF655978	EF655998
Guaiacum guatemalense Planch. Rydb. & Vail	Chase 640 (K)		Y15019	AJ387948
Larrea tridentata (Sessé & Moc. ex DC.) Coville	Chase 636 (K)	N America2000	Y15022	AJ387951
Roepera glauca (F.Muell.) Beier & Thulin (syn. *Zygophyllum glaucum* F.Muell.)	Chase 2204 (K)	Australia	AJ133867	AJ387970
Roepera orbiculata (Welw. ex Oliv.) Beier & Thulin *(*syn. *Zygophyllum orbiculatum* Welw. ex Oliv.)	Craven 5096 (WIND)	S Angola	EF655979	EF655999
Tribulus macropterus Boiss.	Collenette 3/93 (K)	Africa	Y15028	AJ387961
Seetzenia lanata (Willd.) Bullock	Herman 3964 (K)		Y15025	AJ387956
Zygophyllum fabago L.	Chase 516 (K)		Y15030	AJ387968
Zygophyllum kaschgaricum Boriss.	XJBIZLJ012		-	KR001981 KR002000
Zygophyllum xanthoxylum (Bunge) Maxim.	Chase 1700 (K)	China	AJ133872	AJ387975

Appendix 4-2. Voucher information of the samples sequenced for this study. Species name between brackets referred to the name on the herbarium label. Ext. code= DNA extraction code.

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region		
Genus *Cyperus*						ETS1f	*trnH-psbA*	*rpl32-trnL*
Cyperus articulatus L.	Jazan, Saudi Arabia	R. Basahi 21766	2012	KSU	Cym17h	MN885619	×	MN901107
Cyperus bulbosus Vahl	Farasan Alkabir Island	Collenette 8981	1994	E	Cyf89h	MN885618	MN885627	MN901104
	Yemen	Miller , A.G.Long, D.G. 3467	1981	E	CyY34h	×	×	MN901103
Cyperus conglomeratus Rottb.	Farasan group	J. Thomas 22382	2009	KSU	Cyf18h	×	×	×
	Jazan, Saudi Arabia	S. Alharbi 3	2016	RNG	Cym3	MN885622	MN885629	MN901099
	Farasan Alkabir Island	S. Alharbi 139	2016	RNG	Cyf139	MN885621	MN885628	MN901100
	Dawshak Island	S. Alharbi & R. Al Qthanin 104	2016	-	Cyd104	MN885620	MN885630	MN901101
	Eritrea	S Edwards & P Rao 3515	1984	EIT	CyE35h	×	×	×
	Gebel Elba, Egypt	Springuel,I.; Ali, M. & Badri, M. 81A	1994	E	CyEg81h	MN885623	×	MN901102
Cyperus dubius Rottb.	Oman	Knees, MacKinnon, MacLaren & Page 161	2014	E	CyO16h	×	×	MN901098
Cyperus iria L.	Oman	Miller Anthony G. 7261	1985	E	CyO72h	MN885624	×	MN901097
Cyperus rigidifolius Steud.	Yemen	Muller-Hohenstein, K. & Deil, U. 750	1982	E	CyY75h	×	×	×
Cyperus rotundus L.	Sajid Island	S. Alharbi 176	2017	RNG	CyS176	MN885625	MN885626	MN901105
	Oman	MacKinnon L. 368	2013	E	CyO36h	×	MN885631	MN901106
Cyperus rubicundus Vahl.	Eritrea	O Ryding 1316	1988	EIT	CyE13h	×	×	×
Genus *Heliotropium*						ITS1	*trnL*	
Heliotropium arbainense Fresen.	Makkah, Saudi Arabia	S. Alharbi 11	2016	RNG	Hm11	MN886500	MN885574	
Heliotropium bacciferum Forssk	Kuwait	Anisa & Lamia AR1762	1983	RNG	HK17h	MN886485	MN885556	
Heliotropium dentatum Balf.f.	Yemen	Miller , A.G. 8392	1989	E	HY92h	MN886487	MN885565	
Heliotropium ellipticum	Saudi Arabia	John D. Dwyer 13722	1976	RNG	Hm13h	MN886482	MN885572	
Heliotropium europaeum L.	Iraq	Abbass	1989	RNG	HI1h	MN886478	MN885567	
	S. Yemen	L. Kasasian	1980	RNG	HY26h	MN886481	MN885571	
	Morocco	M.Mateos, M.Reina, G. Sangalli, N.Sandon & B. Valides 4614/95	1995	RNG	HMo46h	MN886499	MN885569	
	Pakistan	C. Parker 7404	1994	RNG	HP74h	MN886483	MN885570	
Heliotropium indicum L.	Thailand (H)	R.Nam-Matra & A.Kaisoon 137	2006	RNG	HT13h	MN886471	MN885543	
Heliotropium lasiocarpum Fisch. & C.A.Mey.	Iraq	Abbass	1989	RNG	HI2h	MN886479	MN885568	

Appendix 4-2. Continued**.**

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region	
						ITS$_1$	*trnL*
Genus *Heliotropium*						ITS$_1$	*trnL*
Heliotropium longiflorum Hochst. & Steud.	Farasan Alkabir	S. Alharbi 95	2016	-	Hf95	MN886474	MN885549
	Farasan Alkabir	S. Alharbi 169	2017	RNG	Hf 169	MN886473	MN885546
	Wadi Ash Sahad, Jazan, Saudi Arabia	S. Alharbi 8	2016	RNG	Hm8	MN886476	MN885548
	Salalah, Oman	C. Parker 0.94	1973	RNG	HO94h	MN886475	MN885544
	Somalia	Lavranos 10031A	1971	E	HSo10h	MN886472	MN885545
	N Taif, Saudi Arabia	J. Humbles 10123	1978	RNG	Hm25h	MN886477	MN885547
Heliotropium pterocarpum (DC.) Hochst. & Steud. ex Bunge	Sabya, Saudi Arabia	T. Al-Turki & J. Thomas 20035	2001	KSU	Hm6h	MN886493	MN885554
	Eritrea	Stephenson PRS308	1949	E	HE30h	MN886492	MN885551
	Jazan City, Saudi Arabia	S. Alharbi 4	2016	RNG	Hm4	×	MN885552
	Wadi Baish, Saudi Arabia	S. Alharbi 6	2016	-	Hm6	MN886491	MN885553
Heliotropium ramosissimum (Lehm.) Sieb. ex DC.	Al Hair, Saudi Arabia	J. Thomas 19506	2001	KSU	Hm4h	×	MN885562
	Al Kharj area, Saudi Arabia	C. Parker S.A.37	1970	RNG	Hm37h	MN886490	MN885561
	Canary Islands	A. Zwaenepoel 527	1993	RNG	HC52h	MN886496	MN885550
	Bahrain	C. Parker 1283	1974	RNG	HB12h	MN886486	MN885555
	Sudan	El-Sheikh 1611	1970	E	Hsu16h	MN886497	MN885563
	Farasan Alkabir Island	S. Alharbi 122	2016	-	Hf122	MN886494	MN885559
	Sajid Island	S. Alharbi 145	2016	RNG	Hs145	×	×
	N Taif, Saudi Arabia	J. Humbles 10125	1978	RNG	Hm23h	MN886495	MN885560
	Sarso Island	S. Collenette 9254	1995	E	HSa92h	MN886488	MN885558
(syn. *Heliotropium fartakense* Sw.)	Oman	S.G. Knees 2	2013	E	HO2h	×	MN885557
Heliotropium riebeckii Schweinf. & Vierh.	Yemen	Miller, A.G. 8377	1989	E	HY77h	MN886489	MN885564
Heliotropium supinum L.	Al Mosul, Iraq	S. Sofajy	1985	RNG	HI3h	MN886480	MN885566
Heliotropium zeylanicum (Burm.f.) Lam.	Fayfa	Mekkey Y & Chaudhary, S.12415	1982	KSU	Hm5h	MN886484	×
Heliotropium sp.	Ethiopia	Fanhv 4678	1987	RNG	HE46h	MN886498	MN885573

Appendix 4-2. Continued.

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region		
Genus *Suaeda*						*atp*B-*rbc*L	*psb*B-*psb*H	ITS
Suaeda aegyptiaca (Hasselq,) Zoh.	Qummah Island	S. Alharbi & R. Al Qthanin 108	2016	-	S108	MN885591	MN901091	MN883380
	Shaqra, Saudi Arabia	J. Thomas & Al-Amro 22346	2014	KSU	Sm3h	MN885593	MN901093	MN883382
	Eritrea	O Ryding 1346	1988	EIT	SE13h	×	×	MN883381
	Failakah Island, Kuwait	A.Rawi 10,841	1981	RNG	SK10h	×	×	×
	UAE	A.M.Lorimer 9436	1980	RNG	UAE	MN885594	MN901094	MN883383
Suaeda fruticosa Forssk. ex Grueter	Farasan Alkabir Island	S. Alharbi 13	2016	-	S13	MN885595	MN901095	MN883378
	Sajid Island	S. Alharbi 146	2016	RNG	SS146	MN885596	MN901096	MN883379
Suaeda monoica Forssk.	Jazan, Saudi Arabia	S. Alharbi 2	2016	RNG	Sm2	MN885590	MN901090	×
	Salalah, Oman	C. Parker 0.75	1973	RNG	SO75h	×	×	×
Suaeda sp.	Bahrain	C. Parker 1272	1974	RNG	SB12h	×	×	×
	S.Yemen	L. Kasasian 2614	1980	RNG	SY26h	×	×	×
	Hofof, Saudi Arabia	L. Kasasian 1477	1977	RNG	Sm14h	MN885592	MN901092	×
Genus *Tetraena*						*rbcL*	*trnL-F*	
Tetraena alba (L.f.) Beier & Thulin var. *alba*	Dawshak Island	S. Alharbi & R. Al Qthanin 103	2016	RNG	Td103	MN894179	MN885615	
(*Zygophyllum album* L.f)	Tunisia	J.Lambinon 99/Tu/34	1999	RNG	TT99h	MN894180	MN885616	
	Cyprus	G.Alziar, T.Hedderson et al 229	1991	RNG	TCy22h	×	×	
Tetraena coccinea (L.) Beier & Thulin	Farasan Alkabir Island	S. Alharbi & R. Al Qthanin 11	2016	RNG	Tf11	MN894177	MN885612	
	Qummah Island	S. Alharbi & R. Al Qthanin 107	2016	RNG	Tq107	MN894182	MN885613	
(*Zygophyllum coccineum* L.)	Eritrea	O Ryding 1347	1988	EIT	TE13h	×	×	
	Farasan group	Collenette 6378	1987	E	T8h	MN894183	MN885611	
	Farasan group	Collenette 5598	1986	E	T7h	×	×	
Tetraena cornuta (Coss.) Beier & Thulin (*Z. cornutum* Coss.)	Tunisia	P.Wilkin & E.Wellens 372	1990	RNG	TT37h	MN894178	MN885617	
Tetraena fontanesii (Webb & Berthel.) Beier & Thulin (*Z. fontanesii* Webb & Berthel.)	Tenerife, Canary Islands	C.Jarvis 519	1977	RNG	TC51h	×	×	
Tetraena gaetula (Emb. & Maire) Beier & Thulin (*Z. gaetulum* Emb. & Maire)	Morocco	S.L.Jury & T.Upson 20493	2007	RNG	TMo20h	MN894181	MN885614	
Tetraena mandavillei (Hadidi) Beier & Thulin (*Z. mandavillei* Hadidi)	UAE	A.M.Lorimer 41	1980	RNG	TU41h	×	MN885610	
Tetraena simplex (L.) Beier & Thulin (*Zygophyllum simplex* L.)	Eritrea	S Edwards & P Rao 3508	1984	EIT	TE35h	×	×	
Tetraena simplex (L.) Beier & Thulin	Farasan Alkabir Island	S. Alharbi & R. Al Qthanin 18	2016	RNG	T18B	MN894176	MN885609	
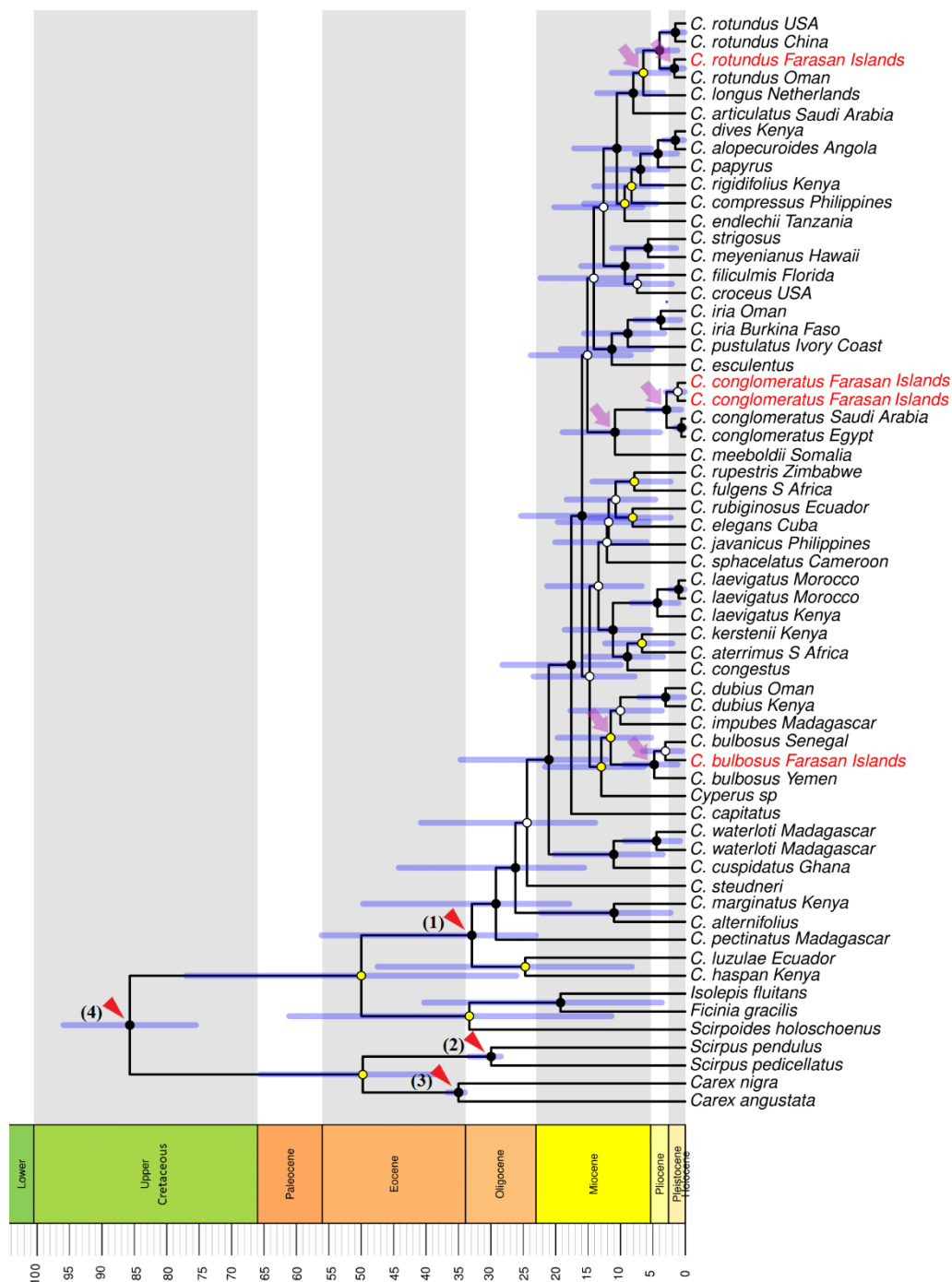
Appendix 4-3a. Maximum clade credibility chronogram of *Cyperus* of combined nuclear and chloroplast data. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥ .75, 0.75>○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 4-4; pink arrows indicate node ages summarised in Figure 4-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *C.* = *Cyperus*

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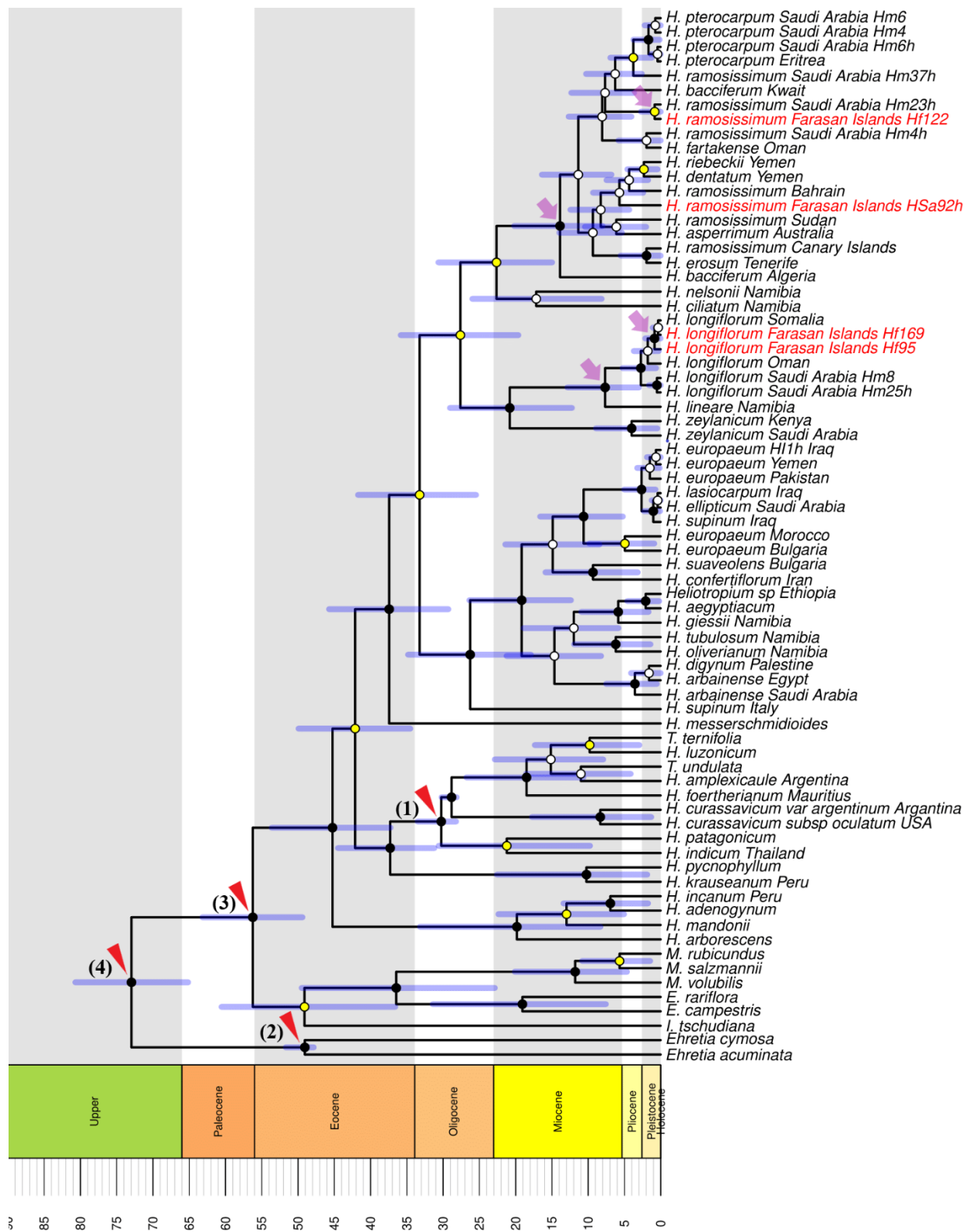
Appendix 4-3b. Maximum clade credibility chronogram of *Heliotropium* of chloroplast and nuclear regions. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 4-4; pink arrows indicate node ages summarised in Figure 4-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *E. = Euploca, H.= Heliotropium, I.= Ixorhea, M. = Myriopus, T. = Tournefortia.*
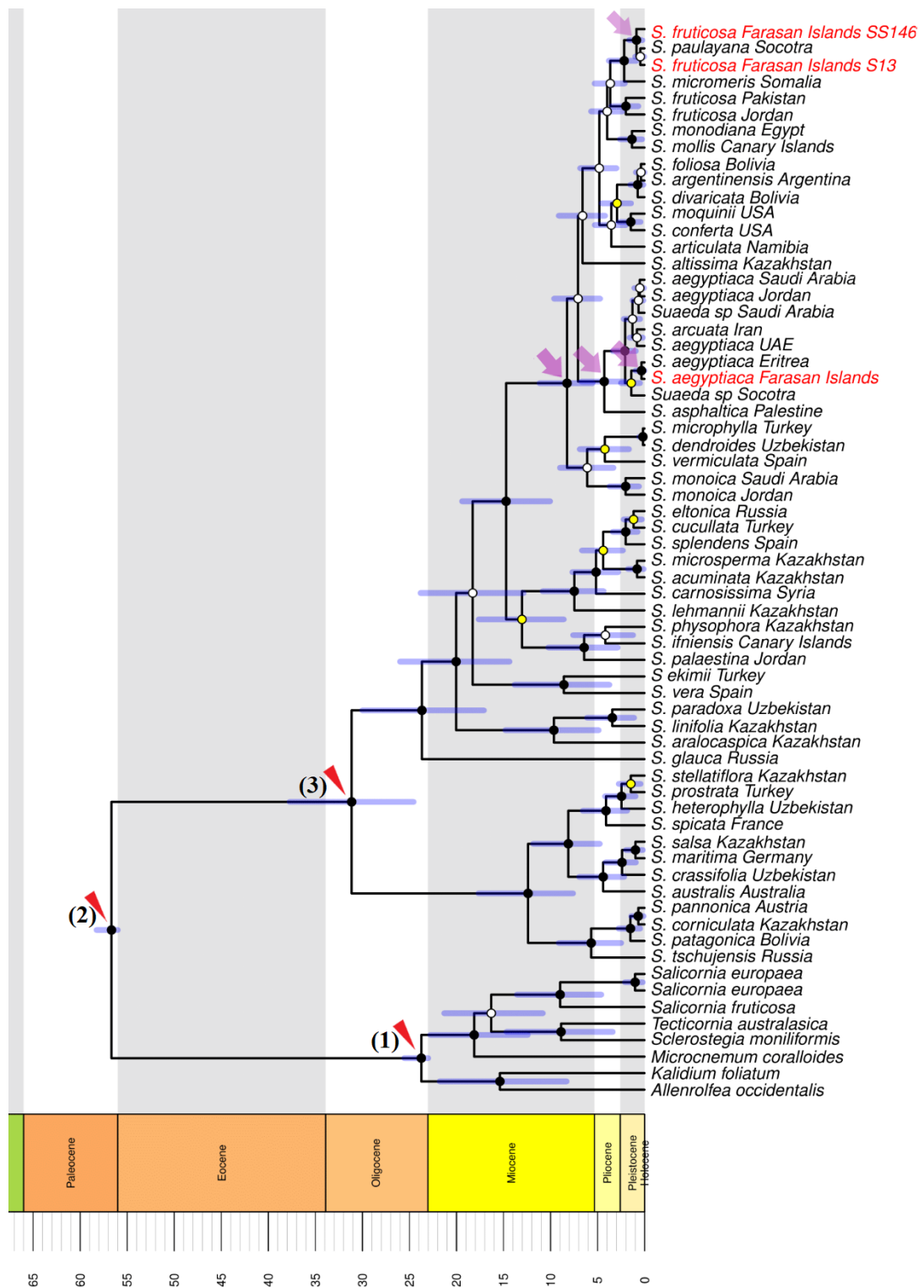
Appendix 4-3c. Maximum clade credibility chronogram of *Suaeda* of combined chloroplast and nuclear data. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 4-4; pink arrows indicate node ages summarised in Figure 4-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *S.=Suaeda*

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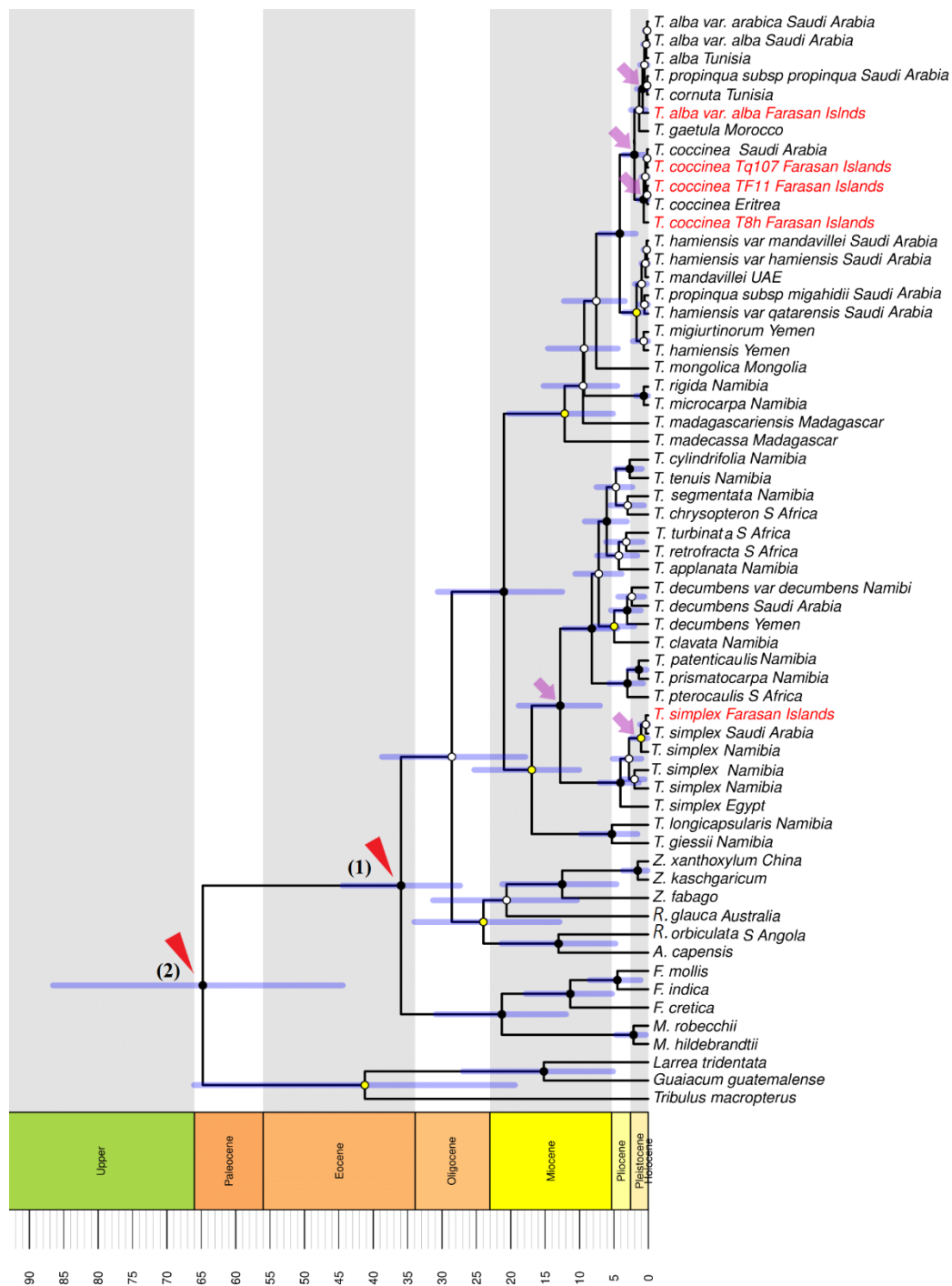
Appendix 4-3d. Maximum clade credibility chronogram of *Tetraena* of chloroplast regions.
Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which marked are with posterior probability:●≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 4-4; pink arrows indicate node ages summarised in Figure 4-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *A. = Augea, F.= Fagonia, M. = Melocarpum, R = Roepera, T. = Tetraena, Z.= Zygophyllum.*

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CHAPTER 5: Phylogenetic affinities and divergence time of the Farasan Islands rocky habitat species of selected genera (*Convolvulus* L., *Euphorbia* L., *Ficus* L., and *Indigofera* L.)

5.1 Introduction

The Farasan archipelago comprises 72 islands and isles (Bruckner et al., 2012). The small islands are dominated by halophytic vegetation (Al Mutairi et al., 2012b), whereas large islands consist of beaches of coral sand or sheer coral cliffs eroded at the bottom by sea water, while the interior part consist of flat or broken fossil coral surfaces, often with large or small fossil coral cave-ins or sinkholes have shrubs and trees (Alwelaie et al., 1993). The fossil coral rocks are the habitat in the archipelago with the most species richness including a great proportion of annuals (El-Demerdash, 1996; Al Mutairi et al., 2012a). This may be because fine soil material accumulates in rock crevices, meaning that most of the rainwater that runs into these clefts is protected against evaporation; thus, under desert conditions cliffs and outcrops are often more favourable to plant life (Zohary, 1973).

The eastern part of Farasan Alkabir Island is the most vegetation-rich area, dominated by *Commiphora gileadensis-Vachellia flava-Salvadora persica* community (Figure 5-1, A). occasionally plants of *Ficus cordifolia* ssp. *salicifolia, Hyphaene thebaica* and rarely *Capparis decidua* are present. Trees and shrubs such as *Maytenus parviflora, Indigofera oblongifolia,* and *Capparis cartilaginea* are also prominent in these areas. There are many climbers over these shrubs and trees such as *Cissus quadrangularis, Convolvulus glomeratus, Rhynchosia minima, Asparagus flagellaris* and *Maerua oblongifolia*. This community is open and supports a well-vegetated ground layer that is dominated by *Tetraena simplex* and *Limonium axillare*. Other herbaceous plants include *Blepharis ciliaris, Abutilon pannosum, Indigofera coerulea, Aerva javanica, Cucumis prophetarum, Cyperus conglomeratus, Kohautia caespitosa, Senna alexandriana* and *S. holosericea* are exist. In the arid plain of the northwest plateau on the Farasan Alkabir and Sajid Islands *Euphorbia collenetteae* form scattered clumps supporting climbing plants such as *Rhynchosia minima, Convolvulus glomeratus, Kickxia corallicola, Maerua oblongifolia* and *Cissus quadrangularis*; together forming the dominant community (Figure 5-1, B). Extensive pockets of *Vachellia flava* woodland exist in the Al-Muharraq area in south-eastern part of Farasan Alkabir, and the Almahsor and Khawlah, on Sajid Island (Figure 5-1, C). At Al-Faqwah coast the huge rocky crevices support the growth of *Ficus glomusa* and *Ficus populifolia* along with thickets of date palms, *V. flava* and *E. collenetteae* (Figure 5-1, D).

This coral rock habitat provides food and shelter for the Idmi gazelle (*Gazella gazella farasani*), the largest natural population of *G. gazella* in Saudi Arabia (Assaeed et al., 1995; Cunningham & Wronski, 2011; Wronski & Schulz-Kornas, 2015), therefore, the Farasan Islands were established as a nature reserve in 1989 (Abuzinada, 2003) for the conservation of *G. gazella.* The coral rocky habitat of Farasan is the only recorded Arabian locality for *Basilicum*

polystachyon, Dinebra somalensis, *Euphorbia coldenetteae*, and *Vahlia digyna*, and the only Saudi Arabian locality recorded for *Micrococca mercurialis* and *Rorida brachystyla*. It is also the home of *Commiphora* aff. *kataf* and *Glossonema* sp. aff. *boveanum,* taxa limited in distribution to some islands of the Farasan Islands (Collenette, 1999; Al-Zahrani, 2010). Furthermore, the Farasan Islands serve as a bioclimatic refuge for Somalia-Masai species such as *Ficus populifolia* and *Grewia tenax* (Hall et al., 2010). Today, this significant biodiversity faces pressing threats. The main island (Farasan Alkabir) is becoming more urbanized, the completion of marine port has increased the number of tourists and road traffic. Factors such as the invasive *Prosopis juliflora* (Figure 5-1, E) and excessive grazing (Figure 5-1, F), increase threats to local biodiversity.
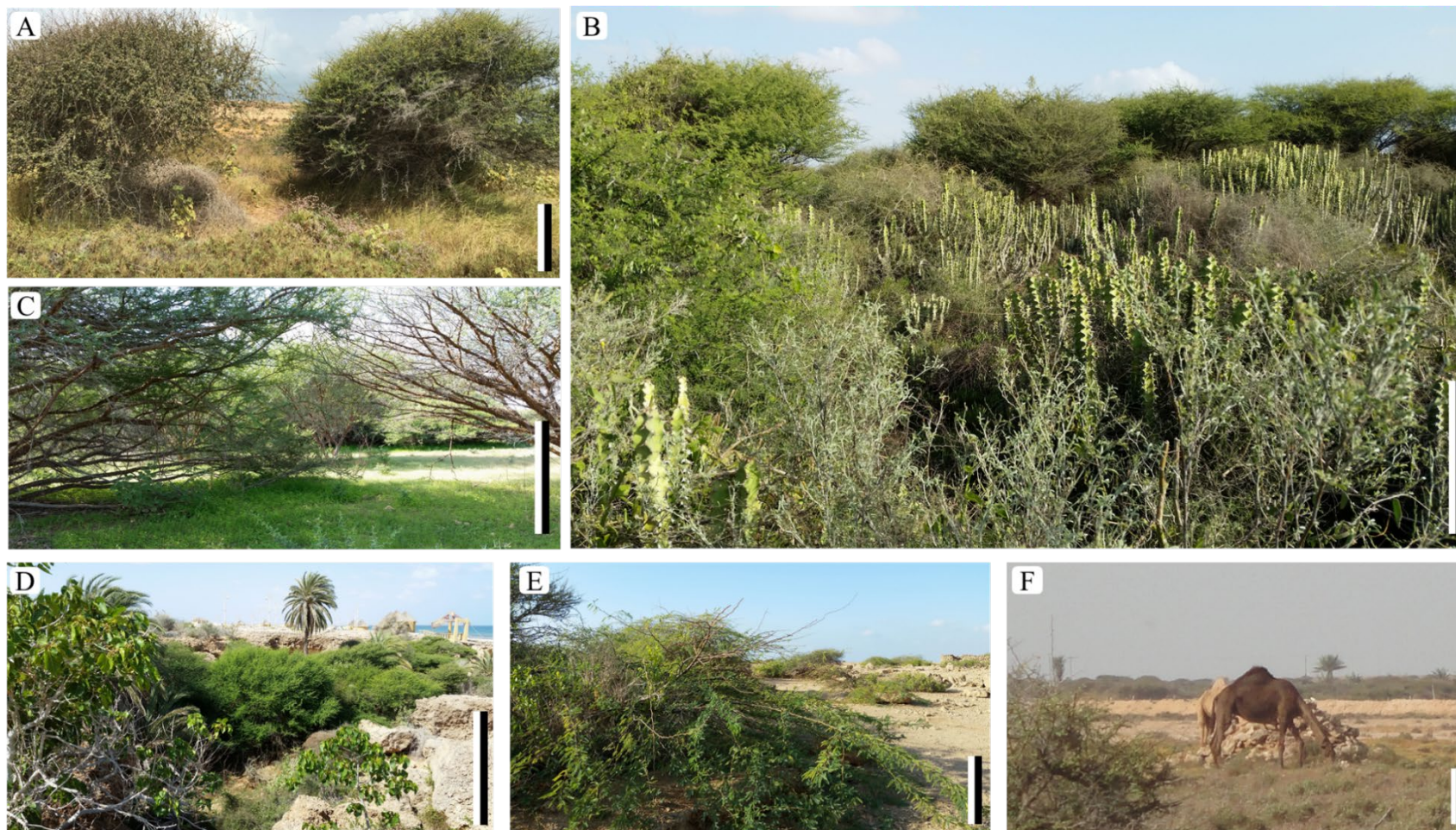
Figure 5-1. Vegetation of coral rocky habitat of the Farasan Islands (A) *Commiphora gileadensis-Vachellia flava-Salvadora persica* community east Farasan Alkabir Island, **(B)** dense pocket of *Euphorbia colletteae* and *V. flava* at Almahsor area, east Sajid Island, **(C)** *V. flava* woodland at Khawlah area, N. Sajid Island, **(D)** rocky crevices at Al-Faqwah coast on Al-Ma'adi Bay, Farasan Alkabir Island, **(E)** the invasive *Prosopis juliflora* penetrating *V. flava* woodland in Al-Muharraq area, south-east Farasan Alkabir, **(F)** vegetation grazed by camels, Sajid Island. The scale bar = 1 m.

The overall vegetation of the Farasan Islands is similar to the Tihama Region of Southwestern Saudi Arabia in both the floral elements and the distribution of natural plant communities (Thomas et al., 2010). Hassan and Al-Hemaid (1996) suggested that the perennial tree component in the Farasan Islands are mostly come from the Arabian Peninsula. This biogeographic hypothesis, however, was inferred based on the short distance between the islands and the Arabian Peninsula. DNA sequence phylogeny of genus *Commiphora* in Saudi Arabia, included Farasan species, supported this hypothesis (Al-Zahrani, 2010). However, this study covers only two species from the Farasan Islands, more molecular phylogenies are needed in different plant groups to fully test the hypothesis. Although the inland species of the Farasan Islands have featured more often in the molecular studies than those in the coastal zone (Chapter 4), these studies mainly focus on population genetics. Different approaches of fingerprinting have been applied in a few numbers of species. Inter-simple sequence repeats (ISSRs) was applied on *Ziziphus spina-christi* (Alansi et al., 2016), Random Amplified Polymorphic DNA (RAPD) technique in *Capparis decidua* (Abdel-Mawgood et al., 2010) and Amplified fragment length polymorphism (AFLP) technique in *Commiphora gileadensis* and *Commiphora* aff. *kataf* (Al-Zahrani, 2010).

The aim of this chapter is to determine the age and biogeographic affinities of the Farasan Islands' interiors species of selected genera (*Convolvulus* L., *Euphorbia* L., *Ficus* L., and *Indigofera* L.). The species will be incorporated in a global phylogenetic context and the timing of diversification events will be estimated in order to answer the following questions: a) is the Arabian Peninsula the main source of the Farasan islands flora? b) does the timing of the Pleistocene land bridges coincide with likely colonisation of plants during the Farasan islands flora formation?

5.2 Materials and Methods

5.2.1 Study group and DNA region selection

Four genera were chosen as an ideal representative of the coral rocky habitat vegetation based on the criteria summarised on Chapter 1. The four genera comprise seventeen species: *Convolvulus* (5 spp.), *Euphorbia* (2 spp.), *Ficus* (3 spp.) and *Indigofera* (7 spp., one of which has two varieties) (Alfarhan et al., 2002; Atiqur Rahman et al., 2002; Hall et al., 2010; Wood et al., 2015). Details including species names, habit, habitat, distribution in the Farasan Islands, dispersal syndrome, flowering and fruiting period and uses are listed in Table 5-1, species global range maps are shown in Figure 5-2.

Table 5-1. List of all recorded native species of *Convolvulus, Euphorbia, Ficus,* and *Indigofera* in the Farasan Islands, including information on the habit, habitat, distribution, fruit type, dispersal syndromes, flowering and fruiting and use. Species marked with an asterisk were sampled for phylogenetic study. F. D = distribution in the Farasan Islands, W. D = world distribution.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Convolvulus arvensis **L.**	Perennial prostrate herb[1]	Weed in cultivated areas and abandoned fields, open Acacia woodland and road margins[2]	**F. D:** Farasan Islands **W.D:** Notorious weed of all temperate regions[1]	Capsule[1]	Zoochorous[31]	All year round[3]	Medicinal herb[4]
Convolvulus glomeratus **Hochst. ex Choisy***	Perennial herb with prostrate, ascending or twining stems[1]	Sandy and saline soils[3]	**F.D:** Widespread in Farasan Alkabir, Sajid and Dumsuk Islands **W.D:** NE. Africa, Middle East, SW Asia[5]	Capsule[1]	Unspecialized[1]	December - August[3]	No data available
Convolvulus piloselliifolius **Desr.**	Perennial prostrate herb[1]	Silty sand[5]	**F. D:** Farasan Alkabir, Sajid and Dumsuk Islands **W.D:** Egypt, Middle East, Pakistan[5]	Capsule[1]	Unspecialized[1]	No data available	Medicinal herb[6]
Convolvulus prostratus **Forssk.[1]**	Perennial prostrate herb[1]	Common on open, waste, dry and sandy soils[3]	**F. D:** Farasan Islands **W.D:** Trop. & N. Africa, Arabian Peninsula to India[1]	Capsule[1]	Unspecialized[1]	Fl.: Jan.-April[3] Fr.: April-May[3]	No data available
Convolvulus rhyniospermus **Choisy***	Annual prostrate herb[1]	Sandy plains and sandy dunes[2]	**F. D:** Sajid Island **W.D:** NE Trop. Africa, Arabia, India, Pakistan[1]	Capsule[1]	Unspecialized[1]	September[3] December	No data available
Euphorbia collenetteae **Al-Zahrani & El-Karemy***	A succulent leafless spiny shrub[7]	Fossil coral substratum and basalt outcrops[7]	**F.D:** Farasan Alkabir, Sajid and Dawshak Islands **W.D:** Farasan Archipelago, Sudan, Eritrea[7]	Schizocarp[11]	Myrmecochory[8]	March-June[7]	No data available
Euphorbia granulata **Forssk. var. granulata***	Annual prostrate herb[9]	Sandy or gravel soils[10]	**F. D.** Farasan Alkabir and Sajid Islands **W.D:** Trop. & N. Africa, Arabian Peninsula, Lebanon-Syria, Palestine-Jordan, Transcaucasia[32]	Schizocarp[11]	Myrmecochory[8] Zoochorous[12]	No data available	Medicinal plant[13]

Table 5-1. Continued.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Ficus cordata subsp. *salicifolia* (Vahl) C.C.Berg*	Small tree[14]	Rocks and cliffs[15]	**F.D:** Farasan Alkabir Island **W.D:** From South Africa and Botswana to the Arabian Peninsula, Socotra Island and Egypt. An outlying population is present in Algeria[16]	Syconium[23]	Zoochorous[17]	All year round[18]	Medicinal plant [13]
Ficus glumosa Del.*	Small tree [14]	Rocky cliffs and slopes[19]	**F. D:** Farasan Alkabir Island **W.D:** Trop. Africa, Arabia[14]	Syconium[23]	Zoochorous[17]	All year round[18]	Edible, of tannin and dye, medicinally used[20].
Ficus populifolia Vahl*	Small tree[14]	Shallow ravines on fossil coral soil[19]	**F.D:** Farasan Alkabir and Qummah Islands **W.D:** SW. Arabia, E. & C. Trop. Africa[14]	Syconium[23]	Zoochorous[17]	All year round[18]	Edible, medicinally used [22,23]
Indigofera coerulea Roxb.var. *coerulea**	Bushy, leafy shrublet[14]	Rocky limestone and in the abandoned field	**F. D:** Farasan Alkabir and Sajid Islands **W.D:** NE. Trop. Africa, from Arabia to India[14], Socotra[24]	Pod[29]	Unspecialized[26]	August-November[3] December, April	Produce the indigo dye (Nil) [25]
Indigofera coerulea Roxb. var. *occidentalis* Gillet & Ali	Bushy, leafy shrublet[14]	Coastal plains and limestone escarpments[24]	**F. D:** Farasan Islands[14] **W. D:** N. & NE. Trop. Africa, Mauritius, Socotra, Arabia and SW. Asia to Pakistan[24].	Pod[29]	Unspecialized[26]	No data available	Produce the indigo dye (Nil)[25]
Indigofera hochstetteri Bak.	Prostrate, decumbent, branched, annual herb[14]	Sand plains[27] and rocky limestone plateau[24]	**F.D:** Farasan Alkabir Island **W.D:** Trop. Africa, Arabia to India[14] Socotra[24]	Pod[29]	Unspecialized[26]	August-October[3]	No data available
Indigofera linifolia (L.f.) Retz.*	Prostrate, much - branched annual herb[14]	Rocky limestone	**F.D:** Farasan Alkabir and Sajid Islands **W.D:** NE Trop. Africa, Trop. Arabia, Afghanistan, India to Australia[14]	Pod[29]	Unspecialized[26]	July-October [3]	No data available
Indigofera oblongifolia Forssk.*	Undershrub spreadingly branched[14]	Rocky limestone and sandy ground	**F.D:** Farasan Alkabir, Sajid, Dumsuk and Zifaf Islands **W.D:** Trop. & S. Africa, Madagascar, Socotra, Arabia to India, introduce to Indonesia and Australia[33].	Pod[29]	Unspecialized[26]	September-November [3] Mach- April[28]	Medicinal plant[13]

Table 5-1. Continued.

Species	Habit	Habitat	Distribution	Fruit type	Dispersal syndrome	Flowering & fruiting	Uses
Indigofera semitrijuga **Forssk.** *	Procumbent, annual herb[14]	Sandy ground	**F.D:** Farasan Alkabir Island **W.D:** NE. Trop. Africa, Arabia[14]	Pod[29]	Unspecialized[26]	December	No data available
Indigofera spinosa **Forssk.** *	Spiny undershrub[14]	Rocky and sand plains[27]	**F.D:** Farasan Alkabir and Sajid Islands **W. D:** NE. Trop. Africa, Arabia[14]	Pod[29]	Unspecialized[26]	March-May[30]	Medicinal plant[13]
Indigofera spiniflora **Boiss.** * **(new record for Farasan Islands)**	Much-branched shrublet[25]	Rocky limestone	**F.D**: Farasan Alkabir and Sajid Islands **W.D:** E. & NE Trop. Africa, Arabia, Socotra[24]	Pod[29]	Unspecialized[26]	December	No data available

1= (Wood et al., 2015) , 2= (Demissew, 1999), 3= (Tropicos. org. Missouri Botanical Garden, 2011) 4=(El-Ghazali et al., 2010) , 5 = (Chaudhary, 2001b) , 6 = (Al-Enazi, 2018), 7= (Al-Zahrani & El-Karemy, 2007), 8= (Horn et al., 2014), 9 = (Chaudhary, 2001c), 10= (Pahlevani & Riina, 2011), 11= (Aldhebiani, 2010) , 12 = (Jordan & Hayden, 1992),13 = (Basahi, 1999), 14= (Alfarhan et al., 2005) , 15 = (Miller & Cope, 1996), 16 = (van Noort & Rasplus, 2019), 17 = (Shanahan et al., 2001), 18 =(Harrison, 2005), 19= (Chaudhary, 1999), 20 = (Jansen & Cardon, 2005), 21 = (Hall et al., 2010), 22=(Beentje, 1988), 23= (Quattrocchi, 2016), 24 = (Cortés-Burns et al., 2002), 25 = (Chaudhary, 2001a), 26 = (Al- (Al-Ghamdi, 2011), 27 = (El-Demerdash et al., 1994), 28= (Zohary, 1972), 29= (Schrire et al., 2009), 30= (Abd El-Ghani, 1997), 31= (Proctor, 1968), 32 = (Euro+Med, 2006), 33 = (POWO, 2019)
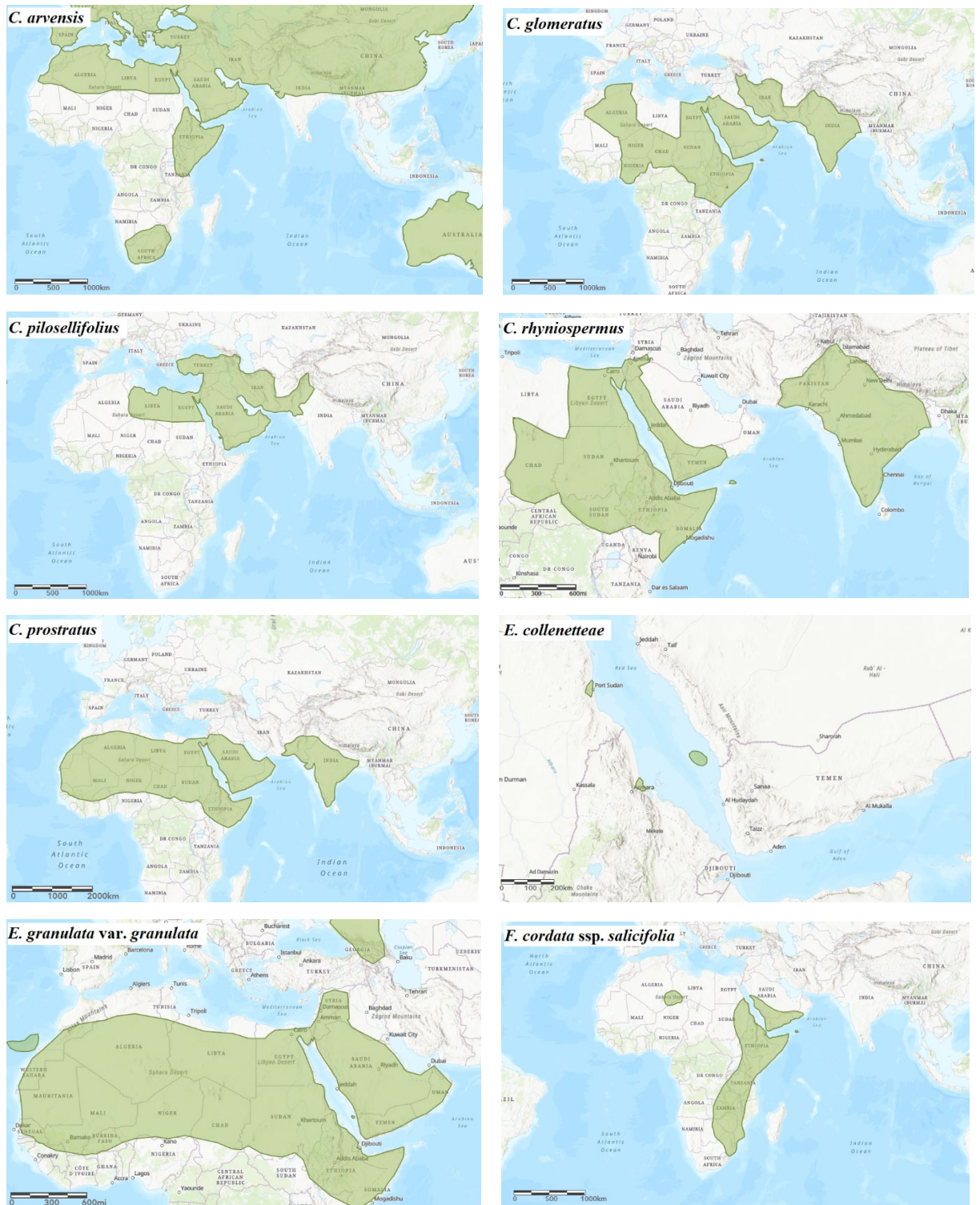
Figure 5-2. Distribution maps of the study group species based on data taken from Alfarhan et al. (2005); African Plant Database (version 3.4.0) (2012); Euro+Med (2006); POWO (2019); Van Noort & Rasplus, (2019), created using ArcGIS Online (Esri, "Topography"). *C = Convolvulus, E = Euphorbia, F = Ficus, I = Indigofera.*
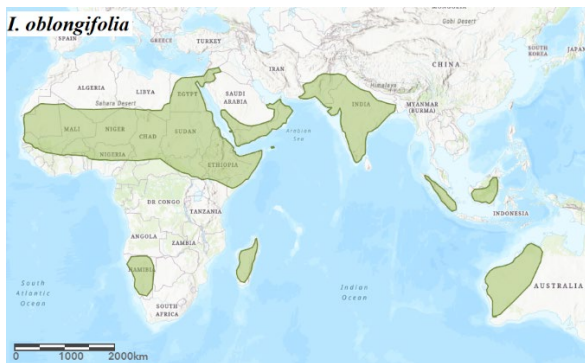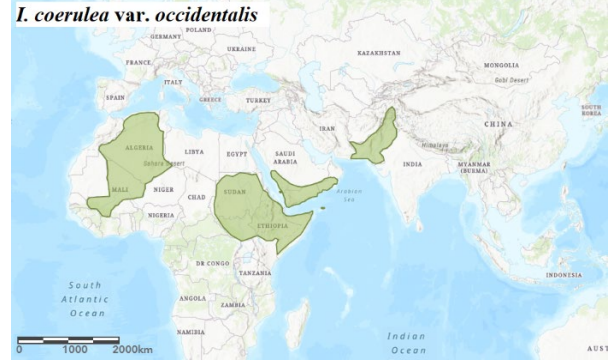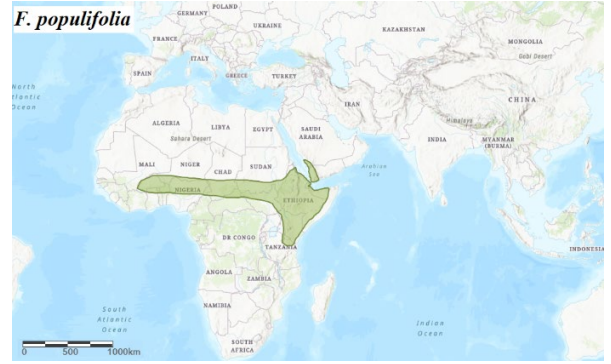
143

Figure 5-2. Continued.

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Brief descriptions of the four selected genera and the available molecular sequence data with the highest taxonomic coverage and relevant source regions are given below.

Convolvulus (Convolvulaceae) is a cosmopolitan genus of around 200 species mostly herbs or undershrubs, almost temperate and subtropical in distribution, rare in the tropics, with the main centre of diversity in the Mediterranean and western Asia (Carine et al., 2003; Tropicos. org. Missouri Botanical Garden, 2011; Wood et al., 2015). Williams et al (2014) sequenced the ITS, *rbcL* and Maturase K gene (*matK*) of 140 species, of which 10 are found within the Red Sea region.

Euphorbia (Euphorbiaceae) is the third largest genus of the flowering plants with approximately 2000 species occurring in both Old and New Worlds, mainly in the tropical, subtropical and warm temperate regions, occupying a wide range of habitats and exhibiting enormous diversity of growth forms (Govaerts et al., 2000). Members of *Euphorbia* are characterised by highly reduced flowers organised into a characteristic inflorescence (the cyathium) (Prenner & Rudall, 2007), and milky latex in their stems (Riina & Berry, 2012). *Euphorbia collenetteae* belongs to subgenus *Euphorbia* section *Euphorbia.* The largest section in the genus includes approximately 360 species of succulent, and photosynthetic stemmed shrubs or trees distributed throughout most of Africa (Dorsey, 2013). Seventy-four species were sequenced for ITS, the chloroplast NADH dehydrogenase F (*ndhF*) gene and *matK* including the partial *trnK* intron by Dorsy et al. (2013). *Euphorbia granulata* var. *granulata* belongs to subgenus *Chamaesyce,* sect. *Anisophyllum,* which composed of annual or perennial herbs (Yang, 2012). Fifteen of the 350 species in the section were sequenced for ITS and *ndhF* mostly from the New World (Yang et al., 2012).

Ficus (Moraceae) is the world's most diverse woody plant genus with around 750 species, distributed in tropical and subtropical regions worldwide; the genus exhibits a variety of growth habits including freestanding trees, shrubs, climbers, hemi-epiphytic stranglers and epiphytes (Berg & Corner, 2005). All species possess a similar obligate pollination mutualism with fig wasps (Agaonidae, Hymenoptera, Chalcidoidea) (Cruaud et al., 2012). Figs are keystone resources in tropical forests, potentially sustaining frugivores through lean periods of low fruit availability (Harrison, 2005). *Ficus glumosa* and *F. populifolia* belong to *Ficus* section *Galoglychia,* which includes 72 species restricted to the African floristic region (Ronsted et al., 2007). Forty-four of the 72 species were the subject of DNA sequencing of nuclear ITS and the ribosomal external transcribed spacer (ETS) (Jousselin et al., 2003; Ronsted et al., 2005; Ronsted et al., 2007; Cruaud et al., 2012). *Ficus cordata* ssp. *salicifolia* belongs to section *Urostigma* subsect. *Urostigma,* which contains 27 species distributed in Africa, Asia, Australia and the Pacific (Chantarasuwan et al., 2015). Twenty-four out of the 27 species were sequenced

for four nuclear DNA markers (ITS, ETS, Glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and glutamine synthetase (ncpGS) (Chantarasuwan et al., 2015).

Indigofera (Fabaceae) is a pantropical genus of small trees or shrubs, comprising about 700 species with centres of diversity in Africa and the Sino-Indian region (Schrire, 1995; Cortés-Burns et al., 2002). Two hundred and sixty-six species were sequenced for the nuclear ITS region, thirty-six species of which sampled from northeast Africa and Arabia (Schrire et al., 2009).

A summary of DNA regions and relevant publications are listed in Table 5-2. Four hundred and twenty-one accessions were downloaded from GenBank for: *Convolvulus* (72); *Euphorbia* (188); *Ficus* (96); Indigofera (65). Details of the GenBank accessions used in this chapter are shown in Appendix 5-1.

Table 5-2. Summary of selective DNA regions for each genus

Genus	Sequence region	Publication
Convolvulus	ITS, *rbcL* and *matK*	Williams et al. (2014)
Subg. *Euphorbia*	ITS, *ndhF* and *matK*/*trnK* intron.	Dorsey et al. (2013)
Subg. *Chamaesyce*	ITS and *ndhF*	Yang et al. (2012)
Ficus	ITS and ETS	Ronsted et al. (2007) Chantarasuwan et al. (2015)
Indigofera	ITS	Schrire et al. (2009)

5.2.2 Taxon sampling

Fourteen species of the Farasan Islands rocky habitat, *Convolvulus* (2 spp.), *Euphorbia* (2 spp.), *Ficus* (3 spp.) and *Indigofera* (7 spp.)*,* were sampled from the field and obtained from the herbaria (outlined in Chapter 2). To supplement the acquired molecular data from GenBank for the chosen genera fifty-eight samples were obtained either from fieldwork or from herbaria, those herbaria were KSU, RIY, E, RNG and EIT; a summary of all voucher specimens whether from Farasan or mainland obtained for this study are listed in Appendix 5-2.

5.2.3 DNA extraction, amplification and sequencing

Methods of DNA extraction and sequencing are detailed in Chapter 4 section 4.2.3. Primer sequences, PCR reactions and cycling conditions for each marker for *Convolvulus, Euphorbia, Ficus*, and *Indigofera* are given in Table 5-3.

5.2.4 Sequence editing and alignment

Outlined in Chapter 3 section 3.2.4. The newly generated sequences were deposited in the GenBank, the accession numbers shown in Appendix 5-2.

5.2.5 Phylogenetic analysis and Bayesian divergence time estimation

Outlined in in Chapter 3 section 3.2.5.

5.2.6 Fossil constraints and secondary calibrations

Because the fossil records for *Convolvulus, Euphorbia, Ficus* and *Indigofera* are poor, node calibration has mainly relied upon the dates estimated in previous studies as temporal constraints. Uncertainty regarding these dates was incorporated by assigning normal prior distributions to these secondary calibration points. Details of primary and secondary constrained nodes, fossil name, organ, offsets and priors for all genera are listed in Table 5-4.

Table 5-3. List of Polymerase Chain Reaction (PCR) Primers, Master Mix recipe and cycling conditions in *Convolvulus, Euphorbia, Ficus,* and *Indigofera.*

Genus	Region ID	Primer sequences 5'-3'	Master Mix recipe	Cycling conditions
Convolvulus	*matK*	3FKIM: CGTACAGTACTTTTGTGTTTACGAG 1RKIM: ACCCAGTCCATCTGGAAATCTTGGTTC (Dunning & Savolainen, 2010)	**29.5 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 14.75 µl 2x BioMix Red 2 µl of each primer (10 mM) 8.25 µl Milli-Q water 0.5 µl BSA Modified from Williams et al. (2014)	95°C for 3 min; 30 cycles of 95°C for 1 min, 50°C for 1 min, and 65°C for 4 min. (Williams et al., 2014)
	rbcL	1F: ATGTCACCACAAACAGAAAC 1460R: TCCTTTTAGTAAAAGATTGGGCC GAG (Savolainen et al., 2000)	**29.5 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 14.75 µl 2x BioMix Red 2 µl of each primer (10 mM) 8.25 µl Milli-Q water 0.5 µl BSA Modified from Williams et al. (2014)	95°C for 3 min; 30 cycles of 95°C for 1 min, 50°C for 1 min, and 65°C for 4 min. (Williams et al., 2014)
	ITS	AB102(26SE): TAGAATTCCCCGGTTCGCTCGCCGTTA C. AB101: ACGAATTCATGGTCCGGTGAAGTGTTC G (Douzery et al., 1999)	**22 µl volume using:** 1 µl of genomic template DNA (10-50 ng) 11 µl 2x BioMix Red 0.99 µl of each primer (10 mM) 7.52 µl Milli-Q water 0.5 µl BSA Modified from Williams et al. (2014)	94°C for 3 min; 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 3min; final extension 72°C for 8 min. (Williams et al., 2014)
Euphorbia	ITS	ITS-I: GTCCACTGAACCTTATCATTTAG (Urbatsch et al., 2000) ITS4: TCCTCCGCTTATTGATATGC (White et al., 1990) ITS-A: GGAAGGAGAAGTCGTAACAAGG	**15 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 7.5 µl 2x BioMix Red 0.5 µl of each primer (10 mM) 4.5 µl Milli-Q water Modified from Dorsey et al. (2013)	95°C for 2 min; 35cycles each had a denaturing step of 30 s at 95°C and an extension step of 45 s at 72°C. The annealing temperature was decreased from 60°C to 50°C as follows: 1 cycle at 60°C,2 cycles at 59°C, 3 cycles at 58°C, 4 cycles at 57°C, 5 cycles at 55°C, 6 cycles at 52°C, and 14 cycles at 50°C. final extension 72°C for 15 min. (Dorsey et al., 2013)
		ITS-A: GGAAGGAGAAGTCGTAACAAGG ITS-B: CTTTTCCTCCGCTTATTGATATG ITS-C: GCAATTCACACCAAGTATCGC ITS-D: CTCTCGGCAACGGATATCTCG ITS-E: CGGCAACGGATATCTCGGCTC (Blattner, 1999) In one part: A+B In two parts: A+C/D+B		95°C for 2 min; 30 cycles of 95°C for 20 sec, 55°C for 30 sec, and 70°C for 1 min; final extension 72°C for 7 min. (Blattner, 1999)
	ndhF	972F:GTCTCAATTGGGTTATATGATG 2110Ri:TCAATTATTCGTTTATCAA 536 F:TTGTAACTAATCGTGTAGGGGA 1318R:CGAAACATATAAAATGCRGTTA ATCC 1318F:GGATTAACYGCATTTTATATGTT TCG 803F:CTATGGTAGCGGCGGGAATTTTT C 972R: CATCATATAACCCAATTGAGAC (Olmstead & Sweere, 1994) In two parts: 972F+2110Ri/ 536F+1318R In three parts: 1318F+2110Ri/ 803F+1318R/536F+972R	**15 µl volume using:** 2 µl of genomic template DNA (10-50 ng) 7.5 µl 2x BioMix Red 0.4 µl of each primer (10 mM) 4.7 µl Milli-Q water Modified from Dorsey et al. (2013)	95°C for 2 min; 35 cycles of 95°C for 45 sec, 53.8°C for 45 sec, and 65°C for 3 min; final extension 65°C for 5 min. (Dorsey et al., 2013)
Ficus	ITS	ITS17SE:ACGAATTCATGGTCCGGTGAA GTGTTCG ITS26SE:TAGAATTCCCCGGTTCGCTCG CCGTTAC (Sun et al., 1994) ITS5: GGAAGTAAAAGTCGTAACAAGG ITS4: TCCTCCGCTTATTGATATGC (White et al., 1990) ITS-A: GGAAGGAGAAGTCGTAACAAGG ITS-B: CTTTTCCTCCGCTTATTGATATG ITS-C: GCAATTCACACCAAGTATCGC ITS-D: CTCTCGGCAACGGATATCTCG (Blattner, 1999)	**20 µl volume using:** 1 µl of genomic template DNA (10-50 ng), 10 µl 2x BioMix Red 2 µl of each primer (10 mM) 6.6 µl Milli-Q water 0.4 µl BSA Modified from Chantarasuwan et al. (2015)	94°C for 2 min; 35 cycles of 94°C for 30 sec, 63°C for 1 min, and 72°C for 1 min; 10 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 1 min; final extension 72°C for 5 min. (Chantarasuwan et al., 2015) 95°C for 2 min; 30 cycles of 95°C for 20 sec, 55°C for 30 sec, and 70°C for 1 min; final extension 72°C for 7 min. (Blattner, 1999)

Table 5-3. Continued.

Genus	Region ID	Primer sequences 5'-3'	Master Mix recipe	Cycling conditions
Ficus	ETS	ETS_Hel1: GCTCTTTGCTTGCGCAACAACT 18S-ETS: ACTTACACATGCATGGCTTAATCT (Baldwin and Markos, 1998) ETS_Fig1_F: GACCCTTGGTTCCTGTGTTGC (Bruun-Lund & Rønsted, unpublished as cited in Chantarasuwan et al. (2015))	**20 μl volume using:** 1 μl of genomic template DNA (10-50 ng), 10 μl 2x BioMix Red 2 μl of each primer (10 mM), 5 μl Milli-Q water 2 μl DMSO Modified from Chantarasuwan et al. (2015)	94°C for 2 min; 45 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 1 min; final extension 72°C for 5 min. (Chantarasuwan et al., 2015)
Indigofera	ITS	ITS18: TCCACTGAACCTTATCATTTAGACC ITS18modified: GTCCACTGAACCTTATCATTTAGAGG (Beyra Matos & Lavin, 1999) ITS26: GCCGTTACTAAGGGAATCCTTGTTAG (Käss & Wink, 1997)	**20 μl volume using:** 1 μl of genomic template DNA (10-50 ng), 10 μl 2x BioMix Red 0.7 μl of each primer (10 mM), 6.6 μl Milli-Q water 2 μl DMSO	96°C for 1 min; 35 cycles of 96°C for 30 sec, 48°C for 30 sec, and 72°C for 1min; final extension 72°C for 4 min. (Schrire et al., 2003)

Table 5-4a. List of fossil priors utilized in BEAST analysis. (Mya= millions of years ago).

Constrained node	Fossil name (Organ)	Age (Mya)	Source	Prior distribution	Offset (Mya)	Mean	Log (SD)
Ficus							
1. Genus *Ficus* (root)	*Ficus* sp. (achene)	Middle Paleocene (60)	Collinson (1989)	Lognormal	60.0	1.0	1.5
Indigofera							
1. *Indigofera* crown	*I.cf. pseudotinctoria* MATS (leaf)	Early Middle Miocene (15.97-13.82)	Liu et al. (1996)	Lognormal	13.82	1.0	0.55

Table 5-4b. List of secondary priors utilized in BEAST analysis. (Mya= millions of years ago).

Constrained node	Age (Mya)	Source	Prior distribution	Mean	Log (SD)
Convolvulus					
1. Red Sea group crown	Late Miocene (c.7.0)	Mitchell et al. (2016)	Normal	7.0	1.25
2. Split between Red Sea and Mediterranean clades	Early late Miocene (c.11.0)	Mitchell et al. (2016)	Normal	11.00	1.5
3. Mediterranean/ North America disjunction	Late Miocene (c.5.92)	Mitchell et al. (2016)	Normal	5.92	1.2
4. *Calystegia* crown (outgroup)	Early Pleistocene (c.2.06)	Mitchell et al. (2016)	Normal	2.06	0.425
Euphorbia					
Sect. *Euphorbia*					
1. Sect. *Euphorbia* crown	Middle Miocene (c.14.0)	Horn et al. (2014)	Normal	14.0	1.8
2. Sect. *Monadenium* crown (outgroup)	Middle Miocene (c.12.49)	Bruyns et al. (2011)	Normal	12.49	3.68
Sect. *Anisophyllum*					
1.Subsect. *Hypericifoliae* crown	Early Middle Miocene (c.15.3)	Horn et al. (2014)	Normal	15.3	2.4
2. Sect. *Anisophyllum* crown (root)	Early Miocene (c.23.6)	Horn et al. (2014)	Normal	23.6	2.7
Ficus					
2. Subsect. *Urostigma* crown	Early Eocene (c.51.31)	Chantarasuwan et al. (2016)	Normal	51.31	5.0
3. Section *Galoglychia*–Section *Americana* crown	Early Oligocene (c.34.75)	Xu et al. (2011)	Normal	34.75	9.0
Indigofera					
2. Tribe Indigofereae (root)	Early Oligocene (32.75)	Schrire et al. (2009)	Normal	32.75	1.0

5.3 Results

5.3.1 Phylogenetic analyses

Sequences were successfully obtained for 82.5% of all samples (Appendix 5-2), given the lack of sequence divergence among individuals of the same species from the same locality in *Ficus populifolia*, *Ficus glumosa* and *Convolvulus glomeratus*, one sequence was selected to represent the taxon from each locality (individuals marked with asterisks, Appendix 5-2). Descriptive statistics for plastid and nucleotide partitions for all genera including amplicon length, alignment length, number of taxa, number of variable sites and the best-fit model of nucleotide substitution are given in Table 5-5.

Table 5-5. Descriptive statistics of nuclear and chloroplast datasets of *Convolvulus, Euphorbia, Ficus,* and *Indigofera.* CP= Chloroplast region, NU= nuclear region

Genus	Sequence region	Amplicon length	Total aligned length (bp)	No. of taxa	Variable sites	Best-fit model of nucleotide substitution
Convolvulus	*matK* (CP)	840	693	28	69 (9.9 %)	GTR+G
	rbcL (CP)	1374	606	27	25 (4.12%)	GTR+G
	cpDNA	-	1298	28	94 (7.2 %)	GTR+G
	ITS (Nu)	764	734	26	191 (26.02 %)	GTR+G
	Nu+CP	-	2033	30	285 (14.01 %)	GTR+I+G
Euphorbia						
Sect. *Euphorbia*	*matK* (CP)	1913	2078	50	216 (10.39%)	GTR+G
	ndhF (CP)	1490	1567	47	190 (12.12%)	GTR+G
	cpDNA	-	3645	52	406 (11.13%)	GTR+G
	ITS	720	664	66	288 (43.37%)	SYM+I+G
	Nu+CP	-	4309	66	694 (16.10%)	GTR+I+G
Sect. *Anisophyllum*	ITS (Nu)	702	718	24	320 (44.5 %)	GTR+ G
	ndhF	1522	1463	25	183 (12.5 %)	GTR+I+G
	Nu+CP	_	2181	25	503 (41.2 %)	GTR+I+G
Ficus	ITS (Nu)	750	733	57	156 (36.02%)	GTR+G
	ETS (Nu)	430	433	57	234 (31.9%)	GTR+G
	Combined data	-	1166	57	390 (33.44%)	GTR+G
Indigofera	ITS (Nu)	688	730	89	413 (56.6%)	GTR+I+G

Convolvulus:

Nuclear ITS and cpDNA (*matK, rbcL*) were combined after the homogeneity partition test indicated that the two data sets are congruent (P> 0.05). The combined data Bayesian inference tree (Figure 5-3) provided better resolution and increased support for the clades compared with separate sequence analyses. *Convolvulus glomeratus* and *C. rhyniospermus* were nested in a monophyletic clade referred to as the Red Sea group by Wood et al. (2015). This group, centred on the Red Sea, comprises species with dense capitulate inflorescences, bluish flowers and/or clavate stigmas and is highly diverse in habit (Wood et al., 2015). *Convolvulus glomeratus* was represented by several samples (Saudi Arabia, Farasan Islands and Ethiopia) that form a strongly supported clade with *Convolvulus* spp. from Saudi Arabia (PP=1.00). However, the relationships among individuals remain unresolved with very short branch lengths. *Convolvulus rhyniospermus* comprises two accessions, from the Farasan Islands and Arabia, which clustered in a clade (PP=1.00).
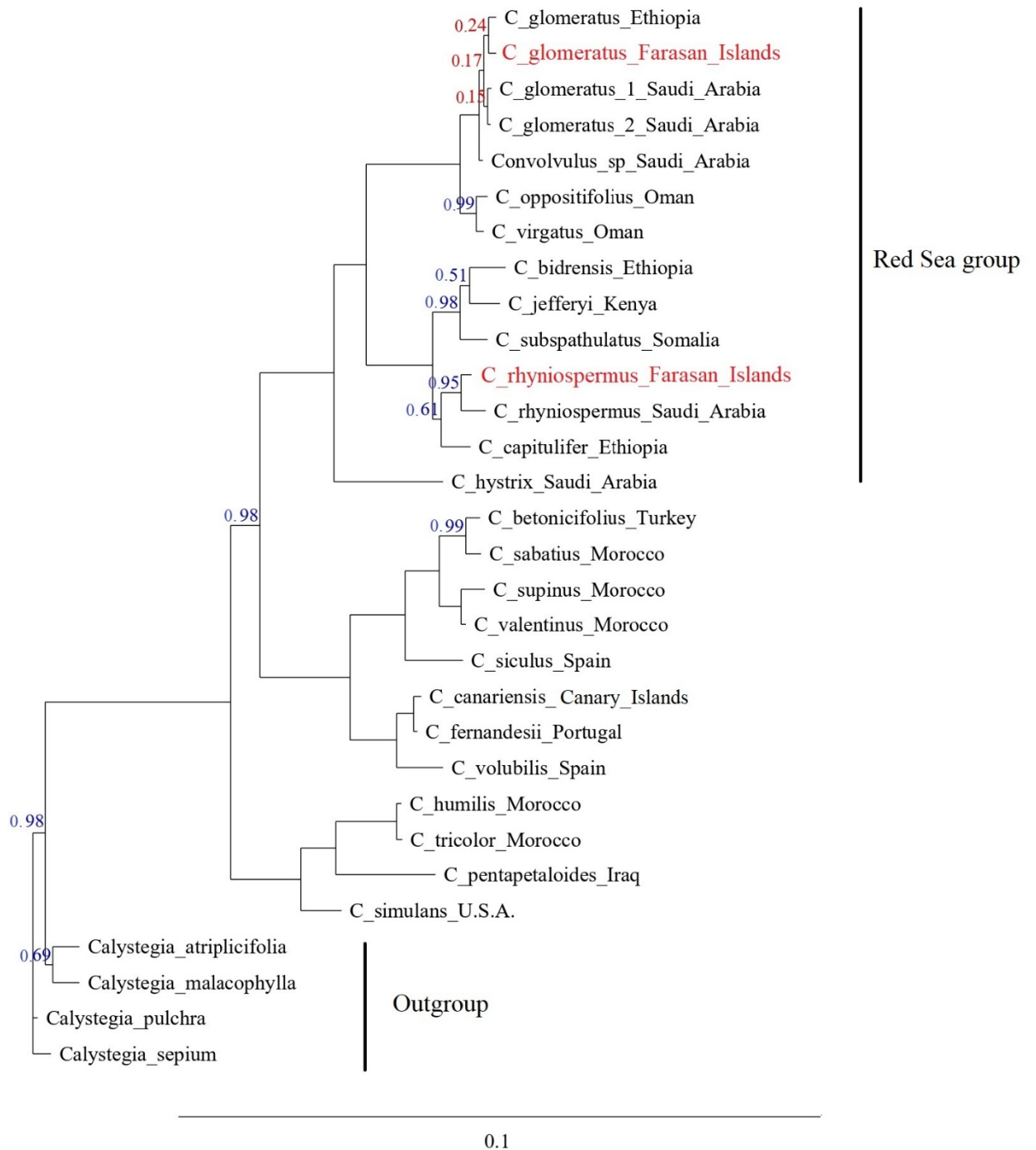
Figure 5-3. Combinable component consensus tree of *Convolvulus* based on combined nuclear ITS and chloroplast *matK* and *rbcL*. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.

Euphorbia:

Separate analyses were conducted for *Euphorbia* subg. *Euphorbia* sect. *Euphorbia* based on ITS and cpDNA (*matK* and *ndhF*), and for *Euphorbia* subg. *Chamaesyce* sect. *Anisophyllum* based on ITS and cpDNA (*ndhF*).

For the sect. *Euphorbia* analyses, no significant posterior probability support for incongruent nodes was evident (exceeding 95%) between the cpDNA (*matK*, *ndhF*) and ITS trees except a few instances of conflict. The main conflict lies in the placement of the monophyletic group comprising the species from *E. neriifolia* to *E. sekukuniensis* (PP=1.00) in the ITS tree; in the cpDNA tree, these species form a grade at the base of the tree sister to the rest of the section (Appendix 5-3, clade A). Another conflicting result involves the distinct positions of the Arabian endemics *E. fruticosa* and *E. seibanica*; ITS analyses place them sister to *E. parciramulosa* (PP=1.00), they are sister to *E. fractiflexa* and *E. collenetteae* (PP=0.95-0.88) in the cpDNA tree (Appendix 5-3, clade B). Given that the few instances of conflict were within a strongly supported clade (sect. *Euphorbia*), in addition to the unambiguous position of the *E. collenetteae* between the trees, all datasets were concatenated for further investigation. The BI analysis of the concatenated data set (Figure 5-4) produced a more resolved phylogeny and was better supported overall than either of the two trees based on ITS or cpDNA gene regions. It shows that *E. collenetteae,* the Red Sea endemic, is strongly supported as sister to the Arabian endemic *E. fractiflexa* (PP=1.00), and both were sister to *E. cactus* from Oman (PP=1.00). Noticeably the Arabian endemic stem-succulent euphorbias do not form a monophyletic group (Figure 5-4, taxa in blue) they are instead scattered among African species.

In subg. *Chamaesyce* sect. *Anisophyllum* nuclear ITS and chloroplast *ndhF* were combined after the ILD test indicated no significant difference (P> 0.05) between the datasets. The BI tree of concatenated regions (Figure 5-5) supports the monophyly of *E.* subsect. *Hypericifoliae* (PP= 1.00). Within this subsection, *E. granulata* from the Farasan Islands and Oman were sister populations (PP= 0.67) and nested among a highly supported group (PP= 0.98) of the Old World species, which consists of prostrate to ascending herbs and subshrubs such as *E. arabica*, *E. humifusa*, *E. riebeckii, E. hispida, E. inaequilatra* and *E. maculata.*

Figure 5-4 (tree labels):

0.84 — E_bougheyi_South_Africa
E_cactus_Yemen
0.42 — E_brevitorta_Kenya
E_bussei_East_Africa
E_magnicapsulala_Ethiopia
0.99 — E_grandicornis_South_Africa
0.96 — E_pseudocactus_South_Africa
0.48 — E_breviarticulata_E_&_NE_Africa
E_pseudoburuana_NE_Africa
E_cooperi_South_Africa
E_virosa_South_Africa
E_enormis_South_Africa
0.68 — E_tortirama_South_Africa
E_collenetteae_Farasan_Islands
0.97 — E_fractiflexa_Arabia
E_cactus_Oman
E_robecchii_NE_Africa
E_qarad_Yemen
0.86 — E_caerulescens
0.77 — E_ledienii
E_keithii
0.96 — E_curvirama
E_limpopoana
E_perangusta
E_avasmontana
E_ammak_Yemen — South Africa
E_clivicola_South_Africa
0.95 — E_otjipembana_Namibia
E_proballyana_Tanzania
0.72 — E_lenewtonii_NE_Africa
E_contorta_South_Africa
0.99 — E_inarticulata_Yeme
0.75 — E_polyacantha_Eritrea
0.82 — E_piscidermis_Eritrea
E_spiralis_Socotra
E_triaculeata_Eritrea
0.57 — E_heterochroma_Kenya
E_heterospina_Kenya
0.96 — 0.89 — E_sudanica_E_&_NE_Africa
E_venenifica_E_&_NE_Africa
E_unispina_W_&_NE_Africa
E_sapinii
0.96 — E_resinifera_N_Africa
E_fruticosa_Arabia
E_seibanica_Yemen
E_parciramulosa_Yemen
0.96 — E_decidua_South_Africa
0.40 — E_tanshawei_Zambia
E_griseola_South_Africa
0.94 — 0.99 — E_abyssinica_E_&_NE_Africa
E_ingens_South_Africa
E_caducifolia
E_teke
E_neriifolia
E_antiquorum — Indian region
E_lactea
E_evansii_South_Africa
0.99 — E_grandidens_South_Africa
0.97 — E_ramipressa
E_tanaensis_Kenya
E_sekukuniensis_South_Africa

E_heteropoda
0.82 — E_invenusta
0.45 — E_bisellenbeckii
E_pseudotrinervis — sect. *Monadenium* (Outgroup)
E_neococcinea

sect. *Euphorbia* / *Euphorbia* subg. *Euphorbia*

0.01

Figure 5-4. **Combinable component consensus tree of *Euphorbia* sect. *Euphorbia* based on nuclear ITS and chloroplast *ndhF* and *matK*.** Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Arabian endemics are coloured blue, Red Sea endemic is coloured red. Scale bar shows the number of substitutions per site.

Figure 5-5 (tree labels):

0.67 — E_granulata_Oman
0.41 — E_granulata_var_granulata_Farasan_Islands
0.26 — E_humifusa_UAE
0.32 — E_arabica_Oman
0.97 — E_riebeckii_Oman
0.71 — 0.68 — E_hispida_Pakistan
E_inaequilatera_Saudi_Arabia
0.98 — E_humifusa_Belgique
E_maculata_Spain
0.90 — 0.98 — E_celastroides_Hawaii
E_thymifolia_Mexico
E_prostrata_Spain
0.61 — E_setosa_Brazil
E_hyssopifolia_Mexico
E_indica_Bahrain
E_peplis_Lebanon
0.96 — E_peplis_Morocco
0.94 — E_polygonifolia_Canada
E_pediculifera_Mexico
E_albomarginata_Mexico
E_serpens_Canary_Islands
0.95 — E_capitellata_Mexico
E_florida_Mexico

E_acuta
E_angusta — subsect. *Acutae* (outgroup)

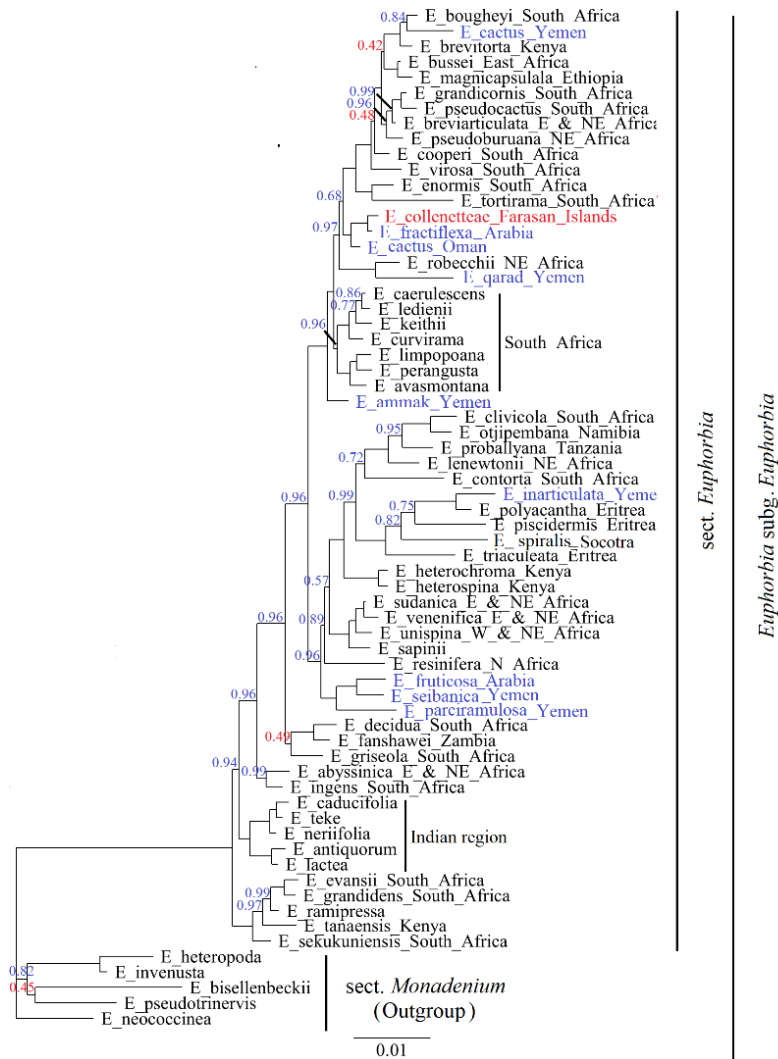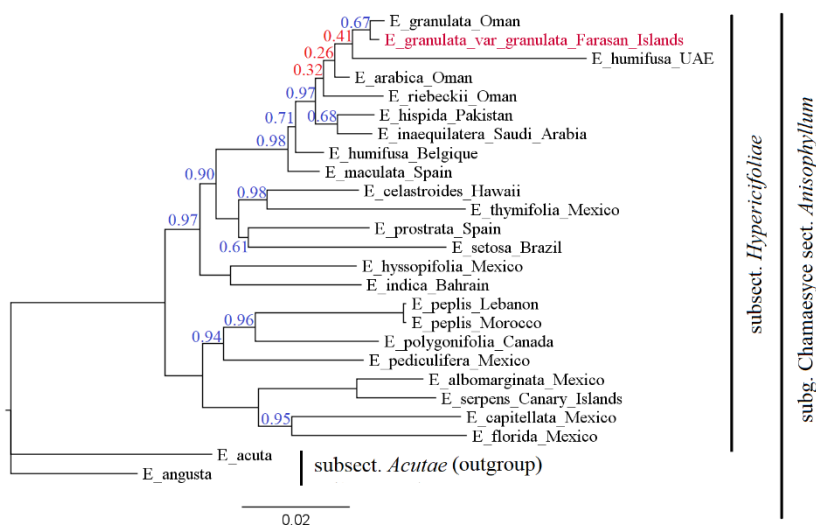subsect. *Hypericifoliae* / subg. *Chamaesyce* sect. *Anisophyllum*

0.02

Figure 5-5. **Combinable component consensus tree of *Euphorbia* sect. *Anisophyllum* based on combined nuclear ITS and chloroplast *ndhF*.** Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.

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Ficus:

Given that the ITS and ETS are part of the same region of the nuclear ribosomal genome (Baldwin & Markos, 1998), and based on previous studies of *Ficus* using ITS and ETS sequence data (Ronsted et al., 2007; Renoult et al., 2009; Chantarasuwan et al., 2015), the two datasets were therefore directly combined into one matrix. All recorded species of *Ficus* in the Farasan Islands belong to subgenus *Urostigma,* which formed a monophyletic group in the Bayesian inference tree (Figure 5-6). *Ficus glumosa* and *F. populifolia* were closely related and nested in subsection *Platyphyllae* of section *Galoglychia*, *F. glumosa* and *F. populifolia* were represented by several samples, each form monophyletic group (with high support). The *F. glumosa* cluster comprises samples from Arabia, Farasan Islands, West and South Africa. Geographical distance shaped the relationships within *F. glumosa* clade, Arabian samples with Farasan formed a strongly supported clade sister to the west African specimen from Cameroun; the South African accession appear basal to them all. While *F. populifolia* was sister to all species of subsection *Platyphyllae* and sampled from Farasan Archipelago and the adjacent areas solely (from Yemen and Somalia), these adjacent populations lack of genetic differentiation in both nuclear segments. In the other hand *F. cordata* ssp. *salicifolia* is nested among section *Urostigma* subsect. *Urostigma*, and is closely related to *F. lecardii* and both formed a strongly supported clade. There was no variation was observed between *F. cordata* ssp. *salicifolia* samples from Farasan Islands and Arabian Peninsula in both ETS and ITS data sets.
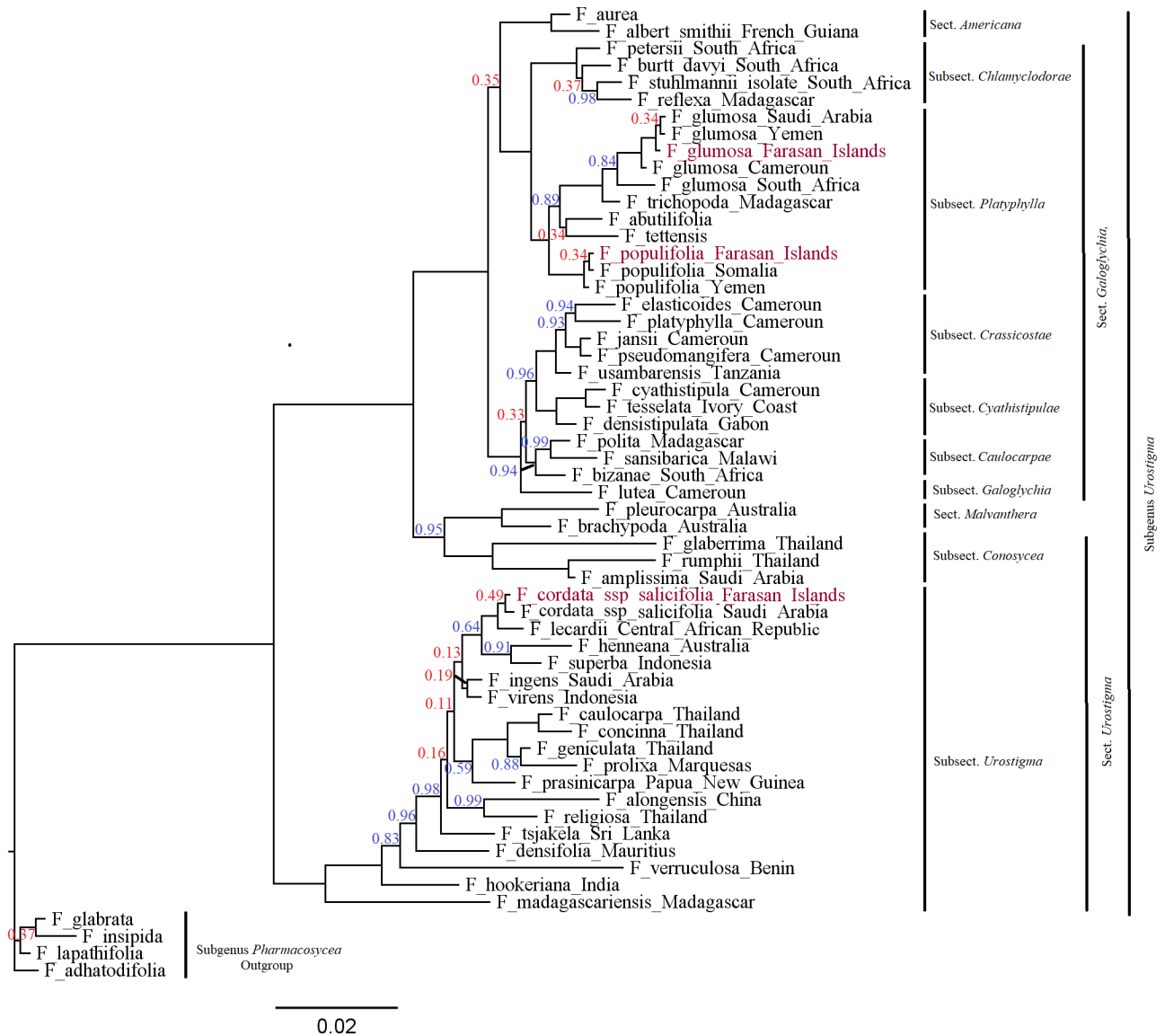
Figure 5-6. Combinable component consensus tree of *Ficus* based on nuclear ITS and ETS. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.

Indigofera:

Indigofera species found in the Farasan Islands fell into two monophyletic clades of genus *Indigofera* out of four clades recognized by Schrire et al. (2003) and Schrire et al. (2009): Palaeotropical, Pantropical, Cape, and Tethyan, which were named according to the biogeographical patterns displayed in each (Figure 5-7). The majority of Farasan species *I. hochstetteri, I. linifolia, I. oblongifolia, I. semitrijuga* and *I. spiniflora* were nested among the Tethyan clade while *I. coerulea* alone was segregate in the pantropical clade.

Indigofera spiniflora and *I. hochstetteri* were sampled from both Farasan and Socotra archipelagos in addition to samples from Arabia or Africa. The accessions of each species formed a monophyletic group (PP=1.00) and (PP=0.85) respectively, in which Farasan individuals were most closely related to the continental lineages, while Socotran populations are distinct found upon long branches located at the base of Farasan/ mainland clade. Within the *I. oblongifolia* clade (PP=1.00) similar geographical distribution was present but with poor resolution observed between Farasan, Socotra and mainland lineages.

For the widely distributed *I. linifolia* representatives were sampled from along the whole geographical distribution: northeast Africa, Asia and Australia. The *I. linifolia* clade was highly structured, Red Sea basin populations (from the Farasan Islands, Eritrea and Jeddah) were distinct from the Asian and Australian lineages and formed a moderately supported clade (PP=0.85). Farasan Islands *I. semitrijuga* was sister to Somalia lineage with very low genetic variation. *Indigofera coerulea* was sister (PP=0.84) to a clade including *I. articulata* and *I. amorphoids* from Saudi Arabia and *I. coerulea* and *I. articulata* from Socotra.
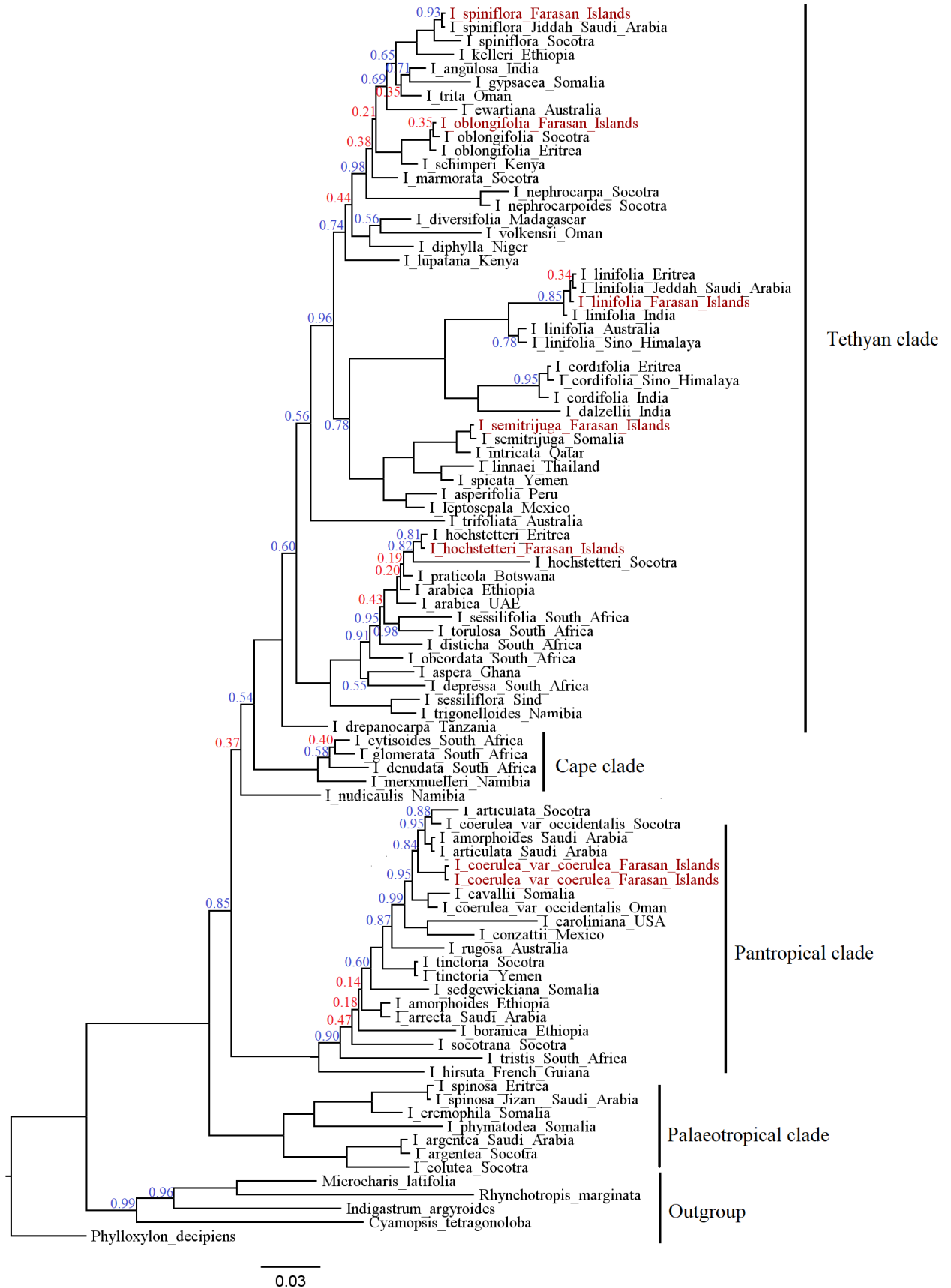
Figure 5-7. Combinable component consensus tree of *Indigofera* based on nuclear ITS. Posterior probabilities less than one are shown near nodes: blue >0.50 support, red ≤0.50. Scale bar shows the number of substitutions per site.
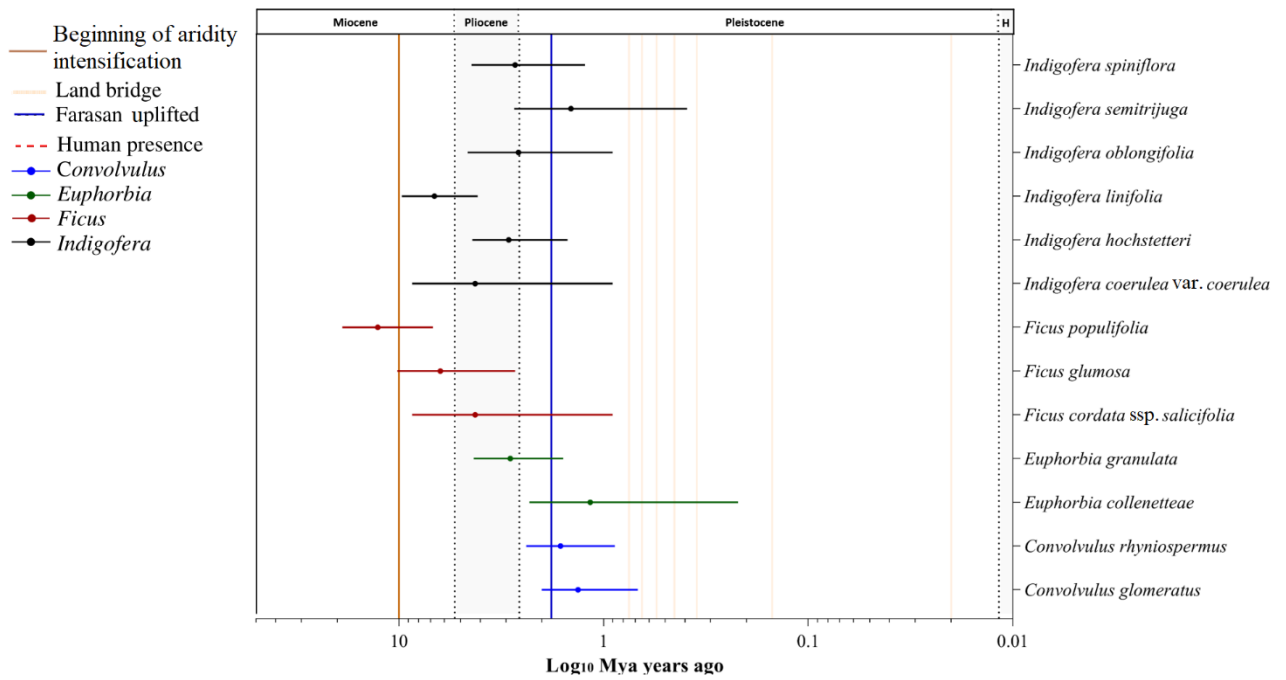
5.3.2 Divergence time estimates

Dated trees produced from BEAST analysis (Appendix 5-4) for any given genus are topologically the same as that produced by MrBayes for all nodes with a posterior probability > 0.50, while clades weakly supported with a posterior probability < 0.50 differed in their topological ordering. The results show that the Farasan Islands coral rocky habitat species under investigation having originated in three successive time frames (Figure 5-12, A): the Miocene, the Pliocene, and the Pleistocene. Divergence of Farasan lineages was during the Middle and early Pleistocene (Figure 5-12, B). Mean ages of species origin and divergence of Farasan lineages with 95% highest posterior density confidence interval HPD for the Farasan Islands species included in this study are listed in Table 5-6 (for *E. collenetteae*, only crown node age is available due to limitations in the data for the Red Sea populations).

Table 5-6. Divergence ages of the rocky habitat species included in this study calculated in BEAST.

Taxon	Species Age (Mya)	Divergence of Farasan lineages (Mya)
Convolvulus glomeratus	1.33 (95% HDP: 0.687 – 2.001)	0.24 (95% HDP: 0.46 – 0.05)
Convolvulus rhyniospermus	1.62 (95% HDP: 0.88 – 2.38)	0.61 (95% HDP: 0.02 – 1.35)
Euphorbia collenetteae	1.16 (95% HDP: 0.2 – 2.3)	-
Euphorbia granulata	2.85 (95% HDP: 1.57 – 4.3)	0.89 (95% HDP: 0.26 – 1.6)
Ficus cordata **ssp.** *salicifolia*	4.23 (95% HDP: 0.90 – 8.6)	1.08 (95% HDP: 0.00013 – 3.289)
Ficus glumosa	6.26 (95% HDP: 2.7 – 10.17)	0.6 (95% HDP: 0.008 – 1.5)
Ficus populifolia	12.66 (95% HDP: 6.8– 18.86)	1.14 (95% HDP: 0.0 – 3.0)
Indigofera coerulea **var.** *coerulea.*	4.23 (95% HDP: 0.9 – 8.6)	0.1 (95% HDP: 0.0000056– 0.59)
Indigofera hochstetteri	2.9 (95% HDP: 1.5 – 4.37)	0.4 (95% HDP: 0.0028 – 1.105)
Indigofera linifolia	6.7 (95% HDP: 4.11 – 9.66)	0.20 (95% HDP: 0.0015 – 0.51)
Indigofera oblongifolia	2.6 (95% HDP: 0.9 –4.6)	0.52 (95% HDP: 0.067 –1.180)
Indigofera semitrijuga	1.44 (95% HDP: 0.39 – 2.73)	0.32 (95% HDP: 0.0055 – 0.82)
Indigofera spiniflora	2.7 (95% HDP: 1.23 – 4.41)	0.21 (95% HDP: 0.000023 – 0.66)
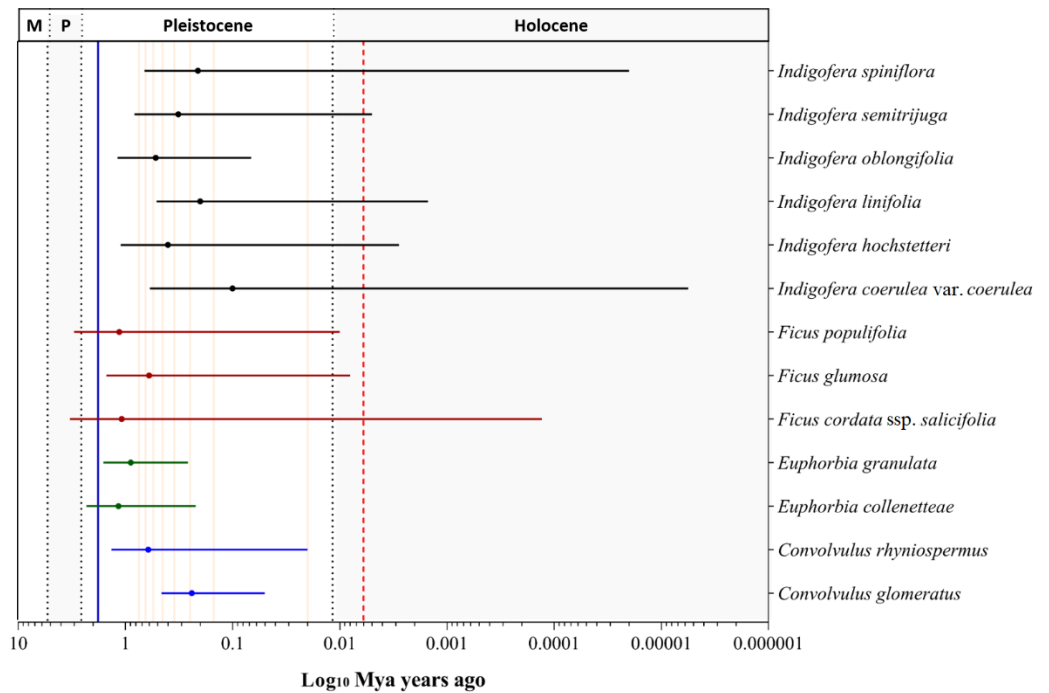
Figure 5-8. Diagram showing estimated lineage divergence times (mean and 95% HPD confidence intervals) for Farasan Islands rocky habitat species dated in this study and indicated in the MCC chronograms depicted in Appendix 5-4. (A) Species origin, (B) divergence of Farasan Islands lineages (crown age of *E. col…nett…eae* put in here is for the purpose of comparison). The age of land bridge formation is based on lowered sea-level (-100m) data from Rohling et al. (2013) and Woodruff (2010).

5.4 Discussion

This study is the first to incorporate the Farasan Archipelago coral rocky habitat species into worldwide phylogenies and to infer molecular divergence age estimates for 14 species belonging to four genera *Convolvulus, Euphorbia, Ficus* and *Indigofera.*

All examined species are drought-adapted plants, with origins spanning three successive time frames (Figure 5-8, A): the Miocene, the Pliocene, and the Pleistocene. Extant research has suggested that from the late Middle Miocene onwards, arid ecosystems expanded in continents' interiors, which is associated with the intensification of aridity (outlined in Chapter 1, Appendix 1) (Kürschner, 1998; Senut et al., 2009; Pound et al., 2012; Sepulchre et al., 2006). The increased availability, and perhaps diversity, of arid niche space during this period may have provided more opportunities for arid plants to develop (Horn et al., 2014). According to previous phylogenetic analysis of Indigofereae (Schrire et al., 2009), *Euphorbia* (Bruyns et al., 2011; Horn et al., 2014) and Convolvuleae (Mitchell et al., 2016), much of the extant diversification of these plant groups occurred during periods of aridity intensification.

Phylogenetic analyses indicate that the global scale affinities of the Farasan Islands lineages belong to the Red Sea region, which corroborates Thomas et al.'s (2010) hypothesis. However, the fine-scale relationships of the species with multiple accessions from the Red Sea, such as *C. glomeratus* (Figure 5-3), *F. glumosa* (Figure 5-8) and *I. linifolia* (Figure 5-7) are unresolved with short branch length. This suggests that the species in the area separated more recently, which is strongly supported by the dated phylogeny. The results of the dated phylogeny suggest that the expansion of the species in the Red Sea region and colonisation of the Farasan Islands occurred during the Pleistocene period (Figure 5-8, B). This process coincided with periodic aridification in the area (Anderson et al., 2013), associated with glaciation at the poles and the uplifting of the Red Sea nearshore islands. These results align with the Pleistocene age estimates of the Farasan coastal species that were investigated in Chapter 4.

The Pleistocene recurring land bridges between Arabia and Farasan could allow overland dispersal of plants with limited seed dispersal mechanisms, such as species of *Convolvulus, Euphorbia* and *Indigofera*, to the islands. The diversification in the Red Sea of the investigated species of *Convolvulus* and *Indigofera* coincides with the formation of land bridges. These species produce seeds that are unadapted for long-distance dispersal (Table 5-1) and are widely distributed in the western and southwestern regions of the Arabian Peninsula (Figure 5-1), which increases the likelihood of their overland dispersal from the Arabian Peninsula. Furthermore, the Red Sea endemic *Euphorbia colleneteae* that is restricted to the Farasan

Islands and a few localities along the eastern coast of Africa are highly supported as sister species to the Arabian endemics *E. fractiflexa* and *E. cactus* (Figure 5-4). *Euphorbia* seeds are mainly ant-dispersed (following ballistic capsule dehiscence), and this syndrome strongly limits dispersal distances and promotes geographic isolation (Horn et al., 2014). *Euphorbia* could benefit from overland dispersal, and this may imply that its speciation was driven by a shift in edaphic preferences (Rajakaruna, 2017) and probably *in situ* evolution on the islands. The chance dispersal of the isolated populations into novel edaphic habitats, due to massive vegetation shifts following climate fluctuation, particularly during the strong climatic fluctuations of the Quaternary, would have created conditions of relaxed competition and facilitated the evolution of edaphic specialisation (Rajakaruna, 2017). The current results corroborates previous phylogenetic analysis of *Commiphora* species conducted by Al-Zahrani (2010) and supports the hypothesis of Hassan & Al-Hemaid (1996) that the Farasan Islands flora are of Arabian origin. However, this study is the first to link Farasan flora formation with the Pleistocene land bridges.

The wetlands of the Farasan Archipelago are an important nesting site for migratory shorebirds (Newton & Symens, 1996; PERSGA, 2004; BirdLife International, 2019), which may have played a significant role in the early stages of the Farasan Islands' flora formation. *Ficus* species and *E. granulata* were among the early colonists of the Farasan interiors (Figure 5-8, B), which were animal-dispersed (zoochorous) plants. *Ficus* is strictly zoochorous (Gillespie & Clague, 2009): figs of the Farasan species are red soft up to 10 mm across (Chaudhary, 1999), and are well known to be dispersed by birds (Lomáscolo et al., 2010). Frugivorous birds were found to have played a pivotal role in the early stages of the islands' forest development, and *Ficus* species are among the early colonists (Gillespie & Clague, 2009). *Euphorbia granulata* seeds are coated with mucilage that can adhere externally to the birds (epizoochory), thus enhancing the likelihood of their long-distance dispersal (Jordan & Hayden, 1992; Steinmann & Porter, 2002).

Humans introduced plants to the Islands as a source of found and for, medicinal and dye properties. In the Canary Islands, 27% of vascular plants are considered introduced (Arechavaleta et al., 2009). Man occupied the Farasan Archipelago 6000 years BP onwards (Bailey et al., 2012). Two species of the study group which have economic importance overlap with the presence of humans: *I. coerulea* and *F. cordata* ssp. *salicifolia. I. coerulea* is well known of the production of the indigo dye (Nil) and was cultivated in Arabia for this purpose (Chaudhary, 2001a). *Ficus cordata* ssp. *salicifolia* is known for its medicinal uses treating vitiligo disease (Basahi, 1999).

5.5 Summary

All the investigated species are drought-adapted plants whose origins coincided with the intensification of aridity in Africa and Arabia from the late Middle Miocene onwards. The expansion of the species in the Red Sea region may have been facilitated by the periodic aridity in the area associated with glacial-interglacial cycles at the poles and may have benefited from the new habitat offered by the Pleistocene uplift of the Red Sea islands, including Farasan Islands.

The island flora appears to have developed via a range of dispersal routes: *Convolvulus, Euphorbia* and *Indigofera* are likely to have colonised the islands from Arabia through overland dispersal via recurring land bridges, while migratory birds that nest in the Islands' wetlands likely transported *Ficus* species and humans probably introduced plants of economic value, such as *I. coerulea.* No single route of dispersal can account for the entire flora.

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Appendices

Appendix 5-1a. Sequences downloaded from GenBank, part of previously published study of genus *Convolvulus* conducted by Williams et al. (2014). Outgroup taxa shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS	GenBank accession *matK*	GenBank accession *rbcL*
Convolvulus bidrensis Sebsbe	D. Sebsebe 2486 (K)	Ethiopia	KC528872	-	-
Convolvulus betonicifolius Miller	Davis 19318 (BM)	Turkey	KC528904	KC529022	KC529182
Convolvulus canariensis L.	M.A. Carine 185 (BM)	Spain	KC528889	KC529024	KC529184
Convolvulus capituliferus subsp. *foliaceus* Verdc.	Ensermu & D. Dessalegn 3679 (ETH)	Ethiopia	KC528876	KC529027	KC529186
Convolvulus fernandesii P.Silva & Teles	A. Pinto & S.N. Cotrim s.n. (BM)	Portugal	KC528895	KC529053	KC529212
Convolvulus glomeratus Choisy,1	W. Zeller 603 (BM)	Saudi Arabia	KC528887	KC529060	KC529219
Convolvulus glomeratus Choisy	T. Mefsin & G.Y. Kagnew 1593 (ETH)	Ethiopia	KC528885	-	-
Convolvulus glomeratus Choisy	D. Hillcoat 369 (BM)	Saudi Arabia(Arabia)	KC528886	KC529061	KC529220
Convolvulus humilis Jacq.	S.L. Jury 19426 (BM)	Morocco	KC528871	KC529068	KC529226
Convolvulus hystrix Vahl.	B. Vincent s.n. (BM)	Saudi Arabia(Saudi)	KC528888	KC529069	KC529227
Convolvulus jefferyi Verdc.	P.A. Luke s.n. (K)	Kenya	KC528875	KC529071	KC529229
Convolvulus oppositifolius Al Alawi	G. Popov 68/46 (BM)	Oman	KC528879	KC529090	KC529247
Convolvulus pentapetaloides L.	R. Wheeler Haines s.n. (E)	Iraq	-	KC529098	KC529255
Convolvulus rhyniospermus Hochst. ex. Choisy	S. Collenette 4740 (E)	Saudi Arabia	-	KC529112	-
Convolvulus sabatius subsp. *mauritanicus* (Boiss.) Murb.	M.A. Carine 282 (BM)	Morocco	-	KC529114	KC529270
Convolvulus simulans L.M.Perry	E.C. Twisselmann 10597 (BM)	U.S.A.	-	KC529122	KC529277
Convolvulus supinus Coss. & Kralik	S.L. Jury 14592 (BM)	Morocco	KC528901	KC529130	KC529285
Convolvulus subspathulatus Vatke	J.J. Lavranos 11250 (E)	Somalia	KC528873	KC529128	KC529283
Convolvulus tricolor L. subsp. *tricolor*	M.A. Carine 250 (BM)	Morocco	KC528870	KC529131	KC529286
Convolvulus valentinus Cav.	M.A., Carine 327 (BM)	Morocco	KC528902	KC529133	KC529288
Convolvulus siculus L. subsp. *siculus,*	M.A. Carine 231 (BM)	Spain	KC528899	KC529121	KC529276
Convolvulus volubilis Link	M.A. Carine 196c (BM)	Spain	KC528898	KC529136	KC529291
Convolvulus virgatus Boiss.	J.P. Mandaville 7113 (BM)	Oman	KC528882	KC529135	KC529290
Calystegia atriplicifolia Hall.f. subsp. *atriplicifolia*	R.R. Halse 3133 (BM)	U.S.A.	KC528915	KC529139	KC529294
Calystegia malacophylla subsp. *pedicellata* (Jeps.) Munz	T. Ross 8545 (BM)	U.S.A.	KC528924	KC529146	KC529301
Calystegia pulChr.a Brummitt & Heywood	J.E. Lousley s.n. (RNG)	England	KC528921	KC529149	KC529304
Calystegia sepium subsp. *americana* (Sims) Brummitt	M.A. Carine 50 (BM)	Portugal	KC528910	KC529150	KC529305

Appendix 5-1b. Sequences downloaded from GenBank, part of previously published studies of genus *Euphorbia*. All data of *Euphorbia* sect. *Euphorbia* belong to Dorsey et al. (2013) except accessions marked with * are taken from Bruyns et al. (2011). Data of *Euphorbia* sect. *Anisophyllum* taken from Yang et al. (2012). Outgroup taxa shaded by gray. L.C.= living collection, E= east, NE= northeast, W=west.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS	GenBank accession *ndhF*	GenBank accession *matK*
Euphorbia subgenus *Euphorbia* sect. *Euphorbia*					
Euphorbia abyssinica J.F. Gmel.	Morawetz, J.J. 372 (EA, K, MICH, MO)	Kenya	KC019616	KC019793	KC019387
Euphorbia ammak Schweinf.	Berry, P.E. 7813 (MICH)	U.S.A. (L.C.), origin Yemen	KC019666	KC019820	KC019427
Euphorbia antiquorum L.	H.-J. & M. van de Bult 08-15 (BKF)	Thailand	KC019593	KC019777	KC019369
Euphorbia avasmontana Dinter	Morawetz, J.J. 284 (MICH)	South Africa	KC019602	KC019783	KC019374
Euphorbia bougheyi L.C. Leach	Berry, P.E. 7788 (MICH)	U.S.A. (L.C.), origin South Africa	KC019658	KC019814	KC019418
Euphorbia breviarticulata Pax	Berry, P.E. 7819 (MICH)	U.S.A. (L.C.), origin E. & NE. Africa	KC019669	KC019823	KC019429
Euphorbia brevitorta P.R.O. Bally	Riina, R. 1734 (MA)	Netherlands (L.C.), origin Kenya	KC019726	-	KC019478
Euphorbia bussei Pax	Morawetz, J.J. 393 (EA, MICH)	Kenya	KC019620	KC019795	KC019388
Euphorbia caducifolia Haines	Berry, P.E. 7759 (MICH)	U.S.A. (L.C.), origin Indian region	KC019648	KC019809	KC019411
Euphorbia caerulescens Haw.	Morawetz, J.J. 273 (MICH, NBG)	South Africa	KC019601	KC019782	-
Euphorbia cactus Ehrenb. ex Boiss.	Morawetz, J.J. 327 (MICH, ON)	Oman, Dhofar	KC019615	-	KC019386
Euphorbia cactus Ehrenb. ex Boiss.	Bruyns 10209 (E)	Yemen	JN207742*		
Euphorbia clivicola R.A. Dyer	Becker, R. 976 (MICH, PRE, UNIN)	South Africa	KC019710	KC019855	KC019465
Euphorbia contorta L.C. Leach	Bruyns 8540 (K)	Mozambique	AM040761*	-	-
Euphorbia cooperi N.E. Br. ex A. Berger	Becker, R. 987 (MICH, PRE, UNIN)	South Africa	KC019714	KC019858	KC019468
Euphorbia curvirama R.A. Dyer	Morawetz, J.J. 309 (MICH)	South Africa	KC019612	KC019791	KC019383
Euphorbia decidua P.R.O. Bally & L.C. Leach	Riina, R. 1719 (MA)	Netherlands (L.C.), origin South Africa	KC019723	KC019864	KC019475
Euphorbia enormis N.E. Br.	Berry, P.E. 7801 (MICH)	U.S.A. (L.C.), origin South Africa	KC019661	KC019816	KC019421
Euphorbia evansii Pax	Morawetz, J.J. 293 (MICH)	South Africa	KC019608	KC019788	KC019379
Euphorbia fanshawei L.C. Leach	Riina, R. 1723 (MA)	Netherlands (L.C.), origin Zambia	KC019724	KC019865	KC019476
Euphorbia fractiflexa S. Carter & J.R.I. Wood	Riina, R. 1752 (MA)	Netherlands (L.C.), origin Arabia	KC019728	KC019870	KC019481
Euphorbia fruticosa Forssk.	Berry, P.E. 7780 (MICH)	U.S.A. (L.C.), origin Arabia	KC019654	KC019812	KC019415
Euphorbia grandicornis Goebel ex N.E	Berry, P.E. 7787 (MICH)	U.S.A. (L.C.), origin South Africa	KC019657	KC019813	KC019417
Euphorbia grandidens Haw.	Morawetz, J.J. 287 (MICH)	South Africa	KC019604	KC019785	KC019376
Euphorbia griseola Pax	Berry, P.E. 7812 (MICH)	U.S.A. (L.C.), origin South Africa	KC019665	KC019819	KC019426
Euphorbia heteroChr.oma Pax	Morawetz, J.J. 402 (EA, MICH)	Kenya	KC019622	KC019797	KC019389
Euphorbia heterospina S. Carter	Berry, P.E. 7875 (MICH)	U.S.A. (L.C.), origin Kenya	KC019693	KC019847	KC019454
Euphorbia ingens E. Mey. ex Boiss.	Becker, R. 985 (MICH, PRE, UNIN),	South Africa	KC019713	KC019857	KC019467
Euphorbia inarticulate Scweinf	Bruyns 10210 (E)	Yemen	JN207780*	-	-
Euphorbia keithii R.A. Dyer	Morawetz, J.J. 290 (MICH)	South Africa	KC019606	KC019786	KC019377
Euphorbia lactea Haw.	Berry, P.E. 7816 (MICH)	U.S.A. (L.C.), origin Indian region	KC019667	KC019821	-
Euphorbia ledienii A. Berger	R. 684 (MICH, PRE, UNIN)	South Africa	KC019707	KC019852	KC019463
Euphorbia lenewtonii S. Carter	Berry, P.E. 7861 (MICH)	U.S.A. (L.C.), origin E. Africa	KC019687	KC019842	KC019448
Euphorbia limpopoana L.C. Leach ex S. Carter	Becker, R. 633 (MICH, PRE, UNIN)	South Africa	KC019705	KC019851	KC019461
Euphorbia magnicapsula S. Carter	Bruyns 11600 (E)	Ethiopia	JN207787*	-	-
Euphorbia neriifolia L.	Berry, P.E. 7776 (MICH)	U.S.A. (L.C.), origin Indian region	KC019653	-	KC019414
Euphorbia otjipembana L.C. Leach	Bruyns 8019 (BOL)	Namibia	JN207799*	-	-
Euphorbia parciramulosa Schweinf.	Bruyns 11755 (E)	Yemen	JN207800*	-	-
Euphorbia perangusta R.A. Dyer	Becker, R. 664 (MICH, PRE, UNIN)	South Africa	KC019706	-	KC019462

Appendix 5-1b. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS	GenBank accession *ndhF*	GenBank accession *matK*
Euphorbia pseudoburuana P.R.O. Bally & S. Carter	Riina, R. 1742 (MA)	Netherlands (L.C.), origin NE. Africa	KC019727	KC019867	KC019480
Euphorbia pseudocactus A. Berger	Morawetz, J.J. 291 (MICH)	South Africa (L.C.)	KC019607	KC019787	KC019378
Euphorbia piscidermis M. Gilbert	ex hort. G. Marx. (BOL)	Ethiopia	JN207801*	-	-
Euphorbia polyacantha Boiss.	Bruyns 10155 (E)	Eritrea	JN207802*	-	-
Euphorbia proballyana L.C. Leach	Bruyns 9670 (E)	Tanzania	JN207804*	-	-
Euphorbia qarad Defl.	Bruyns 11734 (E)	Yemen	JN207807*	-	-
Euphorbia ramipressa Croizat	Berry, P.E. 7820 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019633	KC019824	KC019430
Euphorbia resinifera O. Berg	Berry, P.E. 7817 (MICH)	U.S.A. (L.C.), origin N. Africa	KC019668	KC019822	KC019428
Euphorbia robecchii Pax	Berry, P.E. 7822 (MICH)	U.S.A. (L.C.), origin NE. Africa	KC019671	KC019825	KC019432
Euphorbia sapinii De Wild.	Riina, R. 1756 (MA)	Netherlands (L.C.), origin Africa/Arabia	KC019729	KC019872	KC019482
Euphorbia seibanica Lavranos & Gifri	Riina, R. 1709 (MA)	Netherlands (L.C.), origin Yemen	KC019722	KC019863	KC019474
Euphorbia sekukuniensis R.A. Dyer	Morawetz, J.J. 286 (MICH)	South Africa	KC019603	KC019784	KC019375
Euphorbia spiralis Balf. f.	Bruyns s.n. (E)	Socotra	JN207823*		
Euphorbia sudanica A. Chev.	Berry, P.E. 7810 (MICH)	U.S.A. (L.C.), origin E. & NE. Africa	KC019663	KC019818	KC019425
Euphorbia tanaensis P.R.O. Bally & S. Carter	Morawetz, J.J. 415 (MICH)	Kenya	KC019623	KC019798	KC019390
Euphorbia teke Schweinf. ex Pax	Berry, P.E. 7834 (MICH)	U.S.A. (L.C.), origin Indian region	KC019680	KC019835	KC019442
Euphorbia tortirama R.A. Dyer	Bruyns 6648 (NBG)	South Africa	JN207827*	-	-
Euphorbia triaculeata Forssk.	Bruyns 10152 (E)	Eritrea	JN207829*	-	-
Euphorbia unispina N.E. Br.	Berry, P.E. 7798 (MICH)	U.S.A. (L.C.), origin W. & NE. Africa	KC019660	JN249239	KC019420
Euphorbia venenifica Tremaux ex Kotschy	Berry, P.E. 7868 (MICH),	U.S.A. (L.C.), origin E. & NE. Africa	KC019689	-	KC019451
Euphorbia virosa Willd.	Bruyns 10403 (E)	Angola	JN207834*	-	-
Euphorbia bisellenbeckii Bruyns	Berry, P.E. 7771 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019649	KC019810	KC019412
Euphorbia heteropoda Pax	Berry, P.E. 7750 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019642	KC019807	KC019405
Euphorbia invenusta (N.E. Br.) Bruyns	Berry, P.E. 7747 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019639	KC019805	KC019402
Euphorbia pseudotrinervis Bruyns,	Berry, P.E. 7867 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019688	KC019844	KC019450
Euphorbia neococcinea Bruyns	Berry, P.E. 7749 (MICH)	U.S.A. (L.C.), origin Africa/Arabia	KC019641	KC019806	KC019404
Euphorbia subg. Chamaesyce sect. Anisophyllum					
Euphorbia albomarginata Torr. & A. Gray	Reina-G. 2006-389 (MICH)	Mexico	HQ645221	JQ750762	
Euphorbia capitellata Engelm.	Reina-G. 2006-539 (MICH)	Mexico	HQ645237	JQ750775	
Euphorbia celastroides Boiss.	Berry 7864 (MICH)	Hawaii	JQ750895	JQ750777	
Euphorbia florida Engelm.	Reina-G. 2006-476 (MICH)	Mexico	HQ645268	JQ750794	
Euphorbia hyssopifolia L.	Van Devender 2006-463 (MICH)	Mexico	HQ645283	JQ750818	
Euphorbia pediculifera Engelm.	Reina-G. 2006-483 (MICH)	Mexico	JQ750946	JQ750834	
Euphorbia polygonifolia L.	Berry 7765 (MICH)	Canada	JQ750948	JQ750840	
Euphorbia setosa (Boiss.) Müll.Arg.	Cordeiro 3025 (SP)	Brazil	HQ645346	JQ750853	
Euphorbia thymifolia L.	Van Devender 2006-628 (MICH)	Mexico	JQ750967	JQ750865	
Euphorbia acuta Engelm.	No data available	No data available	AF537450	AF538176	
Euphorbia angusta Engelm.	Yang 41 (MICH)	U.S.A.	HQ645222	JQ750763	

Appendix 5-1c. Nuclear ITS and ETS sequences downloaded from GenBank, part of previously published studies of genus *Ficus* (£Chantarasuwan et al., 2015; **Cruaud et al., 2012; °Jousselin et al., 2003; *Ronsted et al.,2005; §Ronsted et al., 2007; $Renoult et al., 2009). NBG: South African National Biodiversity Institute.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS	GenBank accession ETS
Ficus aurea Nutt.	Rønsted 130 (K)	BG 89-538	EU091598**	EU084431**
Ficus abutilifolia (Miq.) Miq.	Forest 326 (NBG)	NBG 508-77	AY730091*	AY730180*
Ficus alongensis Gagnep	R.C. Ching 1917(P)	China	KJ845963£	KJ845903£
Ficus albert-smithii Standl.	Kjellberg 2006-004	French Guiana	AY730069*	AY730157*
Ficus brachypoda (Miq.) Miq.	Dixon (DNA)	Australia	EF545652**	EF538768**
Ficus bizanae Hutch & Burt-Davy	Kjellberg 1999-27	South Africa	DQ455636§	DQ455670§
Ficus burtt-davyi Hutchinson	Kjellberg 1999-08	South Africa	DQ455647§	DQ455675§
Ficus densifolia Miq.	Baider CB2422 (L)	Mauritius	KJ845984£	KJ845923£
Ficus caulocarpa (Miq.) Miq.	Chantarasuwan 261111-1(L)	Thailand	KJ845954£	KJ845894£
Ficus concinna (Miq.) Miq.	Chantarasuwan 120910-5(L)	Thailand	KJ845991£	KJ845930£
Ficus cyathistipula Warburg	Kjellberg 2007-05-013A	Cameroun	DQ455657§	DQ455679§
Ficus densistipulata De Wildeman	Michaloud 125 27-03-76	Gabon	DQ455659§	DQ455680§
Ficus elasticoides De Wildeman	Kjellberg 2007-05-413	Cameroun	AY730103*	AY730192*
Ficus geniculata Kurz	Chantarasuwan 210910-1 (L)	Thailand	KJ845941£	KJ845883£
Ficus glaberrima Blume	Chantarasuwan 110910-2 (L)	Thailand	KJ845996£	KJ845935£
Ficus glumosa Delile	Kjellberg 1999-09	South Africa	AY063562°	AY063523°
Ficus glumosa Delile	Kjellberg 2007-05-006	Cameroun	GQ504307$	GQ504288$
Ficus henneana Miq.	B. Hyland 8086 (L)	Australia	KJ845968£	KJ845907£
Ficus hookeriana Corner	Hooker&T.Thomson 120 (L)	India	KJ845988£	KJ845927£
Ficus jansii Boutique	Kjellberg 2007-05-034	Cameroun	GQ504310$	GQ504292$
Ficus lecardii Warb.	Harris 2136 (WAG)	Central African Republic	KJ845971£	KJ845910£
Ficus lutea Vahl	Kjellberg 2007-05-029	Cameroun	AY063564°	GQ504289$
Ficus madagascariensis C.C.Berg	P.R. Montagnac 72 (WAG)	Madagascar	KJ845956£	KJ845896£
Ficus platyphylla Delile	Gibernau 1993-02	Cameroun	AY730092*	AY730181*
Ficus petersii Warburg	Kjellberg 1999-06	South Africa	AY730101*	GQ504273$
Ficus pleurocarpa F. Muell.	Cook 9812/CLV441	Australia	AY063568°	AY063529°
Ficus polita Vahl.	Maurin et al. 88 (K)	Madagascar	DQ455642§	DQ455673§
Ficus populifolia Vahl.	Thulin & Warfa 5542 (K)	Somalia	AY730093*	AY730182*
Ficus prasinicarpa Elmer ex C.C.Berg	Nagari 7309 (L)	Papua New Guinea	KJ845948£	KJ845889£
Ficus prolixa G. Forst.	Gillett 2206 (L)	Marquesas	KJ845949£	KJ845890£
Ficus pseudomangifera Hutchinson	Kjellberg 2007-05-034	Cameroun	GQ504309$	GQ504291$
Ficus reflexa Thunberg	No data available	Madagascar	DQ455650§	GQ504278$
Ficus religiosa L.	Chantarasuwan 110910-4 (L)	Thailand	KJ845981£	KJ845920£
Ficus rumphii Blume	Chantarasuwan 120910-4 (L)	Thailand	KJ845993£	KJ845932£
Ficus sansibarica Warburg	Lachaise 1991-05	Malawi	GQ504298$	AY730199*
Ficus stuhlmannii Warburg	Kjellberg 1999-07	South Africa	AY730094*	GQ504274$
Ficus superba (Miq.) Miq.	C. Friedberg 138 (L)	Indonesia	KJ845944£	KJ845886£
Ficus tesselata Warburg	Lachaise 1994-01	Ivory Coast	DQ455662§	DQ455682§
Ficus tettensis Hutch.	Forest 337 (NBG)	NBG 913-96	DQ455665 §	DQ455683§
Ficus trichopoda Baker	Rønsted 118 (K)	Madagascar	DQ455666§	DQ455684§

Appendix 5-1c. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession ITS	GenBank accession ETS
Ficus tsjakela Burm.f	Kostermans 27682 (L)	Sri Lanka	KJ845951£	KJ845892£
Ficus usambarensis Warb.	Wrangham G7111 (K)	Tanzania	DQ455653§	DQ455677§
Ficus verruculosa Warb.	Adjakidje 2779 (WAG)	Benin	KJ845979£	KJ845917£
Ficus virens Aiton	E. Jacobson 2191 (L)	Indonesia	KJ845958£	KJ845898£
Ficus adhatodifolia Schott	Rønsted 148 (K)	BG 2001-0623	EU091563**	EU084404**
Ficus glabrata Kunth.	No data available	No data available	AY063593°	AY063550°
Ficus insipida Willd.	Rønsted 119 (K)	BG 89-523	AY063592°	AY063549°
Ficus lapathifolia (Liebm.) Miq.	Oyama UNAM	Mexico	EU091564**	EU084405**

Appendix 5-1d. Nuclear ITS Sequences downloaded from GenBank, part of previously published studies of genus *Indigofera*. All data belong to Schrire et al. (2009) except accessions marked with * and ** are taken from Vibha et al. (2013) and Zhao and Gao (unpublished) respectively. Outgroup shaded by gray.

Taxon	Voucher (herbarium)	Locality	GenBank accession
Indigofera amorphoides Jaub. & Spach	Gilbert et al. 7373 (K)	Ethiopia	EU729595
Indigofera angulosa Edgw.	Remanandan 4561 (K)	India	EU729682
Indigofera arabica Jaub. & Spach	Gilbert & Thulin 96 (K)	Ethiopia	EU729657
Indigofera argentea Burm. f.	Miller et al. M0137 (E)	Socotra, Ras Hawlaf	AF521785
Indigofera articulata Gouan	Miller et al. 19052 (E)	Socotra, Samha	AF521782
Indigofera aspera Perr. ex DC.	Lock 43970 (K)	Ghana	EU729649
Indigofera asperifolia Bong.	A. Delgado Salinas 2001 (MONT)	Peru	EU729658
Indigofera boranica Thulin	Gilbert et al. 8090 (K)	Ethiopia	EU729583
Indigofera caroliniana Miller	A.B. Pittman 07100210 (MONT)	USA	EU729600
Indigofera cavallii Chiov.	Thulin et al. 6867 (K)	Somalia	EU729596
Indigofera coerulea Roxb. var. *occidentalis* J.B. Gillett & Ali	Miller et al. DA22 (E)	Socotra, Qeyso-Maale	AF521783
Indigofera colutea (Burm. f.) Merr.	Miller et al. 19201A (E)	Socotra, Haggeher Mtns	AF521776
Indigofera conzattii Rose	Hughes 2075 (FHO)	Mexico	AF521585
Indigofera cordifolia Heyne ex Roth	Chauhan & AKPandey 1419	India	JQ945963*
Indigofera cordifolia Heyne ex Roth	Edwards et al. 3700 (K)	Eritrea	AF521741
Indigofera cordifolia Heyne ex Roth	No data available	Sino-Himalaya	KM983162**
Indigofera cytisoides (L.) L.	SChr.ire & Barker 2644 (K)	South Africa	AF521754
Indigofera dalzellii T. Cooke	Vijay Kumar 1278 (K)	India	AF521793
Indigofera denudata L.f.	SChr.ire & Barker 2619 (K)	South Africa	AF521753
Indigofera depressa Harv.	C.H. Stirton 10264 (K)	South Africa	EU729651
Indigofera diphylla Vent.	C. Pase 3132 (K)	Niger	EU729673
Indigofera disticha Eckl. & Zeyh.	SChr.ire & Barker 2615 (K)	South Africa	AF274693
Indigofera diversifolia DC.	Du Puy et al. M66 (K)	Madagascar	EU729675
Indigofera drepanocarpa Taub.	Kahurananga et al. 2776 (K)	Tanzania	EU729632
Indigofera eremophila Thulin	Thulin & Warfa 5999 (K)	Somalia	EU729528
Indigofera ewartiana Domin	G. Chippendale 7088 (K)	Australia	EU729684
Indigofera glomerata E. Mey.	SChr.ire & Barker 2640 (K)	South Africa	EU729626

Appendix 5-1d. Continued.

Taxon	Voucher (herbarium)	Locality	GenBank accession
Indigofera gypsacea Thulin	Thulin et al. 7307 (K)	Somalia	EU729683
Indigofera hirsuta L.	G. Prance 30489 (K)	French Guiana	EU729546
Indigofera hochstetteri Baker	Miller et al. M10182 (E)	Socotra, Hammaderoh	EU729656
Indigofera kelleri Baker f.	Thulin et al. 3781 (K)	Ethiopia	EU729685
Indigofera linifolia (L.f.) Retz	M. Evans 3569 (K)	Australia, N.T., Edith Falls	EU729629
Indigofera linifolia (L.f.) Retz	No data available	Sino-Himalaya	KM983203**
Indigofera linifolia (L.f.) Retz	Chauhan & AKPandey 1441	India, Belgaon	JQ945964*
Indigofera linnaei Ali	Phengklai et al. 4249 (K)	Thailand	EU729670
Indigofera leptosepala Nutt. ex Torrey & A. Gray	Brenan et al. 14327 (K)	Mexico	EU729662
Indigofera lupatana Baker f.	SChr.ire 2561 (K)	Kenya	EU729679
Indigofera marmorata Balf. f.	Miller et al. 19221 (E)	Socotra, Haggeher Mts	AF521779
Indigofera merxmuelleri SChr.eiber	Merxm ü ller & Giess 3445 (K)	Namibia	EU729605
Indigofera nephrocarpa Balf. f.	Miller et al. 14171 (E)	Socotra, Abd al-Kuri	EU729676
Indigofera nephrocarpoides J.B. Gillett	Miller et al. 19202B (E)	Socotra, E Haggeher Mts.	EU729677
Indigofera nudicaulis E.Mey.	Giess & Muller 12253 (K)	Namibia	EU729603
Indigofera obcordata Eckl. & Zeyh.	Acocks 17133 (K)	South Africa	EU729653
Indigofera oblongifolia Forssk.	Miller et al. 19139 (E)	Socotra, Nogad Plain	AF521778
Indigofera phymatodea Thulin	Thulin et al. 7545 (K)	Somalia	EU729515
Indigofera praticola Baker f.	Smith 4437 (K)	Botswana	EU729655
Indigofera rugosa Benth.	Wilson & Rowe 1024 (K)	Australia	AF521773
Indigofera schimperi Jaub. & Spach	SChr.ire 2564 (K)	Kenya	AF274696
Indigofera semitrijuga Forssk.	M. Thulin & Warfa 6014 (K)	Somalia	EU729669
Indigofera sedgewickiana Vatke & Hild.	Gillett & Watson 23455 (K)	Somalia	EU729582
Indigofera sessilifolia DC.	P. Herman 395 (K)	South Africa	EU729654
Indigofera sessiliflora DC.	B.V. Shetty 2327 (K)	Sind	EU729642
Indigofera socotrana Vierh.	Thulin & Gifri 8866 (K)	Socotra, Tinire	EU729559
Indigofera spiniflora Hochst. & Steud. ex Boiss.	Miller et al. DA23 (E)	Socotra, Qeyso-Maale	EU729686
Indigofera tinctoria L.	Miller et al. 19224 (E)	Socotra, Haggeher Mts.,	AF521775
Indigofera torulosa E. Mey.	SChr.ire 2359 (K)	South Africa	AF521774
Indigofera trigonelloides Jaub. & Spach	Liebenberg 4947 (K)	Namibia	EU729643
Indigofera trifoliata L.	J.S. Beard 8326 (K)	Australia	AF521746
Indigofera tristis E. Mey.	SChr.ire 2591 (K)	South Africa	AF521724
Cyamopsis tetragonoloba (L.) Taub.	Reid s.n. (K seed voucher ex G. Reid,),	University of Stirling, UK	AF274687
Indigastrum argyroides (E.Mey.) SChr.ire	Ramdhani & Konje 631 (GRA)	South Africa	EU729488
Microcharis latifolia Benth.	SChr.ire 2571 (K)	Kenya	AF274690
Phylloxylon decipiens Baill.	Serv. Forestier 126 R-6 (K)	Madagascar	EU729485
Rhynchotropis marginata (N.E.Br.) J.B. Gillett	Fanshawe F2458 (K)	Zambia	EU729500

Appendix 5-2. Voucher information of the samples sequenced for this study. Asterisks denoted individuals was included in the analysis to represent the taxon from the locality.Ext. code= DNA extraction code.

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region		
Genus *Convolvulus*						**ITS**	***matK***	***rbcL***
Convolvulus arvensis L.	Dillam, Saudi Arabia	J. Thomas14435	2013	KSU	CM12h	×	×	×
	Eritrea	Zekarias A 35	2000	EIT	CE35h	×	×	×
Convolvulus glomeratus Hochst. ex Choisy	Jazan, Saudi Arabia	R. Basahi 21727	2016	KSU	CM13h	×	×	×
	Sajid Island	S. Alharbi 150	2016	RNG	CS150	MN879546	MN885602	MN885607
	Sajid Island	S. A. Alharbi & R. Al Qthanin 74	2016	RNG	CS74	×	MN885601	MN885608
	Farasan Alkabir Island	S. Alharbi 137	2016	RNG	CF137	MN879548	MN885599	MN885605
	Farasan Alkabir Island	S. Alharbi 185	2016	RNG	CF185*	MN879547	MN885598	MN885603
Convolvulus pilosellifolius Desr.	Saudi Arabia	M.El-Sheikh, M. Shehri 23437	2016	KSU	CM14h	×	×	×
Convolvulus rhyniospermus Choisy	Sajid Island	Alharbi 180	2017	RNG	CS180	MN879544	MN885597	MN885604
Convolvulus sp.	Jazan, Saudi Arabia	S. Alharbi & R. Al Qthanin 7	2016	RNG	CM7	MN879545	MN885600	MN885606
Genus Euphorbia						**ITS**	***ndhF***	***matK***
Euphorbia arabica Hochst. & Steud. ex T.Anderson	Oman	R.Fitzgerald 47	1996	RNG	EO0h	MN886514	MN911447	
Euphorbia canariensis L.	Canary Islands	V.F.Molford 128	1970	RNG	EC12h	×	×	×
Euphorbia colenetteae D.Al-Zahrani & El-Karemy	Farasan Alkabir Island	S. Alharbi 134	2016	RNG	Ef134	MN886502	MN954657	MN901112
Euphorbia granulata Forssk. var. *granulata*	Sajid Island	S. Alharbi & R. Al Qthanin 29	2016	RNG	E29	MN886513	MN911450	
	Baysh, Saudi Arabia	J. Thomas 22890	2012	KSU	Em19h	×	×	
	Oman	C. Parker 0.35	1973	RNG	EH4	MN886508	MN911449	
	Eritrea	S Edwards et al 3800	1985	EIT	EE38h	×	×	
var. *glabrata*	Sudan	C. Parker 1360	Unknown	RNG	ES13h	×	×	
Euphorbia handiensis Burchard	Canary Islands	A.Aldridge 824	1973	RNG	EC82h	×	×	×
Euphorbia hispida Boiss.	Pakistan	S.Husain, R.Lowe, M.Muqarra6 & L.Springate HLMS94.0395	1994	RNG	EP94h	MN886510	MN911444	
Euphorbia humifusa Willd.	Belgium	E.Robbrecht 2963	1986	RNG	EBq29h	MN886512	MN911448	
	UAE	A.M.Lorimer 5	1980	RNG	EU5h	×	MN911443	

Appendix 5-2. Continued.

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	ITS	ndhF	matK
Genus *Euphorbia*						ITS	*ndhF*	*matK*
Euphorbia inaequilatera Sond.	Saudi Arabia	Collenette 9494	1997	E	EM94h	MN886511	MN911445	
Euphorbia indica Lam.	Bahrain	C. Parker E.101	1974	RNG	EB12h	MN886507	MN954658	
Euphorbia maculata L.	Spain	L.Guanson SP-07-14	2008	RNG	ES07h	MN886515	MN954663	
Euphorbia officinarum L.	Morocco	D.Podlech 55247	2000	RNG	Emo55h	×	×	
Euphorbia peplis L.	Morocco	K.Ferguson, R.Parra,M.C. Sanchez & B.Valdes 6640/95	1995	RNG	Emo66h	MN886505	MN954661	
	Lebanon	R.Rutherford 26	1999	RNG	EL26h	MN886504	MN954660	
Euphorbia prostrata Ait.	Spain	L.Guanson SP-91-205	1979	RNG	ES91h	MN886506	MN954662	
Euphorbia resinifera Berg	Morocco	S. Jury, M.Rejdali & .Watson 8732	1987	RNG	Emo87h	×	×	×
Euphorbia riebeckii Pax	Oman	Gallagher, Michael D.6740/1	1983	E	EO67h	MN886509	MN911446	
Euphorbia serpens Kunth	Canary Islands	R.K.Brummitt 18522	1989	RNG	EC18h	MN886503	MN954659	
Genus *Ficus*						ITS	ETS	
Ficus amplissima Sm.	Dhahran, Saudi Arabia	Mandaville, James P. Jr.8868	1991	E	FM88h	MN883368	MN885578	
Ficus cordata ssp. Thunb. *salicifolia* (Vahl) C.C. Berg (Syn. *Ficus salicifolia* Vahl)	Dandah, Saudi Arabia	John D. Dwyer 13567	1976	RNG	FH6	MN883366	MN885575	
	Yemen	Guarino, L. & Balaidi, L.H72	1989	E	FY72h	×	MN885587	
	Abha, Saudi Arabia	Miyazaki 990731R III 21	1999	E	FM21h	×	MN885586	
	Farasan Alkabir Island	S. Alharbi & R. Al Qthanin 112	2016	RNG	Ff112	MN883367	MN885576	
Ficus glumosa Del.	Farasan Alkabir Island	S. Alharbi 164	2016	RNG	Ff164	MN883372	MN885582	
	Taif, Saudi Arabia	Collenette 1772	1980	E	FM72h	MN883369	MN885579	
	Makkah, Saudi Arabia	Lavranos, J.J. & Collenette, S.18527	1980	E	FM18h*	MN883370	MN885580	
	Yemen	Miller, Anthony G. 267	1978	E	FY26h	MN883371	MN885581	
Ficus ingens (Miq.) Miq.	Al-Baha, Saudi Arabia	Fayed1425	1988	E	FM14h	MN883365	MN885577	

Appendix 5-2. Continued.

Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region	
Genus *Ficus*						ITS	ETS
Ficus populifolia Vahl	Farasan Alkabir Island, Farasan group	S. Alharbi & R. Al Qthanin 61	2016	RNG	Ff61*	MN883377	MN885584
		S. Alharbi 184	2016	RNG	Ff184	MN883374	×
	Yemen	Boulos, L.; Rowaished, A.K.; Gifri, A.N.; Saeed, W.A. & Hussein, M.A.16761	1987	E	FY16h	MN883375	MN885583
		Miller, Anthony G. & Long, David G.3484	1981	E	FY34h	MN883373	MN885589
		Miller, Anthony G. & King, Rosemary A. 5170	1984	E	FY51h*	MN883376	MN885585
		Miller, Anthony G. & Long, avid G. 3330	1981	E	FY33h	×	MN885588
Ficus vasta Forssk.	Abha to Jazan, Saudi Arabia	John D. Dwyer 13594	1976	RNG	FH5	×	×
Genus *Indigofera*						ITS	
Indigofera amorphoides Jaub. & Spach	Asir, Saudi Arabia	Miyazaki 991119R2	1999	E	IM99h	MN879521	
Indigofera arabica Jaub. & Spach	UAE	R. Fitzgerald 60 and S. Hoed, Maijcke 60	1996	RNG	IU60h	MN879527	
Indigofera articulata Gouan	Najran, Saudi Arabia	John D. Dwyer 13707	1979	RNG	IH14	MN879520	
	Saudi Arabia	John D. Dwyer 13606	1976	RNG	IH15	MN879519	
Indigofera arrecta Hochst. ex A.Rich.	Jabal Fayfa, Saudi Arabia	Collenette 6797	1988	E	IM67h	MN879514	
Indigofera coerulea Roxb.	Farasan Islands	Sivadasan & J. Thomas 22547	2009	KSU	IF20h	×	
Indigofera coerulea Roxb. var. *coerulea*	Farasan Alkabir Island	S. Alharbi & R. R. Al Qthanin 51	2016	RNG	IF51	MN879517	
Indigofera coerulea Roxb. **var. *coerulea***	Sajid Island	S. Alharbi 143	2016	RNG	IS143	MN879518	
Indigofera coerulea Roxb. var. *occidentalis* Gillet & Ali	Oman	S.G. Knees & L. Mackinno 15	2013	E	IO15h	MN879516	
Indigofera hochstetteri Bak.	Farasan Islands	Unknown	Unknown	RIY	IF0h	MN879529	
	Eritrea	Ghebrehiwet M 2564	2006	EIT	IE25h	MN879528	
Indigofera intricata Boiss.	UAE	R. Fitzgerald 88	1997	RNG	IU88h	MN879525	
	Qatar*	Knees, Sabina Georgina 9011	2013	E	IQ90h	MN879524	
Indigofera linifolia (L.f.) Retz.	Farasan Islands	I.S. Collenette 5004	Unknown	E	IF50h	×	
	Sajid Island	S. Alharbi 175	2017	RNG	IS175	MN879512	
	Eritrea	S Edwards &Tewolde 3712	1985	EIT	IE37h	MN879511	
	Jeddah, Saudi Arabia	Collenette. 4823	1984	E	IM48h	MN879513	

Appendix 5-2. Continued.

| Species	Locality of collection	Collector name & No	Year of collection	Herb. code	Ext. code	Sequenced region
Genus *Indigofera*						ITS
Indigofera oblongifolia Forssk.	Tandah, Saudi Arabia	John D. Dwyer 13569	1976	RNG	IH16	×
	Farasan Alkabir Island	S. Alharbi 136	2016	RNG	IF136	MN879531
	Eritrea	O Ryding 1924	1989	EIT	IE19h	MN879530
Indigofera semitrijuga Forssk.	Farasan Alkabir Island	S. Alharbi 123	2016	RNG	IF123	MN879523
Indigofera sessiliflora DC.	Makkah, Saudi Arabia	Collenette 3937	1982	E	IM39h	×
Indigofera spicata Forssk.	Yemen	Miller, Anthony G. & King, Rosemary A. 5262	1984	E	IY52h	MN879526
Indigofera spiniflora Boiss.	Farasan Alkabir Island, Farasan group	S. Alharbi 153*	2016	RNG	IF153	MN879534
		S. Alharbi 170	2016	RNG	IF170	MN879533
	Between Jeddah & Wadi Khuglais, Saudi Arabia	Collenette 3365	1982	E	IM33h	MN879535
Indigofera spinosa Forssk.	Jazan, Saudi Arabia	R. Basahi 21744	2012	KSU	IM22h	MN879510
	Eritrea	Ghebrehiwet M 177	1995	EIT	IE17h	MN879509
Indigofera tinctoria L.	Yemen	Miller, A.G., Guarino, L., Obadi, N., Hassan, M., & Mohammed, N. M.8224	1989	E	IY82h	MN879515
Indigofera trita L.f.	Oman	McLeish 2539	1993	E	IO39h	MN879532
Indigofera volkensii Taub.	Oman	McLeish 2785	1993	E	IO85h	MN879522
Indigofera argentea Burm. f.	Jazan, Saudi Arabia	S. Alharbi 10	2016	RNG	IM10	MN879508
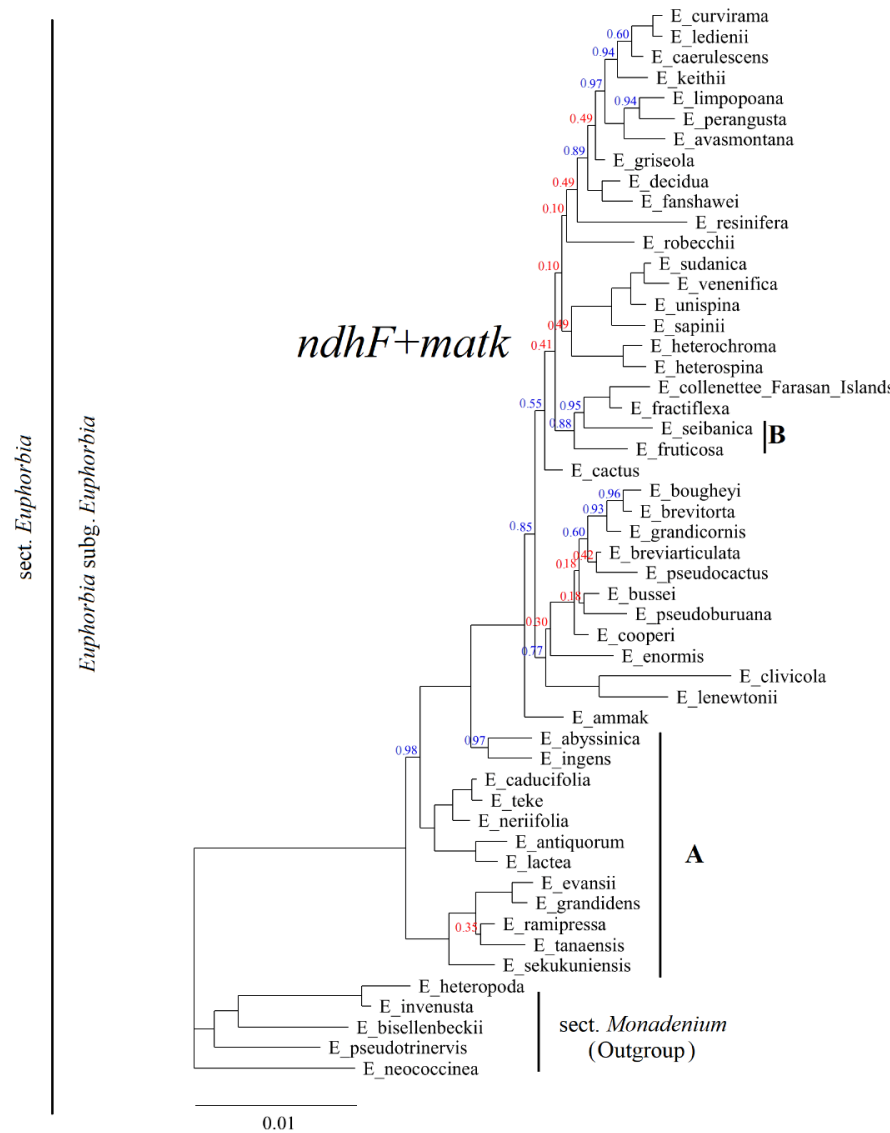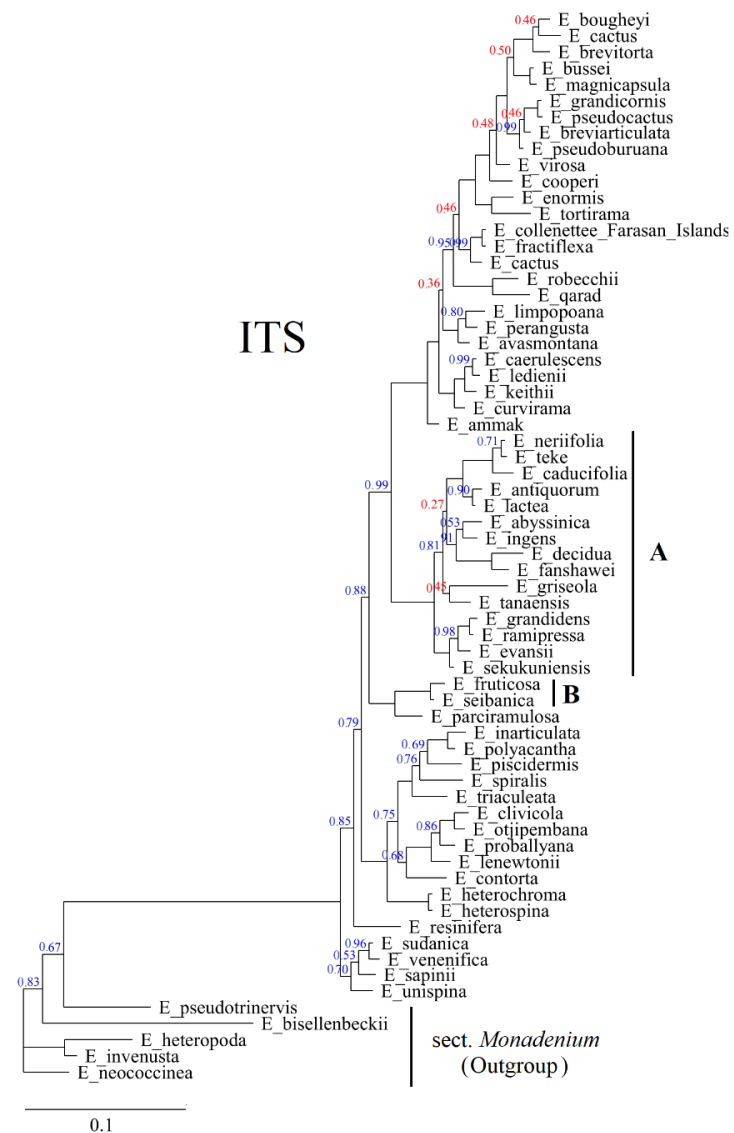
Appendix 5-3. Combinable component consensus trees of _E._ sect. _Euphorbia_ of the nuclear ITS and the combined chloroplast _ndhF_ and _matK_ . Posterior probabilities less than 1.00 are shown above nodes: blue >0. 50 support, red ≤0.50. Scale bar shows the number of substitutions per site. Letters A and B indicate clades that are differently placed between ITS and chloroplast trees.
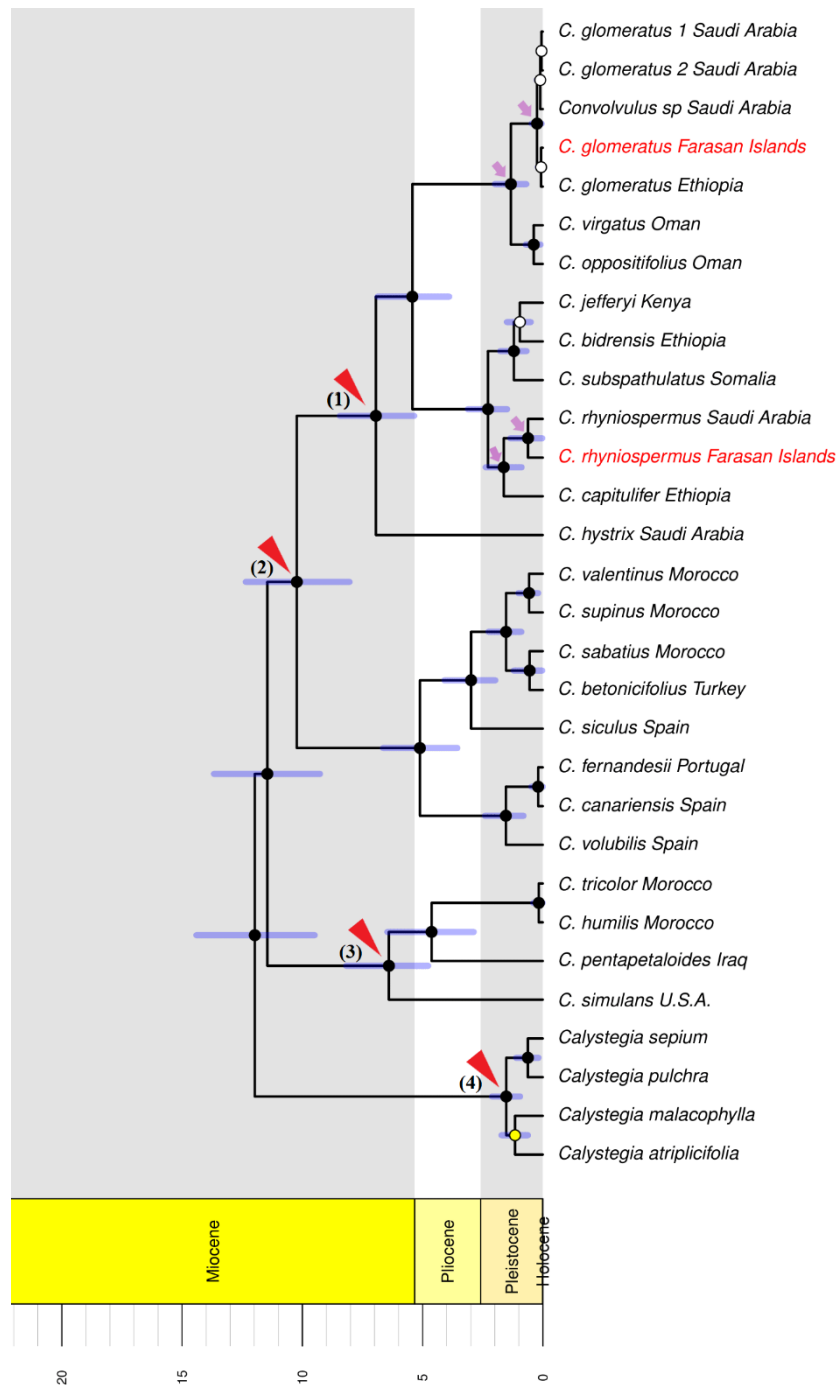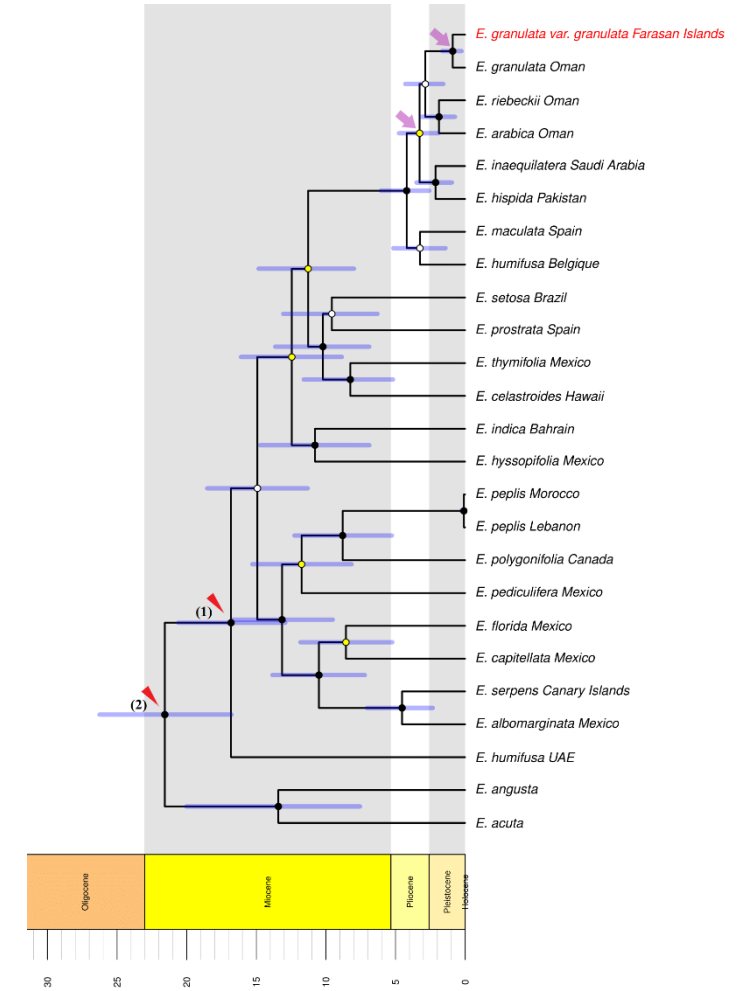
Appendix 5-4a. Maximum clade credibility Chronogram of *Convolvulus* of combined nuclear ITS and chloroplast *matK* and *rbcL* data sets. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ●≥0.95, 0.95>●≥0.75, 0.75>○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 5-4; pink arrows indicate node ages summarised in Figure 5-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *C. = Convolvulus.*
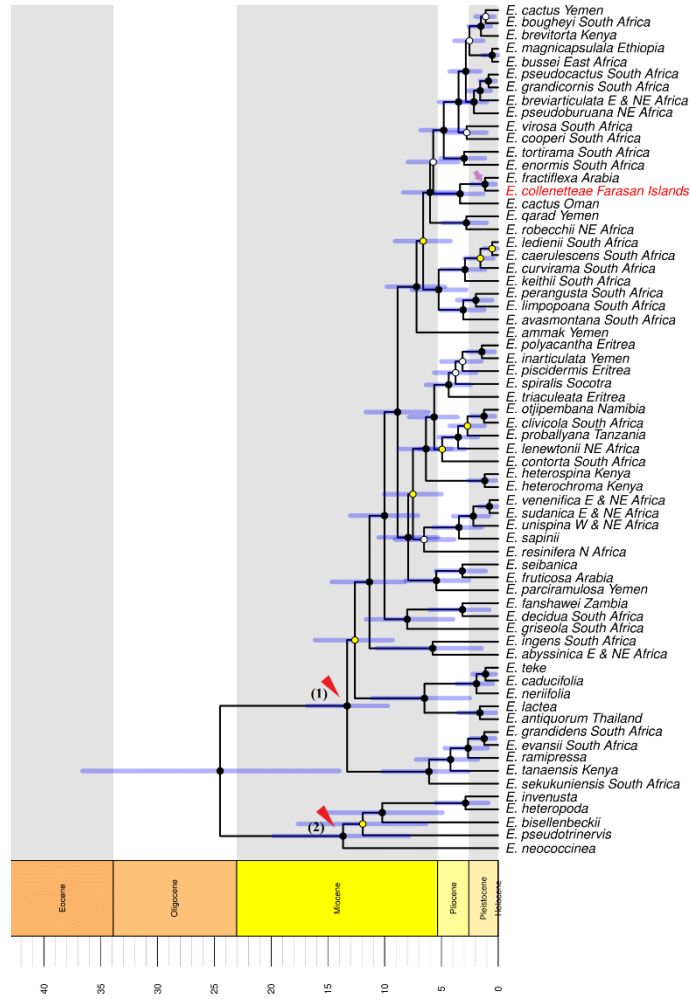
Appendix 5-4b. Maximum clade credibility chronogram of *Euphorbia*: (A) sect. *Euphorbia* based on combined nuclear ITS and chloroplast *ndhF* and *matK* data sets, (B) sect. *Anisophyllum* based on combined nuclear ITS and chloroplast *ndhF*. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability:●≥0.95, 0.95>●≥0.75, 0.75>○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 5-4; pink arrows indicate node ages summarised in Figure 5-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *E.= Euphorbia.*
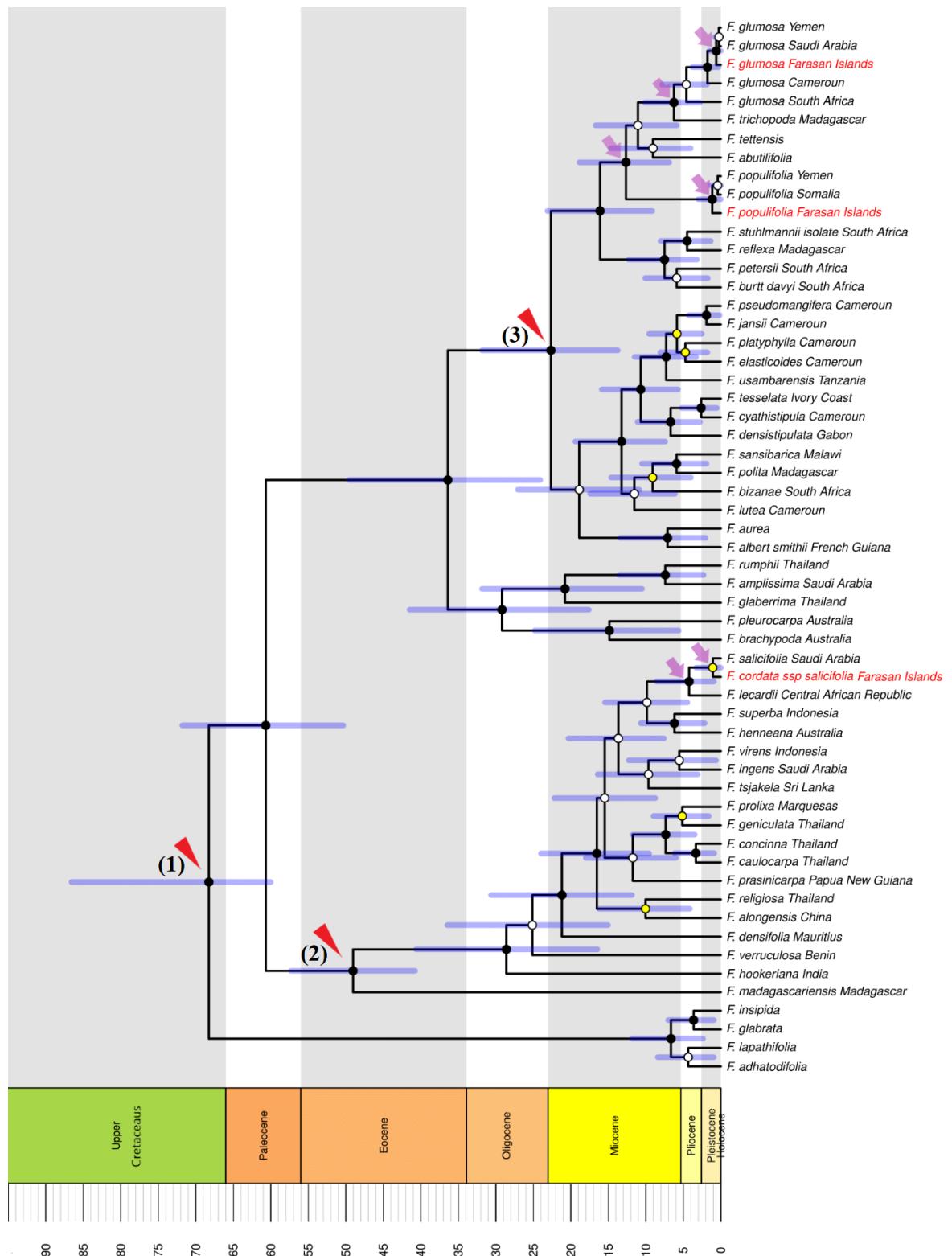
Appendix 5-4c. Maximum clade credibility Chronogram of *Ficus* of nuclear ITS and ETS. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 5-4; pink arrows indicate node ages summarised in Figure 5-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *F. = Ficus.*

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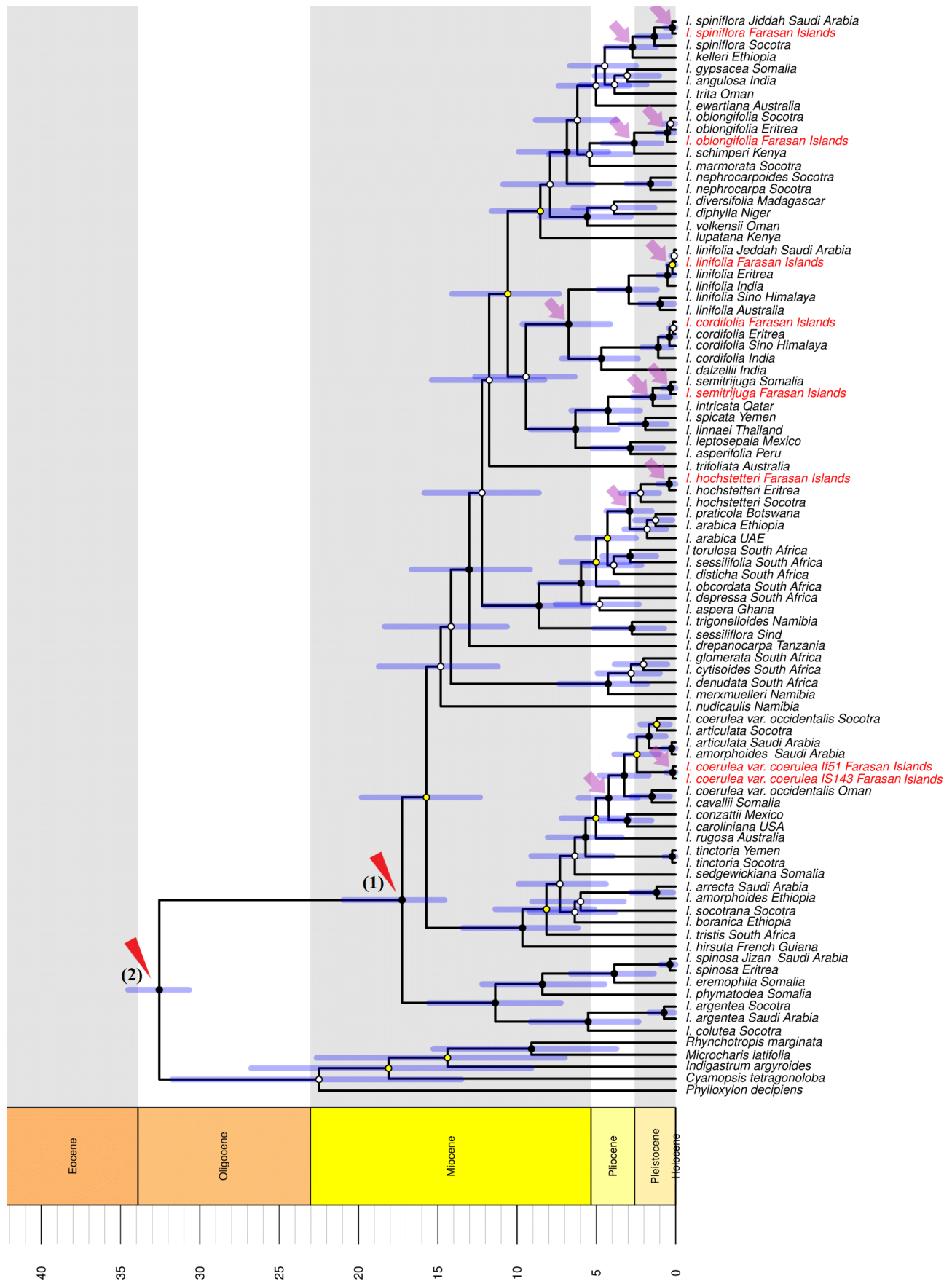
Appendix 5-4d. Maximum clade credibility Chronogram of *Indigofera* of nuclear ITS. Node heights indicate mean ages with blue bars indicating the 95% highest posterior density, the internal nodes of the tree are indicated with circles, which are marked with posterior probability: ● ≥0.95, 0.95> ● ≥0.75, 0.75> ○. Calibrated nodes are marked with red arrows and numbers, which reflect the constrained nods in Table 5-4; pink arrows indicate node ages summarised in Figure 5-8, species stem nodes and the crown nodes of the clades include the Farasan Islands species. *I = Indigofera.*

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CHAPTER 6: Conservation assessment and Red Listing of the Farasan Islands' endemic and non-endemic restricted species

6.1 Introduction

The significance of the Farasan Islands flora to Saudi Arabia lies in the presence of a number of regionally and nationally rare species known thus far in Saudi Arabia on these islands (hereafter Farasan restricted species) (Hall et al. 2010; Thomas et al. 2010). Two Farasan restricted species *Commiphora* aff. *kataf* (Al-Zahrani, 2010) and *Glossonema* sp. aff. *boveanum* (Collenette, 1999), have distinct morphologies from widespread *Commiphora kataf* and *Glossonema boveanum* respectively, which are likely to been endemic elements to the islands (Collenette, 1999; Al-Zahrani, 2010).

Over the past two decades, the Farasan Islands have been recorded as the only Saudi locality of 16 species by several authors (Collenette, 1999; Alfarhan et al., 2002; Hall et al., 2010; Thomas et al., 2010). The revision of available floristic publications of Saudi Arabia (Mandaville, 1990; Chaudhary, 1999, 2001a, 2001b, 2001c, 2001d; Al-Zahrani & El Karemy, 2002; Millennium Ecosystem Assessment, 2010; Daur, 2012; Al-Eisawi & Al-Ruzayza, 2015; Kasem & Marei, 2017) showed that eight of these 16 species have been found in other Saudi localities (Table 6-1, highlighted species). However, this does not detract from their value in the Farasan Islands or internationally because some of them are rare and endangered in Saudi Arabia, such as *Ficus populifolia* and *Nothosaerva brachiata* (Millennium Ecosystem Assessment, 2010). More recently, Basahi & Masrahi (2019) recorded *Blepharis saudensis* as a near endemic taxon to the Farasan Islands. Therefore, thus far, a group of nine taxa in Saudi Arabia can be considered to have restricted range to the Farasan Archipelago, three of which are endemic or near-endemic to the Islands (*B. saudensis, C.* aff. *kataf* and *G.* sp. aff. *boveanum*) (Table 6-1). Furthermore, the Farasan Islands are not the only known Saudi locality of these nine taxa but also the only known Arabian locality of them all except *Micrococca mercurialis* and *Rorida brachystyla*, which occur in Yemen (Mandaville & Bovey, 1978; Batanouny, 1981; El-Ghonemy, 1985; Boulos, 1988; Boulos, 1994; Miller & Cope, 1996; Alkhulaidi & Kessler, 2001; Ghazanfar, 2003; Cope, 2007; Norton et al., 2009; Hall et al., 2010; Hehmeyer & Schönig, 2012; Al-Khulaidi, 2013; Daoud, 2013; Abdullah, 2017). Thus, the Farasan Islands flora are of great value of conservation regionally and nationally.

Most of the Farasan's restricted species are rare and found in only a few small populations (Fisher et al., 1998; Thomas et al., 2010). Although the Farasan Islands have been designated as an Important Plant Area in the Arabian Peninsula since 2010 because of the presence of these plant groups (Hall et al., 2010), none of them are represented in the current conservation framework. An awareness of the need to conserve and manage threatened flora is slowly growing, along with increasing anthropogenic and ecological stress. Conservation efforts in the

Farasan Protected Area have mainly focused on the protection of Idmi gazelle (Abuzinada, 2003) and the mangroves (Gladstone et al., 2003).

The conservation status of Farasan restricted species was assessed by Collenette (1999) and Thomas et al. (2010) (Table 6-2); however, the assessment criteria used are not clear. There is currently an increasing need for up-to-date information and mapping of the distribution of these species. The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species™ can play a pivotal role in meeting this need, which has become a major tool in conservation biology (De Grammont & Cuarón, 2006). The list of threatened species has been used to influence and to inform conservation legislation and policies that identify priority areas for biodiversity conservation, increase public awareness of human impacts on biodiversity and regulate development and exploitation (Possingham et al., 2002; Miller et al., 2007; Hoffmann et al., 2008).

None of the Farasan restricted species are the subject of a Red Listing globally or regionally, and Saudi Arabia has not published a formal national Red List of plant species. However, the IUCN criteria have been applied to assess the conservation status of *Euphorbia collenetteae* and *Blepharis saudensis* by several others. *Euphorbia collenetteae* was assessed as Near Threatened (Al-Zahrani & El-Karemy, 2007) and Least Concern (Pahlevani, 2017); *Blepharis saudensis* was assessed as Endangered (Basahi & Masrahi, 2019). Nevertheless, no detailed information is provided in these literatures regarding which criteria were used or mapping species range in the islands.

The aim of this chapter is to assess the conservation status and produce red list data for the Farasan Island flora, with particular focus on rare species. The study also aims to provide scientifically based information on the trends, status, and threats to the species and populations to increase the awareness of the threatened flora of the Farasan Islands and biodiversity conservation. It is intended that this conservation assessment will promote conservation awareness.

Table 6-1. Review of the Farasan Islands restricted species and available assessment of extinction risk recorded in previous studies. Grey shaded cells=species found in other localities of Saudi Arabia. Species marked with asterisks are occurring in Yemen. NR=not recorded, NA=not assessed, EN=endangered, VU=vulnerable, Loc. EN=locally endangered.

No.	Species name	Family	Authors recorded the species as restricted to the Farasan Islands				Conservation status					Threats	Authors recorded species in another locality in Saudi Arabia
			Collenette (1999)	Alfarhan et al. (2002)	Thomas et al. (2010)	Hall et al. (2010)	Collenette (1999)	Al-Zahrani & El-Karemy, (2007)	Thomas et al. (2010)	Pahlevani, (2017)	Basahi & Masrahi (2019)	Thomas et al. (2010)	
1	*Basilicum polystachyon* (L.) Moench	Lamiaceae	√	√	√	√	EN	NA	EN	NA	NA	Drought, off road traffic	
2	*Blepharis saudensis* Y. Masrahi, & M. Basahi	Acanthaceae	NR	NR	NR	NR	NA	NA	NA	NA	EN	NA	
3	*Commiphora* aff. *kataf* (Forssk). Engl (previously Identified as: *Commiphora erythraea*)	Burseraceae	√	√	√	√	EN	NA	EN	NA	NA	Poor regeneration	
4	*Dinebra retroflexa* (Vahl) Panzer	Poaceae	√	√	√	NR	EN	NA	Loc. EN	NA	NA	Drought	Daur (2012)
5	*Dinebra somalensis* (Stapf) P.M.Peterson & N.Snow (Syn. *Drake-Brockmania somalensis*)	Poaceae	√	√	√	√	EN	NA	Loc. EN	NA	NA	Off road traffic	
6	*Euphorbia collenetteae* D.Al-Zahrani & El-Karemy (syn. *Euphorbia* sp. aff. *fractiflexa*)	Euphorbiaceae	√	NR	√	√	Not EN	NT	Loc. EN	LC	NA	Habitat loss	
7	*Ficus populifolia* Vahl	Moraceae	√	√	√	√	EN	NA	VU	NA	NA	Poor regeneration	Millennium Ecosystem Assessment, 2010
8	*Flueggea leucopyrus* Willd.	Phyllanthaceae	NR	NR	NR	√	NA	NA	NA	NA	NA	Not assessed	Chaudhary (2001b)
9	*Glossonema* sp. aff. *boveanum* (Decne.) Decne	Apocynaceae	√	√	√	NR	EN	NA	EN	NA	NA	Habitat loss	
10	*Indigofera semitrijuga* Forssk.	Fabaceae	√	√	√	NR	EN	NA	EN	NA	NA	Habitat loss	Chaudhary (2001b)
11	*Ipomoea hochstetteri* House	Convolvulaceae	√	√	√	√	EN	NA	EN	NA	NA	Drought and habitat loss	Kasem & Marei (2017)
12	*Limonium cylindrifolium* (Forssk.) Verdc	Plumbaginaceae	√	√	√	NR	NA	NA	VU	NA	NA	Habitat loss	Al-Zahrani & El Karemy (2002)
13	*Micrococca mercurialis* (L.) Benth.*	Euphorbiaceae	√	√	√	NR	EN	NA	EN	NA	NA	Off road traffic	
14	*Nothosaerva brachiata* (L.) Wight	Amaranthaceae	√	√	√	√	EN	NA	VU	NA	NA	Drought	Millennium Ecosystem Assessment, 2010
15	*Rorida brachystyla* (Deflers ex Franch.) Thulin & Roalson (syn. *Cleome noeana* ssp. *brachystyla*)*	Cleomaceae	√	√	√	√	EN	NA	EN	NA	NA	Habitat loss	
16	*Taverniera cuneifolia* (Roth) Arn.	Fabaceae	√	√	√	NR	EN	NA	VU	NA	NA	Drought	Chaudhary (2001b)
17	*Vahlia digyna* (Retz.) O. Kuntze	Vahliaceae	√	√	√	NR	EN	NA	VU	NA	NA	Drought, off road traffic	

6.2 Material and Methods

The Farasan Islands endemic and non-endemic restricted species (Table 6-2) have been selected for Red Listing and conservation assessment. *Commiphora* aff. *kataf* and *Glossonema* sp. aff. *boveanum* were evaluated at the global level. *Blepharis saudensis* was not re-assessed because the current assessment is recent (Basahi & Masrahi, 2019). The remaining species in this study were assessed at the Arabian regional level except for *Micrococca mercurialis* and *Rorida brachystyla*, which were evaluated at the Saudi national level. This is because there is no reliable and adequate data regarding population, habitat and possible threat for these species in Yemen.

Table 6 -2. Farasan Islands species under investigation.

No.	Species name
Endemic	
1	*Commiphora* aff. *kataf*
2	*Glossonema* sp. aff. *boveanum*
Species not known from any part of Arabian Peninsula	
3	*Basilicum polystachyon* (L.) Moench
4	*Dinebra somalensis* (Stapf) P.M.Peterson & N.Snow
5	*Euphorbia colenetteae* D.Al-Zahrani & El-Karemy
6	*Vahlia digyna* (Retz.) Kuntze
Species not known from any part of Saudi Arabia	
7	*Micrococca mercurialis* (L.) Benth.
8	*Rorida brachystyla* (Deflers ex Franch.) Thulin & Roalson

Current threats to the species were recorded based on the field and point distribution data of targeted species that were gathered from three different sources: field observations (outlined in Chapter 2), available scientific literature and data from specimen labels from the Royal Botanic Gardens Edinburgh (**E**) herbarium catalogue (https://data.rbge.org.uk/search/ herbarium/).

These distribution data were then input into the GeoCAT software (Bachman et al., 2011), which in turn calculates two main spatial metrics: the Extent of Occurrence (EOO) and Area of Occupancy (AOO). The EOO was measured by constructing a minimum convex polygon around all the sites of occurrences. The AOO was calculated by overlaying a 2x2 km grid and counting the number of occupied cells (IUCN Standards and Petitions Subcommittee, 2017).

The guidelines for using the IUCN Red List categories and criteria version 13 (IUCN Standards and Petitions Subcommittee, 2017) and the Guidelines for the Application of the IUCN Red List Criteria at the regional and national levels version 4.0 (IUCN, 2012) were followed without deviation or modification to assess the conservation status of the Farasan Islands' targeted species.

To complete the Red List assessment for each species, a wide range of information, including scientific and common names, taxonomic information, distribution, habitats, ecology, threats, stresses, conservation actions, use, mapping and the current status of the taxon outside the region, is required. These data were obtained from various sources, such as field observations, interviews with locals and relevant scientific literature.

Maps of the distribution range of each species were created using ArcGIS Online (Esri, 'Topography').

6.3 Results

The risk of extinction of the Farasan Islands restricted species was evaluated globally and nationally in accordance with the IUCN Red List criteria and categories version 13 (IUCN Standards and Petitions Subcommittee, 2017) and IUCN Red List Criteria at the regional and national levels version 4.0 (IUCN, 2012).

The categories of the IUCN are based on five criteria (A, B, C, D and E) that evaluate the extinction risk of a species based on different ecological and biological factors, such as A) declining population (past, present and/or projected); B) geographic range size and fragmentation, decline or fluctuations; C) small population size and fragmentation, decline or fluctuations; D) very small population or very restricted distribution; and E) quantitative analysis of extinction risk (e.g. Population Viability Analysis) (IUCN Standards and Petitions Subcommittee, 2017). In this study, criterion B was the only used for the species assessment

due to data availability based on the number of locations of the species and distribution range points collected from field visits and existing literature. Criteria A and E were not used for any of the assessed species as the first two requirements—the population reduction rate in the past, present or future and the generation length—could not be met due to the lack of population trend rates and quantitative data. Criterion C was not used due to the lack of an accurate estimation of mature individuals, the rate of decline or the percentage of mature individuals in each subpopulation. Criterion D also was not used due to the lack of data about an absolute plausible future threat that could drive the restricted taxa to become very highly threatened within a very short time.

Each island was considered a single location according to the IUCN location definition (IUCN, 2001; IUCN Standards and Petitions Subcommittee, 2017) because the most plausible threat among the Farasan group is urbanisation.

If the EOO was less than the AOO, the EOO was changed to make it equal to the AOO to ensure consistency with the definition of the AOO as an area within the EOO following the IUCN guideline recommendation (IUCN Standards and Petitions Subcommittee, 2017).

Species conservation assessments

6.3.1 *Basilicum polystachyon* (L.) Moench

[EN° –Endangered, B1ab (v)+2ab (v)]

Family: Lamiaceae

Synonyms: *Basilicum polystachyon* var. *stereocladum* Briq.; *Lehmannia ocymoidea* Jacq. ex Steud.; *Lumnitzera moschata* (R.Br.) Spreng.; *Lumnitzera polystachyon* (L.) J.Jacq. ex Spreng.; *Moschosma dimidiatum* (Schumach. & Thonn.) Benth.; *Moschosma moschatum* (R.Br.) Druce; *Moschosma polystachyon* (L.) Benth.; *Ocimum dimidiatum* Schumach. & Thonn.; *Ocimum moschatum* Salisb., nom. superfl.; *Ocimum polystachyon* L.; *Ocimum tashiroi* Hayata; *Perxo polystachyon* (L.) Raf.; *Plectranthus micranthus* Spreng.; *Plectranthus moschatus* R.Br.; *Plectranthus parviflorus* R.Br., nom. illeg. (Roskov et al., 2019).

Basilicum polystachyon is an annual herb (Alfarhan et al., 2005) that is very rare in the Farasan Islands with a restricted distribution to the Al Muharraq area in Farasan Al-Kabir among the *Vachellia flava* woodlands at 9-12 m **(**Figure 6-1), which is the only known Arabian locality for this plant (Hall et al., 2010). The is widely distributed in tropical to subtropical climates in India, China, Indonesia, Java, Myanmar, Philippines, Sri Lanka, Taiwan, Tropical Hainan, Africa and Australia (Singh et al., 2018).

Basilicum polystachyon is under threat from numerous sources, especially drought, off-road traffic (Thomas et al., 2010) and invasive *Prosopis juliflora.* The population size is small, with the number of individual plants estimated to be between 100 - 500 (Thomas et al., 2010), and the estimated EOO and AOO is 8 km2. The current survey of this species, undertaken during the course of this study, did not identify any populations, suggesting a possible decline in the population size. *Basilicum polystachyon* was initially assessed as CR; however, the probability of the species re-colonizing outside the region is likely due to the proximity of the Farasan Islands to Africa. Therefore, the preliminary regional category was downlisted to EN.
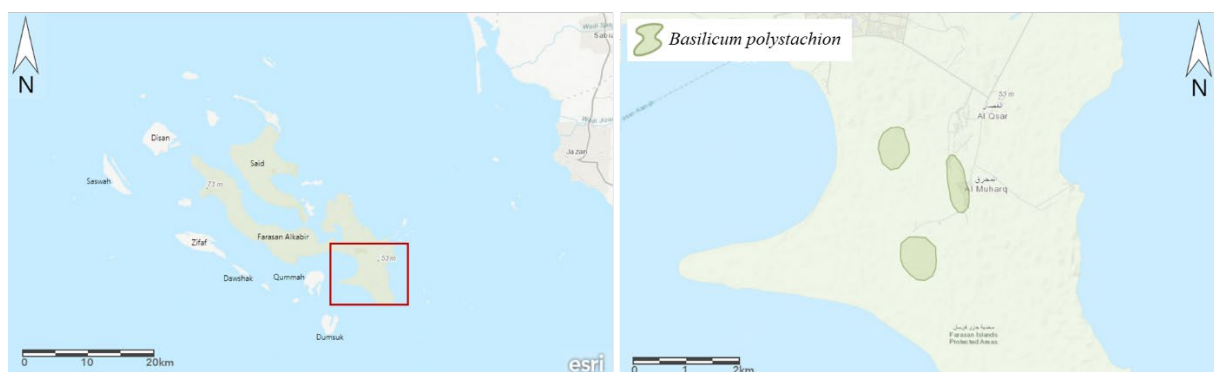
Figure 6-1. *Basilicum polystachyon* distribution.

6.3.2 *Commiphora* aff. *kataf*

EN, Endangered B1a (i, ii) +B2a (i, ii)

Family: Burseraceae

Previously identified as: Commiphora erythraea (Ehrenb.) Engl., Collenette, Fl. Saudi Arabia, 88 (1985); *Commiphora erythraea* (Ehrenb.) Engl., Collenette, Fl. Saudi Arabia, 97 (1999); *Commiphora erythraea* (Ehrenb.) Engl., Chaudhary, Fl. Saudi Arabia, 235 (1999); *Commiphora erythraea* (Ehrenb.) Engl., Chaudhary, Fl. Saudi Arabia, V2: P1, (2000) (Al-Zahrani, 2010).

Commiphora aff. *kataf* is restricted to the small uninhabited islands Dumsuk and Dawshak of the Farasan Islands (Figure 6-2) (Al-Zahrani, 2010). The plants grow in areas of relatively well-developed soils within the raised coral platform at 4.5-13.7 m and often form a sparse open scrub with an estimated population of 200 individuals across Dumsuk Island (Hall et al., 2010). The phylogenetic and morphological study of Saudi *Commiphoras* showed that *C.* aff. *kataf* is very closely related to *Commiphora kataf*, the largest *Commiphora* species in Saudi Arabia (Al-Zahrani, 2010); however, *C.* aff. *kataf* can be found in two forms: the common form is a small, spreading bushy tree up to 4.5 m tall (Figure 6-3, A), and the second form is the prostrate habit up to 1 m tall and 4 m wide (Figure 6-3, B). For *C.* aff. *Kataf* both the leaves and petiole are always hairy, covered with velutinous hairs; pseudoaril is red, large, cup-like with two broad triangular lobes. While leaves and petiole of *C. kataf* are glabrous or covered with pilose hairs, pseudoaril reddish orange and basal cup-shape with two broad triangular arms (Al-Zahrani, 2010).

Commiphora aff. *kataf* is under the threat of poor regeneration (Thomas et al., 2010) and drought. The taxon is restricted to Dumsuk and Dawshak Islands of the Farasan Archipelago with a very small population size of less than 500 individuals. The estimated EOO is 50.653 km^2, and the estimated AOO is 36 km^2. The population size is likely to decrease, given the current threats. Thus, *Commiphora* aff. *kataf* has been assessed as EN.
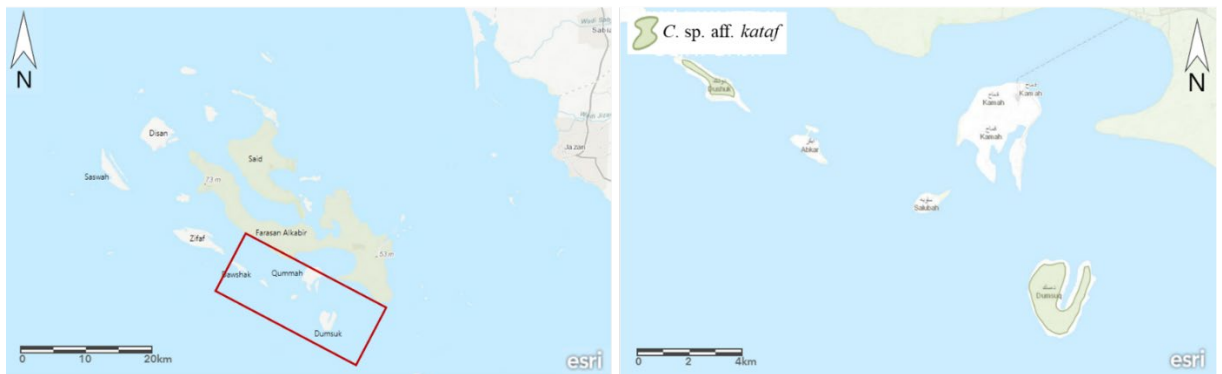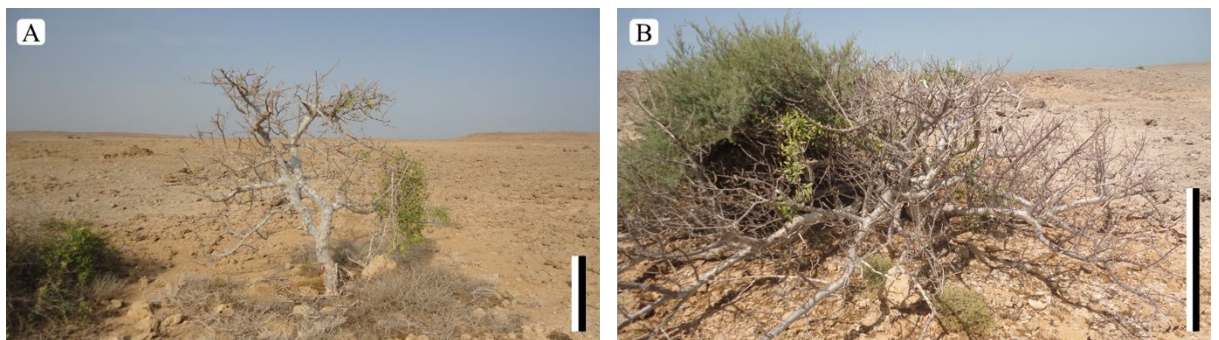

Figure 6-2. *Commiphora* aff. *C. kataf* distribution.

Figure 6-3. *Commiphora* aff. *kataf (***A)** erect form in Dumsuk Island, **(B)** prostrate form in Dawshak Island. Scale bar = 1 m.

6.3.3 *Dinebra somalensis* (Stapf) P.M.Peterson & N.Snow

VU°- Vulnerable, B1ab (i, ii, iii, v)+2ab (i, ii, iii, v)

Family: Poaceae

Synonyms: Drake-brockmania somalensis Stapf; *Eleusine somalensis* Hack*. (Roskov et al., 2019)

Dinebra somalensis is a mat-forming annual grass spread by stolons. It occupies seasonally flooded locations in silty and saline soils. The species is endemic to the Somalia Masai regional centre of endemism distributed from Tanzania to Northeast Africa (Somalia, Sudan, Ethiopia; excluding Uganda) (Ghazanfar & Beentje, 2010). The Farasan Islands is the only recorded Arabian locality of this species with a restricted distribution range on Farasan Alkabir Island. It can be found in a salty clay pan among *Salvadora persica* trees 10 km northwest of the Farasan village (Figure 6-4) at 5-13 m (Collenette, 1999).

Dinebra somalensis is under numerous threats, especially off-road driving (Thomas et al., 2010), drought, infrastructure development and urbanisation. The species is rare and has a restricted geographical distribution to Farasan Alkabir Island. The species has small population size, and could decrease, given the threats to the habitat. The number of individuals estimated to be between 100-500 individual plants (Thomas et al., 2010). The estimated EOO and AOO is 2.0 km^2. Thus, *Dinebra somalensis* is assessed as EN; however, the species is likely to re-colonize the Farasan Islands from Africa due to the proximity. Thus, the assessment was downlisted to VU.
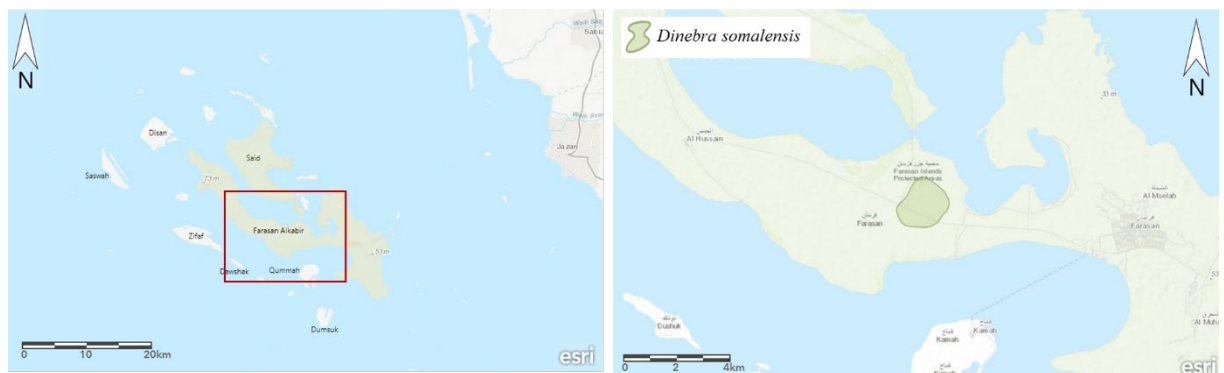

Figure 6-4. *Dinebra somalensis* distribution.

6.3.4 *Euphorbia colletteae* D.Al-Zahrani & El-Karemy

Family: Euphorbiaceae.

Common names: Marar, Saab, Scharath (Al-Zahrani and El-Karemy, 2007).

Euphorbia colletteae is a spiny succulent shrub endemic to the Red Sea region with a restricted distribution to the Farasan Archipelago (Figure 6-8) and the coastal plains of Sudan (Port Sudan) and Eritrea (Archico Bay) (Al-Zahrani & El-Karemy, 2007). It is represented on the islands by a very low number of individuals of less than 500 shrubs (Thomas et al., 2010). It is common in Farasan Alkabir Island, particularly the arid plain of the NW plateau, and Sajid Island, where it forms scattered clumps. It flowers and fruits from March to June (Al-Zahrani & El-Karemy, 2007).

Euphorbia colletteae is closely related to *E. fractiflexa* (Arabian endemic) according to the phylogenetic results of Chapter 5 and the morphological traits (Aldhebiani, 2010); however, it is distinguished from *E. fractiflexa* by its characteristic habits and habitat. *Euphorbia colletteae* are more stout, dark blue-green in colour and up to 4 m in height. The stems are almost erect with low branching, slightly zigzagged with 3-8 angles and segmented (pear-shaped segments). It occurs in the cracks and faults of the fossil coral rocks of the coastal areas as well as on basalt outcrops at 0.5-75 m (Al-Zahrani & El-Karemy, 2007). Whilst *E. fractiflexa* is green to greyish-green, up to 2.5 m tall, with stems of three angles and strikingly zigzagged, it is not segmented and grows in granite rocks, gravel mounds and coastal plains (Al-Zahrani & El-Karemy, 2007). *Euphorbia fractiflexa* occur in Yemen and Southwestern Saudi Arabia (Chaudhary, 2001d) at elevations ranging between about 150–550 m altitude (Al-Zahrani and El-Karemy, 2007).

Euphorbia colletteae is under the threats of habitat loss (Thomas et al., 2010), expansion of cultivation in the region (Figure 6-6, A), infrastructure development and urbanisation (Figure 6-6, B). The species is common in Farasan Alkabir and the Sajid Islands but rare in Dumsuk and Dawshak Islands. The population size is decreasing with a small number of individuals estimated to be less than 500 shrubs. The estimated EOO is 831.329 km^2, and the estimated AOO is 216.0 km^2. The species is thus initially assessed as EN. The probability of re-colonisation the islands from the small populations in Sudan and Eritrea is very low because *Euphorbia* seeds are mainly ant-dispersed (following ballistic capsule dehiscence). This

strongly limits dispersal distances and promotes geographic isolation (Horn et al., 2014). Therefore, the preliminary category is left unchanged.

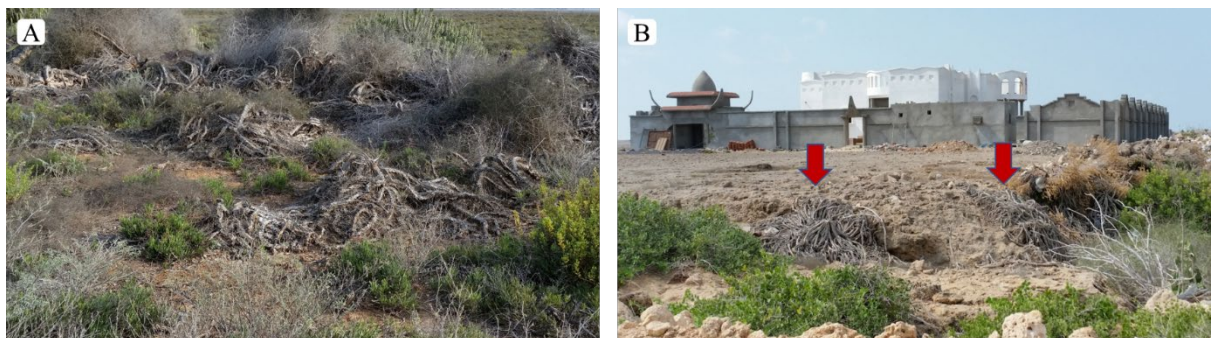**Figure 6-5.** *Euphorbia collenetteae* distribution.

Figure 6-6. (A) Removed *E. collenetteae* to convert land for agricultural use in Almahsor area (Sajid island); **(B)** removed plants for housing construction in Sier Dist. NW Farasan Alkaber island. Red arrow marked the removed plants.

6.3.5 *Glossonema* sp. aff. *boveanum*

EN- Endangered B1ab (i,ii,iii,v)+ 2ab (i,ii, iii,v)

Family: Apocynaceae

Common names: Kabesh (Arabic) (Atiqur Rahman et al., 2002).

Glossonema sp. aff. *boveanum* is a bushy and leafy perennial herb at 20 cm tall known only from the Farasan Islands (Collenette,1999). Unlike the widespread *G. boveanum* distinguished by greyish leaves and pale-pink, narrow flowers of 4 mm wide, *G.* sp. aff. *boveanum* has bright-green leaves and relatively small white flowers (1 cm wide). The species is fairly common in the Farasan Alkabir, Sajid and Dumsuk Islands (Figure 6-7) in fossil coral rock at 2.5 - 19 m (Collenette,1999). It is typically found fruiting after the rainy season in December and January.

Glossonema sp. aff. *boveanum* has threats of off-road traffic, urbanisation, drought and infrastructure development. The population size is small and is estimated to be less than 500 individuals (Thomas et al., 2010), and could decrease, given the threats to the habitat. It has a restricted distribution in three Islands among the Farasan group. The estimated AOO is 16 km^2, and the estimated EOO is 367.64 km^2. Thus, *Glossonema* sp. aff. *boveanum* is assessed as EN.
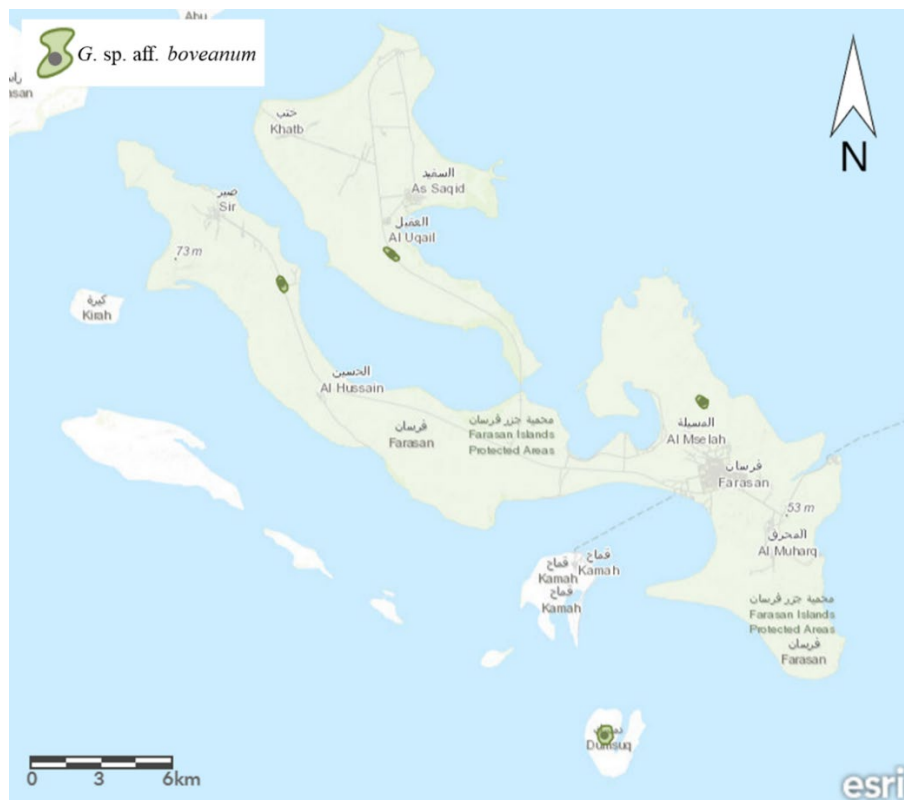

Figure 6-7. *Glossonema* sp. aff. *boveanum* distribution.

6.3.6 *Micrococca mercurialis* (L.) Benth.

EN° –Endangered, B1ab (v)+2ab (v)

Family: Euphorbiaceae*.*

Synonyms: Claoxylon mercuriale (L.) Thwaites; *Mercurialis abyssinica* Hochst. ex Pax & K.Hoffn.; *Mercurialis alternifolia* Lam*.; Microstachys mercurialis* (L.) Dalzell & A.Gibson; *Tragia mercurialis* L. (Roskov et al., 2019)

Micrococca mercurialis is a very rare annual herb known to occur in Al Muharraq area in damp sand among the palm trees in Farasan Alkabir Island (Figure 6-8) at 9-12 m (Collenette, 1999). This location is the only Saudi Arabian locality of this species (Alfarhan et al., 2002). *Micrococca mercurialis* occurs throughout tropical Africa, Yemen, India, Sri Lanka, Western Malaysia and Northern Australia, growing in open places in woodlands and bushlands, along rivers and shores, commonly in ruderal habitats and sometimes as a weed from the sea-level up to 1700 m in altitude (Grubben & Denton, 2004).

Micrococca mercurialis has the threat of off-road traffic (Thomas et al., 2010). It is very rare and is restricted to one locality on Farasan Alkabir Island. The population size is small with a number of individuals estimated to be between 100-500 plants (Thomas et al., 2010). The estimated EOO and AOO is 4 km^2. The current survey during the course of this study for this species did not identify any populations, suggesting possible decline in the population size. *Micrococca mercurialis* is thus assessed as CR. Nevertheless, the probability of species re-colonisation from Yemen is likely due to the proximity, and therefore the assessment has been downlisted to EN.
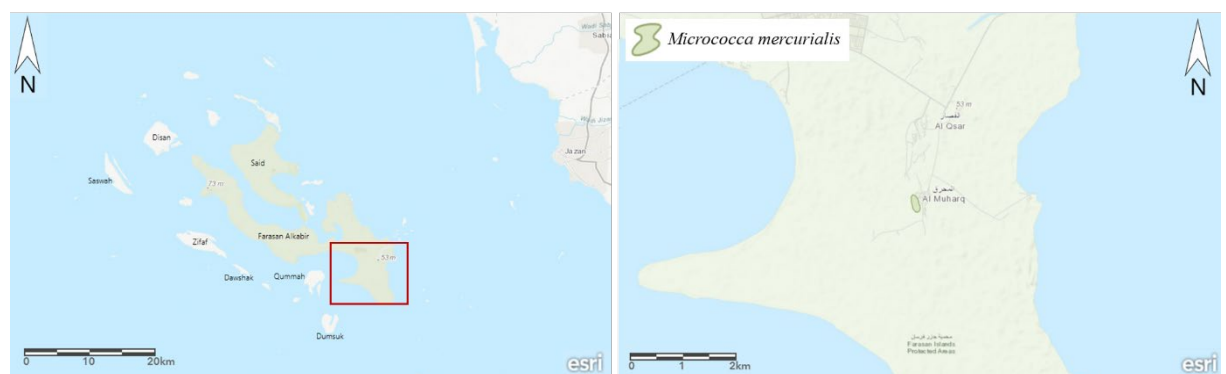

Figure 6-8. *Micrococca mercurialis* distribution.

6.3.7 *Rorida brachystyla* (Deflers ex Franch.) Thulin & Roalson

VU°, Vulnerable, B1ab (i, ii)+2ab (i, ii)

Family: Cleomaceae.

Synonyms: Cleome brachystyla Deflers ex Franch; *C. noeana* Boiss. subsp. *brachystyla* (Deflers ex Franch.) D.F.Chamb. & Lamond; *C. fimbriata* Vicary subsp. *brachystyla* (Deflers ex Franch.) Govaerts (Thulin & Roalson, 2017).

Rorida brachystyla is a woody, stemmed, bushy, glandular hairy herb (Collenette, 1999) restricted to Khallah Bay on Farasan Al-Kabir Island (Figure 6-9), the only Saudi Arabian locality for the species. It can be found growing in cracks on cliffs in deep fossil coral ravines at 9 m in altitude (Collenette, 1999). *Rorida brachystyla* is known to occur in Yemen, Djibouti and Somalia, growing in deserts or semideserts on gravelly or rocky ground at 10–1,000 m (Thulin & Roalson, 2017).

Rorida brachystyla is under the threat of habitat loss (Thomas et al., 2010) and drought. The species is very rare on the Farasan Islands with a restricted geographical range to one locality. The population size is small, which is estimated to be between 100-500 individuals (Thomas et al., 2010) and likely to decrease, given the threats to the habitat. The estimated EOO and AOO is 12 km^2. Thus, *Rorida brachystyla* is assessed as EN; however, the likelihood of species immigration from Yemen is probable. Therefore, *Rorida brachystyla* has been downlisted to VU.
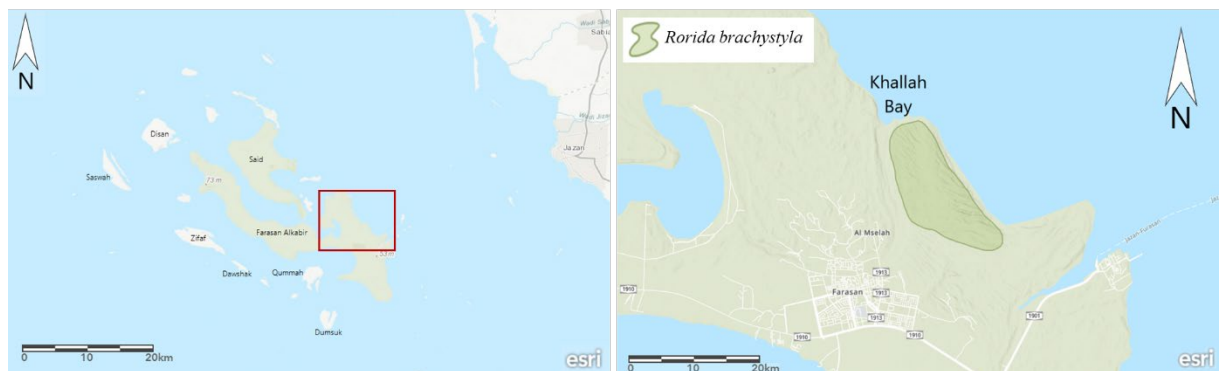

Figure 6-9. *Rorida brachystyla* distribution.

6.3.8 *Vahlia digyna* (Retz.) Kuntze

EN° –Endangered, B1ab (v)+2ab (v)

Family: Vahliaceae.

Synonyms: Bistella digyna (Retz.) Bullock; *Haloragis jerosioides* Perrier; *Oldenlandia decumbens* Spreng.; *Oldenlandia digyna* Retz.; *Oldenlandia sessiliflora* Sm.; *Vahlia menyharthii* Schinz; *Vahlia ramosissima* A. DC. ex DC.; *Vahlia sessiliflora* DC.; *Vahlia viscosa* Roxb. (Roskov et al., 2019).

Vahlia digyna is a regionally rare annual herb restricted to the *Vachellia* woodlands in the Al Muharraq area on Farasan Alkabir Island (Figure 6-10), which is the only Arabian locality of this species. It can be found in the clay pan among *Vachellia* trees at 6 m (Collenette, 1999). The species is widely distributed from India to tropical Africa (Alfarhan et al., 2005).

Vahlia digyna is under numerous threats, especially drought, off-road traffic (Thomas et al., 2010) and invasive plants. The species is very rare on the Farasan Islands with a restricted geographical range. The population size is small with an estimated number of individuals between 100-500 plants (Thomas et al., 2010). The current survey during the course of this study for this species did not identify any populations, suggesting possible decline in the population size. The estimated EOO and AOO is 8 km^2. The *Vachellia* woodlands in Al Muharraq has been intensively invaded by the exotic tree *P. juliflora*, which negatively affects the native plants (Thomas et al., 2016; El-Shabasy, 2017). Therefore, *Vahlia digyna* has been assessed as CR. The probability of re-colonisation from outside the region is likely. Thus, the assessment has been downlisted to EN.
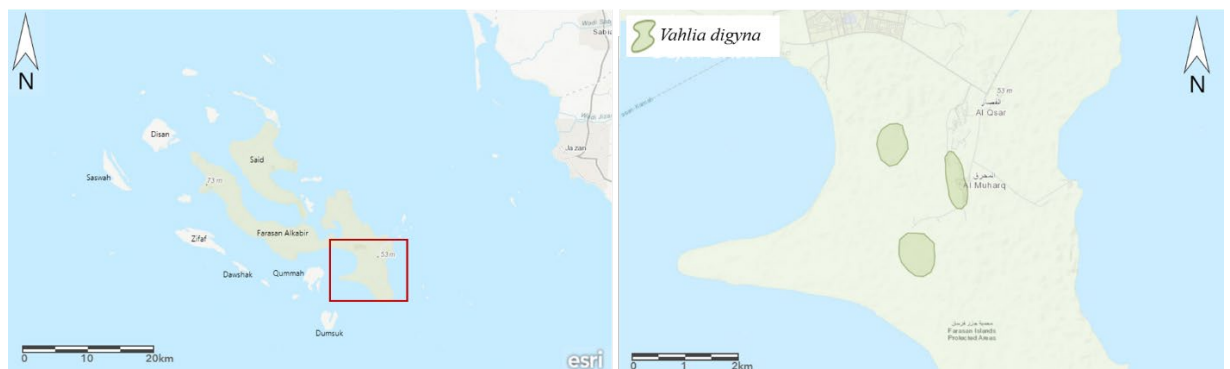

Figure 6-10. *Vahlia digyna* distribution.

Overall, all species were classified under threatened categories, including EN and VU. Six taxa were classified as Endangered and two as Vulnerable (Table 6-3). Mapping of species distribution demonstrated the areas with the highest importance for conservation (Figure 6-11). The highest number of these rare species occurs on the main island Farasan Alkabir with eight taxa, followed by Dumsuk Island with three taxa and then Sajid and Dawshak Islands with two taxa each.

Table 6-3: Red Listing status of the Farasan Islands' endemic and non-endemic restricted species. EOO, extent of occurrence; AOO=Area of Occupancy; Loc.=IUCN location not locality; IUCN= International Union for Conservation of Nature.

| Taxon Name	EOO (km^2)	AOO (km^2)	Altitude (m)	Loc.	IUCN category	IUCN criteria
Basilicum polystachyon	8.00	8.00	9-12	1	EN°	B1ab (v)+2ab (v)
Commiphora aff. *kataf*	50.65	36.00	4-14	2	EN	B1ab (i, ii)+2ab (i, ii)
Dinebra somalensis	12.00	12.00	5-13	1	VU°	B1ab (i, ii, iii, v)+2ab (i, ii, iii, v)
Euphorbia collenetteae	831.33	216.00	0.5-75	4	EN	B1ab (i, ii, iii, v)+2ab (i, ii, iii, v)
Glossonema sp. aff. *boveanum*	367.63	16.00	2.5-19	3	EN	B1ab (i,ii,iii,v)+ 2ab (i,ii, iii,v)
Micrococca mercurialis	4.00	4.00	9-12	1	EN°	B1ab (v)+2ab (v)
Rorida brachystyla	12.00	12.00	9	1	VU°	B1ab (i, ii)+2ab (i, ii)
Vahlia digyna	8.00	8.00	6	1	EN°	B1ab (v)+2ab (v)
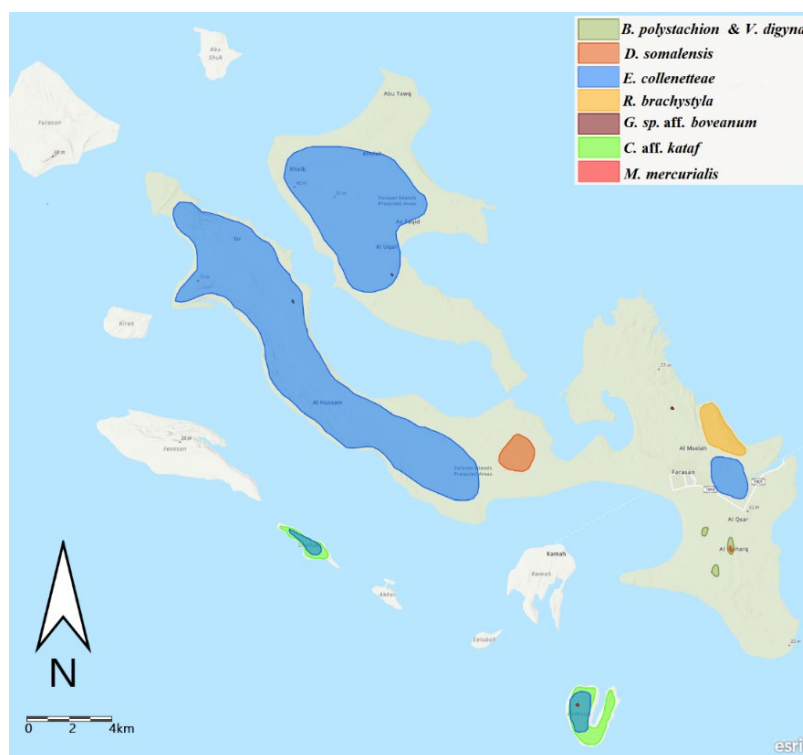
Figure 6-11. Farasan Island map showing the distribution of all investigated species.

6.4 Discussion

The Farasan Islands flora includes nine species unknown in any other part of Saudi Arabia, three of which are endemic or near-endemic to the Islands and four of which are not known in any other part of the Arabian Peninsula. In this study, the threatened status of eight species (Table 6-2) was assessed in the global and regional contexts because most of these species have not previously been the subject of IUCN Red Listing. *Commiphora* aff. *kataf* and *Glossonema* sp. aff. *boveanum* were assessed at the global level using the IUCN Red List categories and criteria version 13 (IUCN Standards and Petitions Subcommittee, 2017). *Basilicum polystachyon, Dinebra somalensis, Micrococca mercurialis, Rorida brachystyla* and *Vahlia digyna* were assessed at the regional and national levels using the Guidelines for the Application of the IUCN Red List Criteria version 4.0 (IUCN, 2012). *Blepharis saudensis* was not re-assessed because it was recently assessed as Endangered (Basahi & Masrahi, 2019).

The current Red List of endemic and non-endemic restricted species of the Farasan Islands at the species level illustrates a high risk of extinction for this rare flora (75% Endangered, 25% Vulnerable). Based on this assessment it is clear that there is an urgent need for conservation actions for these species. At the beginning of this decade, the conservation assessment of Farasan flora and rare species, in particular, was carried out by Hall et al. (2010) and Thomas et al. (2010). A number of major threats to plant conservation were recorded, including off-road driving, the impact of invasive species (*Prosopis juliflora)*, overgrazing, agricultural intensification, and development of the islands for tourism. Currently, the same threats are ongoing, and no observed actions have been taken to minimise the risk. In contrast, there has been accelerated development in urbanisation and developmental projects, such as constructing an airport (SCTH, 2017). The endangered species have small populations, therefore any further disturbance to their habitats would result in their extinction from the study area (Thomas et al., 2010). Conservation policies should be broader to include plants, animals and land use, which is currently focussed upon protecting Idmi gazelle (Abuzinada, 2003) and the largest populations of mangrove. Conservation planning also should take into consideration all that islands housing the rare species (Figure 6-11) for effective conservation.

Internationally, the conservation of endemic or near-endemic Farasan Archipelago elements are perhaps of the highest importance and should be the focus of priority actions. Three taxa are endemic or near-endemic to the Farasan Archipelago (*Blepharis saudensis*, *Commiphora* aff.

C. *kataf* and *Glossonema* sp. aff. *boveanum*), which are assessed as Endangered. Further taxonomic work is necessary to confirm the novelty of *Commiphora* aff. C. *kataf* and *Glossonema* sp. aff. *boveanum*, which would provide them with more value for conservation assessments and for including them on the Global Red List.

Euphorbia collenetteae is the best-assessed species among the group, this study highlights the increased risk to the island flora over the past two decades. The species is a Red Sea endemic taxon that occurs in the Farasan Archipelago with the largest population in the area. In 1999, no threat was observed to the Islands' population (Collenette, 1999), but eight years later, in 2007, it was assessed as Near Threatened (Al-Zahrani & El-Karemy, 2007). However, by 2010 it was categorised as Locally Endangered (Thomas et al., 2010). For this study, in 2019, it has been assessed as an Endangered species. The criteria used in the previous assessments are unclear; however, the indications of an increasing risk in these assessments cannot be ignored. The LC status that has been given to the species by Pahlevani (2017) was not taken into consideration because the assessment relied on the literature only. It is critical for the survival of the species that the population is monitored, considering the Farasan Islands account for a high proportion of the world population.

The conservation status has been down listed in this study for five species: *Basilicum polystachyon, Dinebra somalensis, Micrococca mercurialis, Rorida brachystyla* and *Vahlia digyna.* Given that large islands near the mainland is expected to have high immigration rates (MacArthur & Wilson, 1967), the populations within the Farasan Islands may experience a 'rescue effect' (Brown & Kodric-Brown, 1977) from populations in the nearest mainlands (Africa and Yemen). The category in *E. collenetteae* has been left unchanged due to the limited dispersal of *Euphorbia* (Horn et al., 2014).

Basilicum polystachyon, Micrococca mercurialis and *Vahlia digyna*, which are categorised as Endangered, are of interest because they have always been rare and are restricted to the Al-Muharraq area on the Southeast Farasan Alkabir Island. These species occur in small native ranges and are often of greatest conservation concern. No species of the Farasan Islands have yet been proven to be extinct, but a recent survey during the course of this study for these species did not identify any populations. More survey work is needed to determine whether they are Endangered as assessed in this study or regionally extinct. The Al-Muharraq area suffered from a severe drought during the course of this study, and drought in consecutive years poses a threat to vulnerable plants. If the plants suffer from water stress at seedling stage, growth will be stunted, few flowers produced, and few viable seeds (Alzan, 2013). Furthermore, the

invasive species *Prosopis juliflora* is one of the most pressing threats in this area (Hall et al., 2010). *Prosopis juliflora* has been established at the expense of the native *Vachellia* woodlands and is spreading into the largest populations of the *V. flava* woodlands in this area, the only known locality for these species. It has been proven that *P. juliflora* has significantly greater negative impacts on the density, frequency and richness of the associated native species, particularly the annuals (El-Keblawy & Al-Rawai, 2007). The possible mechanisms for this inhibition include the reduction in the water table and the release of allelopathic chemicals (Samuel et al., 2012). A recent study on the Farasan Islands indicated that *P. juliflora* has negatively affected the growth of the native *V. flava* woodlands (El-Shabasy, 2017). If no urgent action is taken to monitor the growth of *P. juliflora*, it is likely to spread rapidly throughout the Farasan Islands as has occurred elsewhere in Saudi Arabia (Hall et al., 2010).

Since 1989, the Farasan Archipelago has been established as a National Protected Area (Abuzinada, 2003). Animal wildlife have the priority of conservation legislation compared to plant wildlife. This legislation only provides protection for the largest stands of mangroves in Zifaf Island and Khawr Al-Qandal Northeastern Farasan Alkabir Island. The awareness of the need to conserve and to manage threatened flora is growing slowly, but it is vital because most of the species listed in this chapter are not represented in the current conservation framework. The slow start in addressing threatened flora conservation in the Farasan Islands cannot be attributed to a lack of political willpower or limited resources. The major hindrance has been the lack of up-to-date, detailed, baseline data of the threatened species, their biology, their distribution and the reasons for their decline. This need has begun to be addressed with the designation of the Farasan Islands as an Important Plant Area in the Arabian Peninsula (Hall et al., 2010) and the establishment of an electronic flora of the Farasan Islands (http://ffa.myspecies.info/) as a field guide (Al Qthanin, 2019); however, increased public awareness is still needed. This study provides a significant contribution to filling this gap by providing up-to-date, scientifically based information on the distribution, status, trends and threats to endangered species.

6.5 Conservation Implications

"Establishment of protected areas and community-based management areas for *in situ* conservation is essential to mitigate threats to plant biodiversity" (Caujape-Castells et al., 2010). The current basic framework for Farasan Islands conservation focuses on the Idmi gazelle (Abuzinada, 2003) and mangrove populations (Gladstone et al., 2003). The conservation policies should be broadened to include terrestrial plants and land use. *Euphorbia collenetteae* population in Farasan Alkabir and Sajid Islands may have to receive a higher priority in future protection before it is too late. The Al-Muharraq area should hold special importance in conservation terms, as it is a concentrated pool of the endangered species and occupied by *Vachellia flava* woodland, the major food of the gazelle (Wronski and Schulz-Kornas, 2015).

Conservation action in Al-Muharraq must focus on removing the *Prosopis juliflora* which has already invaded the *Vachellia* woodland. There are several control methods for *Prosopis* including mechanical removal, biological controls, herbicidal application, fire and restriction of dispersal (Gallaher and Merlin, 2010). Control methods have been employed in South Africa, Australia, Hawaii and elsewhere with various degrees of success (Pasiecznik et al., 2001). The most successful mechanical control methods require cutting the stem or root at least 10–20 cm below ground to remove dormant buds (Osmond et al., 2003a; Shiferaw et al., 2004; van Klinken et al., 2006). Seedlings may be eradicating through fire or the use of a foliar herbicide such as picloram or triclopyr (Osmond et al., 2003b; Geesing et al., 2004; van Klinken et al., 2006).

The investment in *ex situ* conservation, such as conservation of plants and seeds in botanic gardens and seed banks, is an urgent complementary measure to *in situ* conservation for Farasan endangered species. The combination of *in situ* and *ex situ* protection strategies was applied successfully in two Canarian endemic Fabaceae from La Palma island, *Lotus eremiticus* and *L. pyranthus*, which consist of fewer than 10 and 20 individuals, respectively (Caujape-Castells et al., 2010). The conservation plan comprises "the protection and management of their habitats, the maintenance of clones of most individuals in the Cabildo's plant nursery to complement the *in situ* conservation measures and the genetic characterisation of all the individuals" (Caujape-Castells et al., 2010).

In addition, protection of the Farasan-restricted species needs close collaboration with relevant regional organisations in the Red Sea participating countries that share the subpopulation of

those species. This research calls for the design of a regional biodiversity strategy to deal with the various threats facing the rare plant species in the Red Sea Basin, such as the current collaboration of protection of the marine habitat in the Red Sea and Gulf of Aden managed by the Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden PERSGA (Gladstone et al., 2003).

The Farasan Islands have long been a popular destination for domestic tourism. The 2030 vision of Saudi Arabia aims to develop the Farasan Islands and increase the number of tourists (SCTH, 2017). The balance between the development of the Farasan Islands and protected areas is the only opportunity for the long-term sustainability of tourism in the area. Marine Protected Areas can be beneficial economically through well-managed eco-tourism (PERSGA/GEF, 2004). An example of the successful integration of environmental protection and sustainable development is provided by Egypt along the Gulf of Aqaba. "Entrance fees for protected areas and guided mangrove tours (e.g. in the mangroves of the Nabq Managed Resource Protected Area) are some of the measures implemented to date. Additional means of generating revenue include the establishment of non-profit conservation funds and private-sector grants"(PERSGA/GEF, 2004). The Farasan Islands Marine Protected Area has a high diversity of marine habitats, including seagrass beds, mangroves and extensive areas of fringing reef dominated by a diverse coral community or a mixture of coral and macroalgae (Gladstone, 2000). These habitats support a wide range of associated fauna including marine mammals, turtles and seabirds (Gladstone, 2000). The fascinating nature of the Farasan Islands can provide employment opportunities for the locals and sustainable finances through well-managed eco-tourism based on the entrance fees and tour guided groups.

The most conspicuous impact of tourism in the Farasan Islands is the off-road traffic, which is the leading contributor to habitat degradation in the main islands, Farasan Alkabir and Sajid, and one of the major threats to the rare species. Management policies need to direct towards controlling off-road vehicle driving, restoring the affected areas and maintaining ecosystem function in these islands. Reducing vehicle disturbances on roads has been proposed to maintain local biodiversity in arid landscape (Gelbard and Harrison, 2003). The recovery and succession of native vegetation were documented in the United States desert as a result of roads abandonment (Bolling and Walker, 2000; Holl et al., 2000).

6.7 Summary

The current Red List of endemic and non-endemic restricted species of the Farasan Islands at the species level illustrates a high risk of extinction for this rare flora (75% Endangered, 25% Vulnerable). While economic development, invasive species and drought have been identified as major factors in the decline of the threatened native flora of Farasan Islands, it is also possible to associate a general ignorance of the importance of the Islands' vegetation with this decline. This study provides the first Red List of the Farasan Islands flora according to IUCN categories and criteria. This is an important initiative step toward the protection of these species by providing up-to-date, scientifically based information on the species distribution, status and possible threats. The results will be submitted directly to decision-makers in SWA to catalyse actions for biodiversity conservation.

The conservation implications proposed in this study include giving *Euphorbia collenetteae* population in Farasan Alkabir and Sajid Islands priority in future protection, removing the *Prosopis juliflora* from Al-Muharraq area, investment in *ex situ* conservation for endangered species as a complementary measure to *in situ* conservation and controlling off-road vehicle driving. The study recommends the design a regional biodiversity strategy to protect rare plant species in the Red Sea Basin and calls for eco-tourism investment in the Farasan Islands.

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CHAPTER 7 General Discussion and Conclusion

7.1 Discussion

The Farasan Islands is of particular interest due to its location in the southern Red Sea and is one of only two major island groups in the area. The Farasan Islands flora has a potentially great value to conservation both for the plant species present and the habitats those plants provide. This thesis has explored some of the phylogenetic affinities of the flora with the intention to discover the timescale for colonisation and extent to which the species present are parts of a more general widespread subtropical flora or whether they are more closely linked to adjacent mainland floras. Some of the species on the islands are foodstuff of the vulnerable endemic subspecies *Gazella gazella farasani* (Assaeed et al., 1995; Cunningham & Wronski, 2011; Wronski & Schulz-Kornas, 2015) so have an important role in conservation of this gazelle. In addition to this, the ecological importance of the mangroves on the island include protecting the coast from erosion and trapping sediments. As a result, they also protect coral reefs, consolidate shore habitats, provide nesting, roosting and breeding sites for several birds, provide nursery and shelter for a number of marine organisms, and enrich the marine food web in the surrounding oligotrophic water (PERSGA, 2004). These Islands are a concentrated pool of group of rare species in Arabian Peninsula (Hall et al., 2010) and are now the best studied in the area.

The Red Sea has over a thousand islands with the two largest groups lying in the south, the Farasan Islands (Saudi Arabia) in the east and the Dahlak islands with over 350 islands (Eritrea) in the western Red Sea (Rasul & Stewart, 2015). Although the majority of these large islands are an uplifted fossilised coral reef in origin (Almalki & Bantan, 2015), the fact that they are quite close to mainland coasts makes them appear like continental archipelagos (Masseti et al., 2015). The Farasan Islands flora is well studied compared with Dahlak Islands which vegetation remains largely unknown.

Using phylogenies and molecular dating has enormously improved the study of island floras and provide insights into the evolution of continental island vegetation that may guide the conservation of island plants. This study is the most comprehensive phylogenetic study to date carried out on Farasan Islands plant species covering ten angiosperm genera including 25 species, which represent the main habitat types of the islands. The novelty of this thesis is in the investigation of the common features and differences among these species in terms of phylogenetic age and geographical affinities with respect to the Farasan Islands lineages.

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The phylogenetic analyses have demonstrated that the Farasan Islands flora, as expected for a continental shelf island, is linked to the nearest mainland floras of Arabia and Africa (Table 7-1) and most of the species are probably more closely tied to the large species pool of adjacent Arabian flora (Figure 7-2, A & Table 7-1). Current phylogenetic results support previous hypotheses on the Farasan flora proposed by Thomas et al. (2010) and Hassan & Al-Hemaid (1996). The study also shows that Farasan flora is very recent, the islands populations have a very close link and may still interact with the mainland populations, although this would need much more in-depth population analysis to resolve the detail. Plant colonisation is probably not just from the mainland to the islands it could be vice versa, and the flow of propagules maintained both sides and a larger effective population than there would otherwise be. Thus, we cannot look to these islands in isolation they are part of a bigger picture of the Red Sea flora. In contrast to the oceanic islands where most of the plants are endemics, the Farasan flora shows no true endemism. However, the Farasan Islands are important as a carrier of substantial parts of some species populations since lots of species on these islands are growing on mainland areas that are very politically unstable such as Yemen, Somalia and Sudan where there is not the political room to focus on conservation measures. This adds a particular importance to the Islands' flora as a sort of local ark and given the nature of Farasan, as islands, that makes them easier to control and protect than the mainland where many more people live.

Without doubt, one of the key ecological groupings are the mangrove species of *Avicennia marina* and *Rhizophora mucronata* that are widely distributed in the Indo West Pacific region (Duke, 2017). However, based on our phylogeny, the Red Sea lineages are genetically distinct and join up with other immediately geographic close lineages. The Red Sea forms a unique habitat which is the warmest (exceeding 35°C) and most saline (up to 46 ppt) seawater in the world (Bruckner et al., 2012). This is partially due to the absence of permanent rivers or streams flowing into the sea, low annual rainfall, and high levels of evaporation (Bruckner et al., 2012). Mangroves in the Red Sea are adapted to temperature conditions that actually might be more prevalent with climate change in the oceans of the future. Global ocean surface temperate having increased, and these changing are ongoing (Hoegh-Guldberg & Bruno, 2010). Heat adapted mangroves of the Red Sea could be really important for the broader conservation programme of estuarine coastlines. These mangrove populations might be used to replant other areas where the population has died out because of increased sea temperature due to climate change.

The Farasan islands have the most conservable populations of mangroves in Arabian Peninsula. However, conservation planning action of Farasan mangroves needs to integrate with action on the mainland. The island populations are not in isolation and the very close phylogenetic link to the mainland populations suggests they might be acting as one population in one integrated area. Degradation or destruction of the source habitat will, in turn, impact the dependent sink populations (Tittler et al., 2006). Some countries in the Red Sea have included some of their mangroves within established reserves or marine protected areas, mainly Djibouti, Saudi Arabia and Egypt (PERSGA, 2004). However, the vast majority of the mangrove swamps in these countries and in other parts of the region, including all mangrove areas in Yemen and Sudan, lack any legal protection for conservation purposes (PERSGA, 2004). Political cooperation and efficient coordination are needed between the different authorities in the Red Sea to conserve the mangrove in the basin.

The Farasan Archipelago provides a safe migratory route and nesting sites for birds in the islands' wetlands (PERSGA, 2004). Birds are key long-distance dispersal vectors for many plants (Heleno & Vargas, 2015; Viana et al., 2016) and this study evidences that birds may play a key role in the Farasan Islands flora formation, particularly of coastal plant species. Around 48% of the study group species are either strictly bird dispersed plants (12%), or their dispersal could be mediated by birds (36%) (Figure 1-2, B). Viana et al. (2016) have proved empirically that migratory birds mediate regular seed dispersal, on the order of millions of seeds, over the ocean between Europe and Africa and into the Canary Islands. Such studies are needed in the Farasan Islands to understand the role of migratory and local birds of the seed dispersal at the local scale.

The dated phylogenies produced in this thesis support a mixed model of colonisation, with some species spreading over land links during periods of lower sea level while others have more plausibly been dispersed by wind, water or animals (Figure 7-1). Without doubt the flora's closest links are with the Arabian peninsula, the closest mainland, and the young age of the islands would support the idea that colonisation of the Arabian Peninsula from Africa and Asia was followed by the relatively short dispersal to the Farasan Archipelago (Figure 7-2, A). Over the 25 species studied in the thesis (Figure 7-2, B) there is a wide range of putative dispersal mechanisms.

The Arabian link of the Red Sea endemic *Euphorbia col;lenetteae* provide an insight into possible *in situ* evolution on the islands. The harsh conditions of high levels of aridity, salinity, temperature and irradiance with limited water availability prevailing in the Farasan Islands (El-

Demerdash, 1996; Ibrahim, 2008) may have contributed to the evolution of this succulent species. All these conditions coupled with the unique habitat of fossil coral rocks could promote local adaptation or even speciation driven by a shift in edaphic preferences (Rajakaruna, 2017). Population genetics study to compare the Farasan population with those from East Africa would be of great value for ascertaining the genetic divergence of the Arabian and African populations. This would give an indication of the importance of the genetic diversity found in the islands' population and would provide evidence on the duration of separation between these populations and also on plant colonisation events in the Red Sea area.

Given the conservation rationale behind this study the IUCN Red List Categories and Criteria have been applied for the first time to exemplar the Farasan islands endemic and non-endemic restricted species that can guide identifying priority areas for conservation. The importance of the Farasan Islands flora in Saudi Arabia is not in terms of its endemism, which is low, but in being a concentrated pool of individual species that are rare elsewhere in the Arabian Peninsula and the Red Sea. These particular species are distributed in a number of islands among the archipelago, the high diversity and large population size occur in the large island Farasan Alkabir. All these islands need to be taken into consideration for effective conservation. Isolated uninhabited island such as Dawshak, Dumsuk and Zifaf could be good nature reserves, and the focused concern needs to be on the populations that occur on the main inhabited islands Farasan Alkabir and Sajid. Accelerating development and spread of the invasive species coupled with climate change are increase stress on the main islands' populations. According to the Red List assessment carried out in this study, all the rare species are threatened with extinction (75% Endangered, 25% Vulnerable). The high percentage of extinction risk among these species places a significant conservation responsibility on Farasan Protected Area administrators to include them in conservation policies which should be broadened to include plants, animals and land use. The up-to-date, detailed, baseline data provided in the study are a valuable source that can guide to identifying priority areas for conservation.

Table 7-1. List of 25 native species belonging to ten genera from the Farasan Islands that were included in the phylogenetic study, including information on the affinities estimated in this study, seed types, dispersal syndromes and potential dispersal vectors. Colour shaded cells determine fine-scale affinities as following: green= Arabian, light green= more likely Arabian, grey= African, unshaded= unresolved relationships

Area	Species name	Phylogenetic affinities		Fruit type	Dispersal syndrome	Possible dispersal vector
		Global scale	Fine scale			
Mangrove	*Avicennia marina*	Red Sea	Unresolved	crypto-viviparous	Hydrochorous	Sea current
	Rhizophora mucronata	Red Sea	Unclear/data limitation	Viviparous	Hydrochorous	Sea current
Coastal area	*Cyperus bulbosus*	Unclear	Unresolved	Achene	Epizoochorous, endozoochorous	Birds (mud, ingestion)
	Cyperus conglomeratus	Red Sea	Unresolved	Achene	Epizoochorous, endozoochorous	Birds (mud, ingestion)
	Cyperus rotundus	Unclear/data limitation	Unclear/data limitation	Achene	Epizoochorous, endozoochorous	Birds (mud, ingestion)
	Heliotropium ramosissimum	Arabia	Arabia	Nutlet	Anemochorous	Wind
	Heliotropium longiflorum	Horn of Africa/Arabia	Africa	Nutlet	Anemochorous	Wind
	Suaeda fruticosa	Horn of Africa	Unclear/data limitation	Utricles	Epizoochorous, endozoochorous	Birds (mud, ingestion)
	Suaeda aegyptiaca	Red Sea/Arabian Sea	Unresolved	Utricles	Epizoochorous, endozoochorous	Birds (mud, ingestion)
	Tetraena alba var. *alba*	Red Sea/Mediterranean	Unresolved	Mericarp	Anemochorous, epizoochorous	Wind, birds
	Tetraena coccinea	Red Sea area	Unresolved	Mericarp	Anemochorous, epizoochorous	Wind, birds
	Tetraena simplex	Unclear	Unclear	Mericarp	Anemochorous, epizoochorous	Wind, birds

Table 7-1. Continued.

| Area	Species name	Phylogenetic affinities		Fruit type	Dispersal Syndrome	Possible Dispersal vector
		Global scale	Fine scale			
Islands' interior	*Convolvulus glomeratus*	Red Sea area	Unresolved	Capsule	Unspecialized	Unknown
	Convolvulus rhyniospermus	Red Sea area	Unclear/data limitation	Capsule	Unspecialized	Unknown
	Euphorbia collenetteae	Red Sea area	Arabia	schizocarp	Myrmecochory	Ant
	Euphorbia granulata	Red Sea area	Unclear/data limitation	schizocarp	Epizoochorous	Birds
	Ficus cordata ssp. *salicifolia*	Red Sea area	Unclear/data limitation	syconium	Endozoochorous	Birds
	Ficus glumosa	Red Sea area	Unresolved	syconium	Endozoochorous	Birds
	Ficus populifolia	Horn of Africa/Arabia	Unresolved	syconium	Endozoochorous	Birds
	Indigofera coerulea var. *coerulea.*	Horn of Africa/ Red Sea area	Unresolved	Pod	Unspecialized	Unknown
	Indigofera hochstetteri	Red Sea area	Unclear/data limitation	Pod	Unspecialized	Unknown
	Indigofera linifolia	Red Sea area	Unresolved	Pod	Unspecialized	Unknown
	Indigofera oblongifolia	Horn of Africa/ Red Sea area	Unresolved	Pod	Unspecialized	Unknown
	Indigofera semitrijuga	Horn of Africa	Unclear/data limitation	Pod	Unspecialized	Unknown
	Indigofera spiniflora	Red Sea area	Unclear/data limitation	Pod	Unspecialized	Unknown
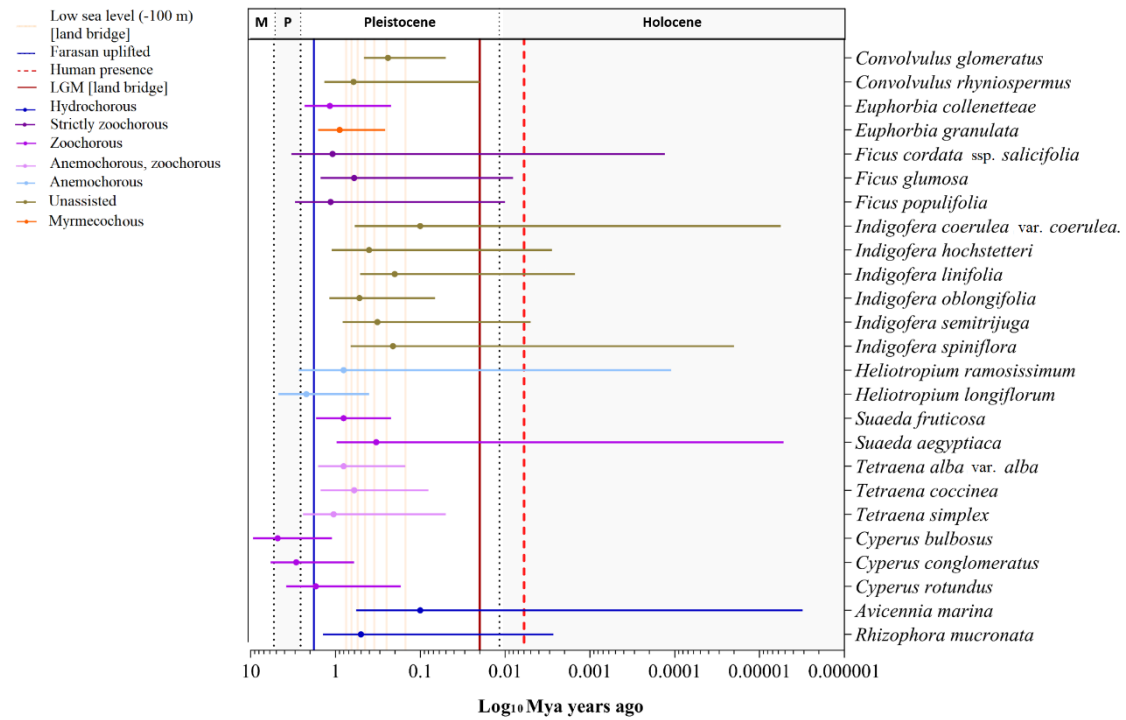
Figure 7-1. Diagram showing estimated lineage divergence times (mean and 95% HPD confidence intervals) for Farasan Islands study group species dated in this study and indicated in the MCC chronograms depicted in Chapters 3 to 5. (*E. collenetteae* is narrow endemic and arguably has not expanded; the date put in here is for the purpose of comparison). The age assigned for *C. bulbosus* are of the crown node included (Farasan, Yemen & Senegal) due to unresolved relationships. Lowered sea-level (-100m) data from Rohling et al. (2013) and Woodruff (2010). Colour of error bars are coded by dispersal syndromes. LGM= Last Glacial Maximum, M=Miocene, P=Pliocene.
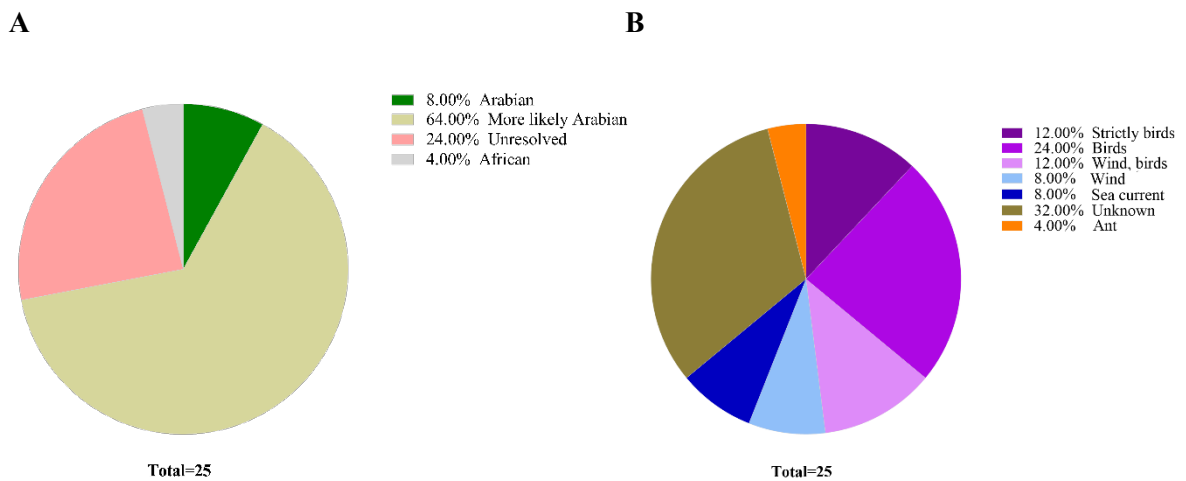

Figure 7-2. Pie chart showing the percentage of potential: **(A)** geographic origins for Farasan Islands flora, **(B)** dispersal vectors that contributed to Farasan flora assembly before human presence.

7.2 Conclusion

The substantial floral diversity of the Farasan Islands of the Red Sea Basin is under serious threat of loss. This study aims to understand when and from where this flora has formed, using sequence-based phylogenies and molecular dating. Integrate molecular method with Red List assessment can provide a complete framework to identify the priority areas of conservation inside and outside the islands.

Molecular phylogenies including the use of molecular dating are important tool to identify the source regions of islands floras and how isolated and distinctiveness of these lineages are. This is very important in terms of the continental islands that their floras are a very close link with the mainland populations. Identify the source regions that could be integrated into conservation planning of the islands' threatened populations is of great value to full conserve these populations.

The phylogenetic evidence shows that flora Farasan Islands is closely part of the Red Sea flora mainly from Arabia. Key change of this thesis is that Farasan Island conservation need to be integrated with the conservation of the mainland populations. The main island Farasan Alkabir has the priority in conservation action due to the presence of all rare species and increase the stresses.

The Dahlak Archipelago (Eritrea) is more or less similar to the Farasan Islands but remains almost unstudied. Similar study needs to be conducted in Dahlak Islands which may contain a lot of important populations. A key element of conserving the southern Red Sea flora is to conduct research on these Eritrean islands to allow a coordinated approach to conservation. This would require a joint academic research between Saudi Arabia and Eritrea and an integrated conservation plan.

Further phylogenetic studies and population genetic studies for key species are needed for other plant groups in the islands.

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