University of Tartu

Faculty of Science and Technology

Institute of Ecology and Earth Sciences

Department of Botany

Laura Suur

**PHYLOGENY AND MYCORRHIZAL ASSOCIATIONS OF NYCTAGINACEAE**

Bachelor's Thesis

Supervisor: Prof. Urmas Kõljalg

Tartu 2013

# Contents

# Terminology

**Accessory fruit** – fruit, in which some part of the flesh has developed from adjacent tissues exterior to the ovary; eesti k. rüüsvili, ebavili.

**Achene** – a simple, dry, one-seeded indehiscent fruit in which the seed coat is not adhesive to the pericarp; eesti k. seemnis.

**Anthocarp** – a false fruit consisting of the true fruit and the base of the perianth; eesti k. õiekatte putkeosaga ümbritsetud vili.

**Arbuscular mycorrhiza** – mutualistic symbiosis between a fungus and a plant where the hyphae of the mycobiont penetrate and forms arbuscules in phytobiont's cortical cells, but does not cover the root with a mantle; eesti k. arbuskulaarne mükoriisa.

**Bayesian posterior probability** – the probability of an event A estimated after the evidence created by the event A has been taken into account; eesti k. aposterioorne tõenäosus.

**Bootstrap analysis** – a computer-based method to assess the accuracy of a statistical estimate, gives a numerical value to assess the support of the phylogenetic nodes on phylogenetic trees (bootstrap value), which expresses the percentage of bootstrap trees that also resolved the clade; eesti k. bootstrap analüüs.

**Calyx** – the sepals collectively; the outermost flower whorl; eesti k. tupplehed.

**Corolla** – the petals collectively; usually the colored whorl; eesti k. kroonlehed.

**Decay index** – Bremer support; the number of steps needed to lose the clade on a phylogenetic tree, the higher the number the stronger support for the node; eesti k. laguindeks, Bremeri toetus.

**Diclesium** – achene or nut surrounded by a persistent calyx; eestikeelne vaste puudub.

**Ectomycorrhiza** – mutualistic symbiosis between a fungus and a plant where the hyphae of the mycobiont does not penetrate into but forms a Hartig net between phytobiont's epidermal and cortical cells, while it forms a fungal mantle over the plant root; eesti k. ektomükoriisa.

**Free-central placentation** – the ovules are not connected to the ovary wall, but position on a central column of tissue; eesti k. tsentraalne platsentatsioon.

**Inflorescence** – a flower cluster, with a definite arrangement of flowers; eesti k. õisik.

**Involucre** – a whorl of bracts around or beneath a condensed inflorescence, such as a capitulum or umbel. It resembles and performs the function of the calyx of a single simple flower; eesti k. üldkatis.

**Maximum likelihood** – a method of statistical analysis that estimates the value of one or more parameters to maximize the likelihood of the data set to occur given a statistical model; eesti k. suurima tõepära hinnang.

**Maximum parsimony** – a criterion for estimating a parameter from observed data based on the principle of minimizing the number of events needed to explain the data. In phylogenetic analysis, the optimal tree under the maximum parsimony criterion is a three tree that requires the fewest number of character-state changes; eesti k. säästuprintsiip, parsimoonia.

**Paris type arbuscular mycorrhiza** – the fungal hyphae grow directly from one host plant cell to another and form intracellular structures; eesti k. paris-tüüpi arbuskulaarne mükoriisa.

**Perianth** – the petals and sepals taken together; eesti k. õiekate.

**Pericarp** – the fruit wall which develops from the mature ovary wall; eesti k. viljasein.

**Perisperm** – food-storing tissue derived from the nucellus (megasporangium) that occurs in the seeds of some flowering plants; eesti k. perisperm.

**Phylogram** – a branching or a tree diagram representing the estimated evolutionary relationships among a set of taxa. Branch lengths are proportional to the inferred genetic or evolutionary distance; eesti k. fülogramm.

**RFLP analysis** – restriction fragment polymorphism analysis, where DNA is restricted by enzymes and resulting pieces are separated by gel electrophoresis; gives a profile according to the lengths of the fragments; eesti k. PCR-i restriktsiooni fragmendi pikkuse polümorfismi analüüs.

**Sieve tube element** – a phloem cell that is involved in the long-distance transport of food substances; eesti k. sõeltoru rakk.

**Utricle** - an indehiscent bladder-like fruit, a type of achene; eesti k. seemnis.

# Introduction

Nyctaginaceae Juss. is an angiospermous family, which belongs to the clade eudicots, order Caryophyllales Berchtold & J. Presl. There are around 350 species of trees, shrubs and herbs in approximately 30 genera mainly in tropical, subtropical, and warm temperate regions on the American continents (Mabberley, 2008). The largest variety at generic and specific levels has been described in Neotropics and in arid western North America, whereas nearly half of the recognized genera of the family are found in the latter (Douglas & Spellenberg, 2010). *Pisonia grandis* R. Br. (tribe Pisonieae Meisn.) has a quaint range of distribution almost entirely restricted to small "bird-islands" in the Western Indian Ocean and Eastern Pacific (Ashford & Allaway, 1982; Suvi et al., 2010).

There are few agriculturally and horticulturally important species of Nyctaginaceae: *Mirabilis expansa* (Ruiz & Pav.) Standl. (known as mauka or chago, grown as a root vegetable in the Andes), *Mirabilis jalapa* L. (known as the four o'clock flower, a wide-spread ornamental plant), *Bougainvillea* Comm. ex Juss. species (hybrids and cultivars grown as decorative outdoor and indoor plants).

Nyctaginaceae has been considered a largely non-mycorrhizal family (Janos, 1980; Haug et al., 2005), although a significant number of studies about arbuscular mycorrhiza (Becerra et al., 2007; Sigüenza et al., 1996; Suvi et. al. 2010) and ectomycorrhiza-forming species have been published (Ashford & Allaway 1982, 1985; Haug et al., 2005; Suvi et. al. 2010). *Pisonia grandis* exhibits a completely unique type of ectomycorrhiza with transfer cells and underdeveloped or absent Hartig net (Ashford & Allaway 1982, 1985), whereas associating only with a restricted range of mycobionts (Chambers et al., 1998, 2005; Suvi et al., 2010; Hayward & Horton, 2012).

The aim of this literature review is to provide an overview of the taxonomy and phylogeny of Nyctaginaceae, mycorrhizal taxa of the family, and the ecology and mycorrhizal associations of *P. grandis*. The first part discusses the classification within the family, the historical classification system based on morphological features and the changes after the implementation of molecular markers, and the position of Nyctaginaceae in the plant kingdom. The second part concentrates on mycorrhizal

species of Nyctaginaceae identified so far and will attempt to provide hypothesis about the evolution of the mycorrhiza-forming ability in the family. The last part focuses on *Pisonia grandis* and gives an overview of its ecology, unique "pisonioid" mycorrhiza and the range of mycobionts with illustrative material from UNITE database.

All of the figures used in this overview have been taken from scientific sources and have not been modified because of lack of access to specific information (none of the sequence matrices for generating the phylogenetic trees represented in this paper had been uploaded to TreeBASE) or not to lose exemplifying features (coloration of the photos and figures).

# 1. Taxonomy and phylogeny of Nyctaginaceae

## 1.1 General features of Nyctaginaceae

The greatest variety of Nyctaginaceae genera has been found in the tropics and subtropics of the American continents. However there are genera with a broader range of distribution. A few genera are represented globally in the tropical and subtropical regions (*Acleisanthes* A. Gray*, Boerhavia* L., *Commicarpus* Standl., *Pisonia* L.*, Mirabilis* L.) whereas one monospecific genus (*Phaeoptilum* Radlk.) is restricted to Africa (Fig. 1a) (Douglas, 2007).

The morphological diversity in Nyctaginaceae is remarkable: fibrous, fleshy or tuberous roots; thin or thick, fleshy or succulent, plane or undulate leaves; unisexual or bisexual flowers, etc. (Mabberley, 2008; Spellenberg, 2004). Two synapomorphies have been suggested to support the monophyly of the family: the absence of a corolla, which is replaced by petaloid sepals in the simple flower (Fig. 1b), which are often times clustered in inflorescences (Fig. 1c), and anthocarpic fruit-type (an achene or utricle enclosed in perianth tissue) (Fig. 1d, e) (Douglas & Manos, 2007). However, Levin (2000) indicates that there have been conflicts in the definitions of the anthocarp by different authors. The term is traditionally applied to the accessory fruit (Spellenberg, 2004) and even though the fruit of Nyctaginaceae is technically a diclesium (Douglas & Manos, 2007), the term anthocarp has been persistent in the literature (therefore to avoid confusion, I will utilize it throughout this review).

The fruit structure is commonly used as an identification feature. There are genera which are recognized by the absence of the anthocarp (*Boldoa* Cav. ex Lag., *Cryptocarpus* Kunth, *Salpianthus* Humb. & Bonpl.), whereas in the rest of the taxa the anthocarp exhibits various forms: not completely enclosed by the anthocarp (*Andradea* Allemäo, *Leucaster* Choisy, *Reichenbachia* Spreng.), ribbed and winged (*Phaeoptilum*, *Grajalesia* Miranda*, Tripterocalyx* (Torr.) Hook., *Abronia* Juss., and some *Colignonia*, *Acleisanthes*, and *Boerhavia*) (Fig. 1d), covered with viscid glandular hair or warts (*Pisonia*, *Pisoniella* (Heimerl) Standl., *Cyphomeris* Standl., *Commicarpus*, some *Boerhavia* and *Acleisanthes*), fleshy (*Neea* Ruiz & Pav., *Guapira* Aubl.), boat-shaped (*Allionia* L.) or unelaborated (*Mirabilis*, *Anulocaulis* Standl., *Nyctaginia* Choisy, some

*Colignonia* and *Boerhavia*) (Fig. 1e). Depending on the fruit structures, the dispersal of the seeds is suggested to be mediated by wind, birds, or gravity (Douglas & Manos, 2007).

Additionally, there are several unusual characteristics which occur with a notable frequency. Firstly, cleistogamous (closed, self-fertilizing) flowers are produced in addition to chasmogamous (open) flowers in four genera of Nyctaginaceae: *Acleisanthes*, *Cyphomeris*, *Nyctaginia*, and some *Mirabilis*. Secondly, at least 25 species in seven genera are characterized as gypsophiles or gypsum-tolerant. Thirdly, a number of genera flower during the evening time, hence its common name – the Four'o'clock family (Douglas & Manos, 2007).
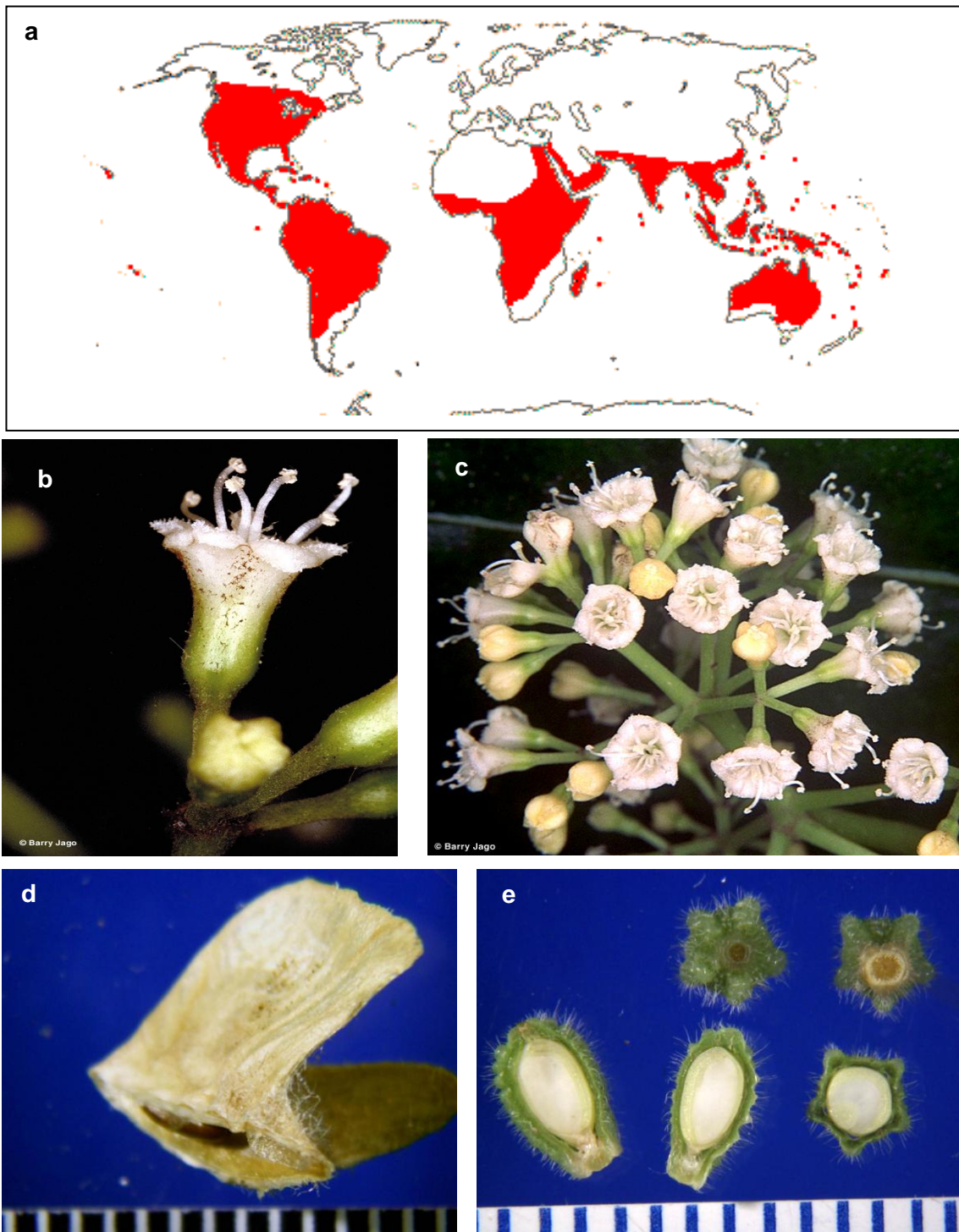
*Figure 1.* a. The range of Nyctaginaceae distribution (by Missouri Botanical Garden). The coloration depicts only the local presence of Nyctaginaceae genera, but not the diversity or species richness. b. The flower of *Pisonia umbellifera* (J. R. Forster & G. Forster) Seemann (by Barry Jago). c. Flower and buds of *Pisonia umbellifera* in an inflorescence (by Barry Jago). d. The winged fruit of *Abronia villanosa* S. Watson (by Ivy Nickels)*.* e. The whole fruits, cross- and half-sections of fruits of *Mirabilis nyctagineae* Michx (by D. L. Nickrent). The scale bar on the bottom on 1d and 1e marks millimeters.

## 1.2 Intrafamilial relationships in Nyctaginaceae

Nyctaginaceae was first described in "Genera Plantarum" by Antoine Laurent de Jussieu in 1789 (Douglas & Spellenberg, 2010). The first groundbreaking works of the phylogeny of the family were done by Heimerl (1889, 1934), who discussed Nyctaginaceae in *Die Natürlichen Pflanzenfamilien*, and by Standley in several works (1909, 1911, 1918, 1931) (as cited in Douglas, 2007). Heimerl (1934) created a supergeneric classification based on plant habit, linear vs. capitate stigma, straight vs. curved embryo, sex distribution, pollen grain morphology, and the occurrence of bracts or involucres (Table 1) (as cited in Douglas & Manos, 2007). However, there were dissensions about the number of tribes between Heimerl (1934), who recognized five worldwide, and Standley (1918), who recognized six solely in North America (as cited in Spellenberg, 2004). Moreover, Hutchinson (1959) recognized eight tribes worldwide (as cited in Spellenberg, 2004). Bittrich and Kühn (1993) revised Heimerl's classification on tribal and subtribal level and incorporated genera described after 1934 and recognized six tribes: Leucastereae Benth. & Hook., Boldoeae, Abronieae S. Watson, Nyctagineae (subtribes Colignoniinae, Boerhaviinae, Nyctagininae, Phaeoptilinae), Bougainvilleeae Choisy, and Pisonieae Meisn. (Table 1) (as cited in Douglas & Manos, 2007).

Table 1

*The historical classification schemes of Nyctaginaceae by Heimerl (1934) and Bittrich and Kühn (1993).*

| Heimerl, 1934 | Bittrich and Kühn, 1993 | Genera |
|---|---|---|
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Boerhavia* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Okenia* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Nyctagininae** | *Nyctaginia* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Anutocautis* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Cyphomeris* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Nyctagininae** | *Allionia* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Commicarpus* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Nyctagininae** | *Mirabilis* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Boerhaviinae** | *Acleisanthes* |
| Mirabileae-Boerhaaviinae | **Nyctagineae-Nyctagininae** | *Cuscatlania* |
|  | **Nyctagineae-Boerhaviinae** | *Caribea* |
| Mirabileae-Abroniinae | **Abronieae** | *Tripterocalyx* |
| Mirabileae-Abroniinae | **Abronieae** | *Abronia* |
| Pisonieae | Pisonieae | *Cephalotomandra* |
| Pisonieae | Pisonieae | *Pisonia* |
| Pisonieae | Pisonieae | *Neea* |
| Pisonieae | Pisonieae | *Guapira* |
|  | **Pisonieae** | *Neeopsis* |
|  | **Pisonieae** | *Grajalesia* |
| Pisonieae | **Nyctagineae-Colignoniinae** | *Pisoniella* |
| Colignonieae | **Nyctagineae-Colignoniinae** | *Colignonia* |
| Mirabileae-Bougainvilleinae | **Bougainvilleeae** | *Bougainvillea* |
|  | **Bougainvilleeae** | *Belemia* |
| Mirabileae-Phaeoptilinae | **Nyctagineae-Phaeoptilinae** | *Phaeoptilum* |
| Leucastereae | Leucastereae | *Leucaster* |
| Leucastereae | Leucastereae | *Ramisia* |
| Leucastereae | Leucastereae | *Andradea* |
| Leucastereae | Leucastereae | *Reicheobachia* |
| Boldoeae | Boldoeae | *Boldoa* |
| Boldoeae | Boldoeae | *Cryptocarpus* |
| Boldoeae | Boldoeae | *Salpianthus* |

*Notes.* Italicized names represent genera, hyphenated names correspond to tribe-subtribe levels of classification, bold names represent names changed or added in the system by Bittrich and Kühn (1993).

The classification within Nyctaginaceae based on morphology has been problematic due to great diversity of features, which was indicated above. Douglas and Spellenberg (2010) indicated that incomplete differentiation of populations in relatively rapidly changing environmental pressures (North America) and the changing species concept have caused additional disarray within the classification. Levin (2000) pointed out that similar morphological features might have evolved multiple times independently, which has created inaccuracy in classifications that were proposed before the availability of molecular markers.

Levin (2000) was the first to take a phylogenetic approach to the study of Nyctaginaceae, however in his first work he analyzed only the relationships of the subtribes Nyctagininae and Boerhaviinae of Nyctaginaceae *sensu* Bittrich and Kühn (1993). He reconstructed phylogenetic relationships within and among three genera: *Mirabilis* (Nyctagininae), *Selinocarpus* A. Gray (Boerhaviinae), and *Acleisanthes* (Boerhaviinae) based on chloroplast (ca. 90 bp at the 3' end of *rbcL*, the intergenic region between the 3' *rbcL* gene and the *accD* gene) and nuclear DNA sequences (ribosomal ITS region). Four species from genera *Allionia, Boerhavia, Abronia,* and *Pisonia* were used as outgroups (Fig. 2).

It had been suggested by previous authors that based on highly similar morphological features *Selinocarpus* is the closest relative of *Acleisanthes* (Levin, 2000). Levin (2000) found that the molecular evidence supported the monophyly of *Acleisanthes-Selinocarpus* group, but not the monophyly of the two genera separately. One of the *Selinocarpus* species, *S. diffusus* A. Gray*,* nested within a clade of four species of *Acleisanthes,* closest to *A. wrightii (*A. Gray) Benth. & Hook. f. ex Hemsl.. The revealed phylogenetic relationships within *Acleisanthes* were in accordance with the morphological features: the basal clade lacks resinous glands on anthocarps, others with the exception of *A. crassifolia* A. Gray (and *S. diffusus*) have resinous glands. The relationships within *Selinocarpus* are less clear, however based on the molecular data all *Selinocarpus* species were transferred to *Acleisanthes*, which left the group monophyletic (Levin, 2002). Therefore *Selinocarpus* is synonymous to *Acleisanthes.*
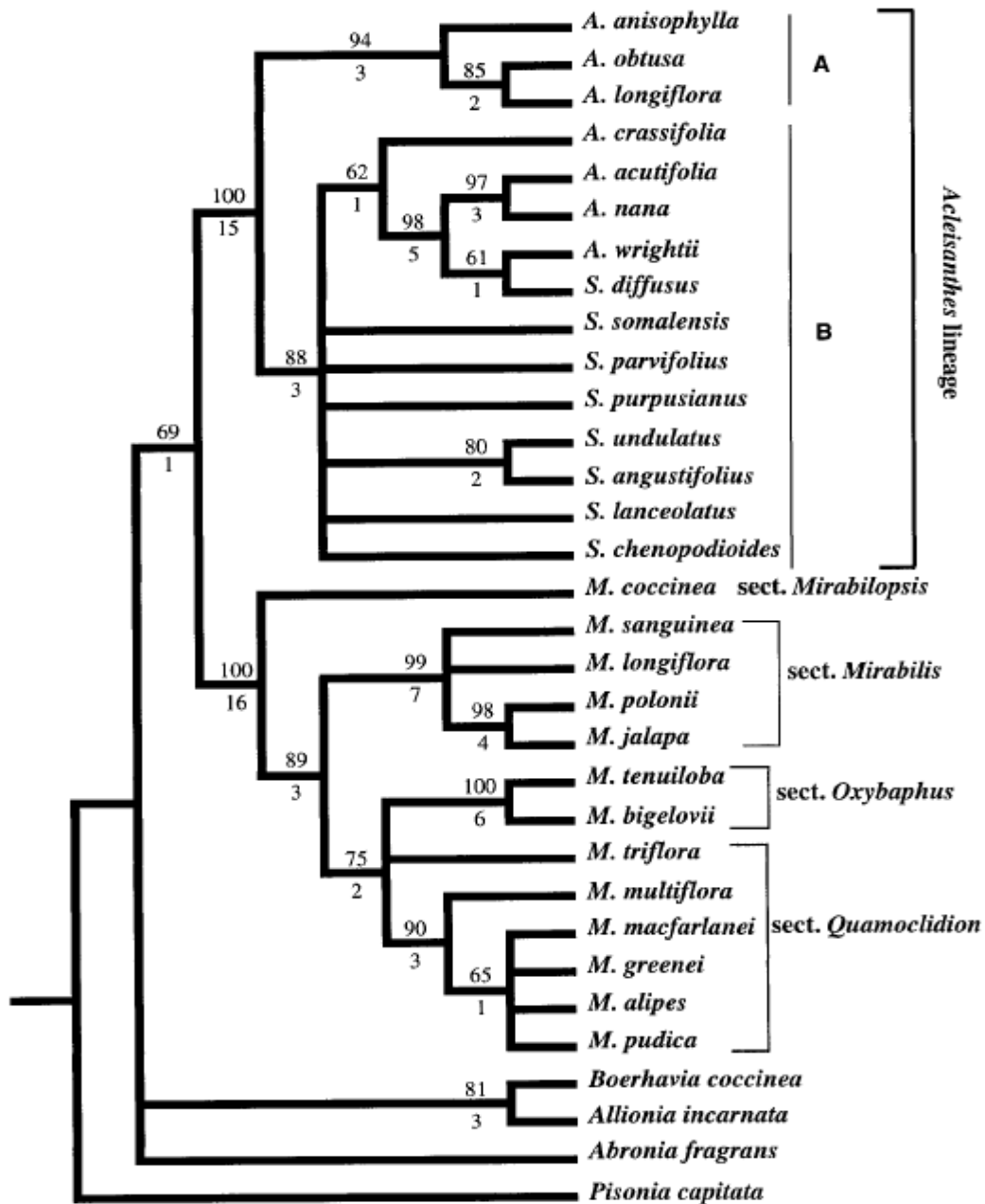
*Figure 2.* The strict consensus of 260 most-parsimonious trees based on the ITS data of sampled *Acleisanthes*, *Selinocarpus,* and *Mirabilis* genera. Bootstrap values above the branches, decay indices below the branches. A and B indicate the *Acleisanthes* A and *Acleisanthes* B clades within the *Acleisanthes* lineage. *Pisonia capitata* S. Watson emerged as the most distant outgroup. Adapted from Levin (2000).

Levin (2000) also found that the molecular analysis of the nuclear and chloroplast sequences verified the monophyly of *Mirabilis*, which is consistent with the morphology. However, the molecular data leaves uncertainties in the phylogenetic arrangement in the sections of the genus *sensu* Bittrich and Kühn (1993).

Levin (2000) published the first work which questioned the warranty of the classification of Nyctaginaceae based on morphological features and the first to indicate that the morphological categorization of species into genera might not correspond to true phylogenetic relationships in this family.

Douglas and Manos (2007), who were interested in the evolution of independent and contingent characters as well as the biogeographic history of the family, conducted a more comprehensive study with 51 species from 25 genera (all had representatives in North America) and reconstructed phylogeny based on three chloroplast loci (*ndhF*, *rps16*, *rpl16*). Outgroups were selected from outside the family: Phytolaccaceae R. Br. and Sarcobataceae Behnke (also belong to Caryophyllales). To test the monophyly of Nyctaginaceae, more distant Aizoaceae Martynov, Molluginaceae Bartl., and Stegnospermataceae Nakai from Caryophyllales were included.

The study found that the subtribal classifications of Nyctaginaceae that was based on pollen morphology and the development of involucre are likely unreliable due to high degree of homoplasy (Fig. 3). In addition to Bougainvilleeae and Nyctagineae, genera *Neea* Ruiz & Pav. and *Guapira* Aubl. (Pisonieae) *sensu* Bittrich and Kühn (1993) appear to be paraphyletic. This was already anticipated by earlier authors and Standley (1931a, p. 73) has even noted that "I know of few groups of plants in which specific differences are so unstable and so baffling . . . particularly in *Neea*, *Torrubia* Vellozo [= *Guapira*] and *Mirablis*, no single character seems to be constant" (as cited in Douglas & Manos, 2007). Additionally, accordingly to the molecular analysis the genera *Pisoniella* stands as a sister taxon to Pisonieae (had previously been included in the tribe by Heimerl (1934), but placed into Colignoniinae by Bittrich and Kühn (1993)) (as cited in Douglas & Manos, 2007).
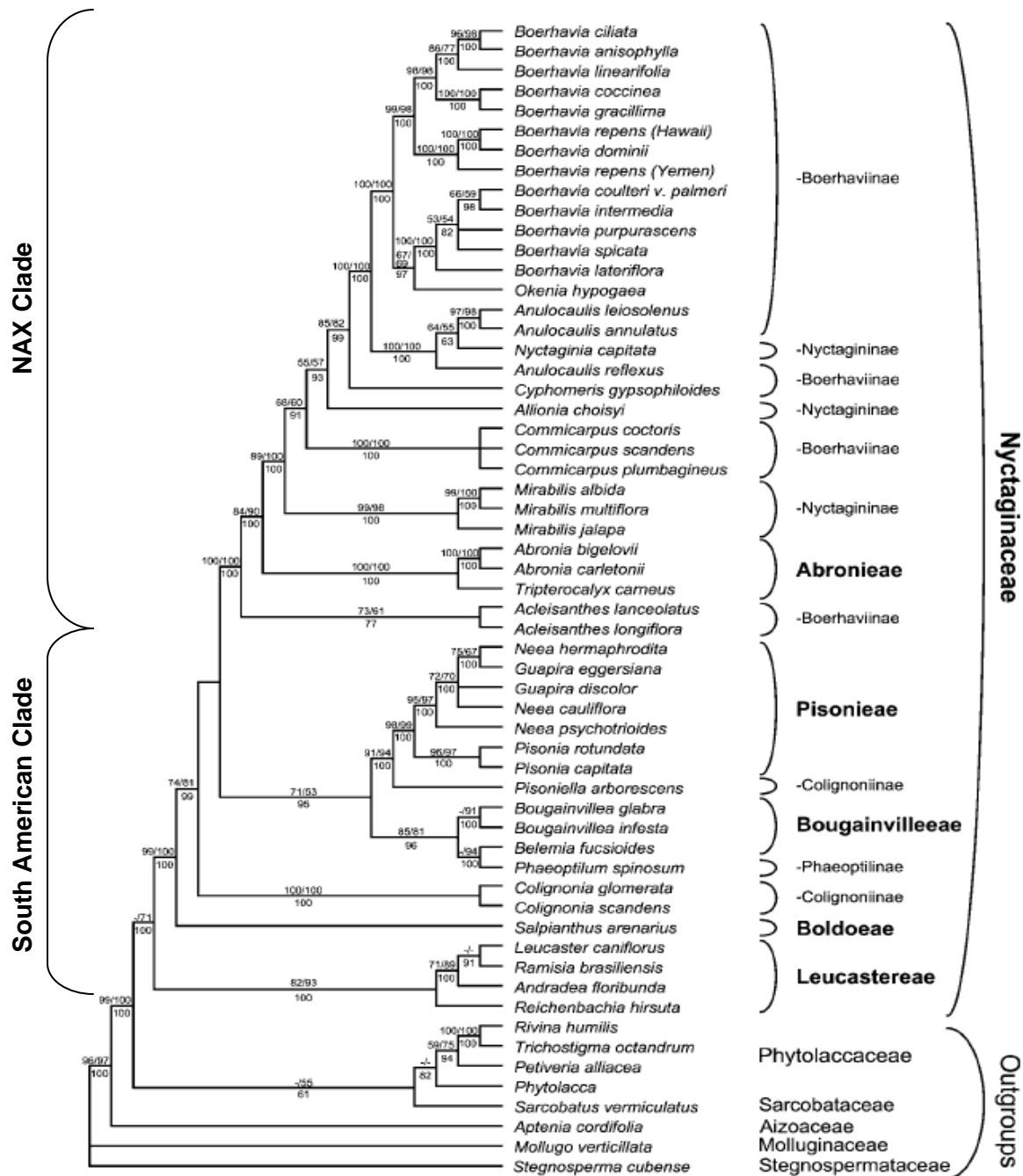
*Figure 3*. Maximum-likelihood topology of Nyctaginaceae. Parsimony bootstrap/maximum likelihood bootstrap support values above branches, Bayesian posterior probability below branches. "-" indicates boostrap support value below 50. Nyctaginaceae tribes *sensu* Bittrich and Kühn (1993) in bold, "-" before unbold name stands for Nyctagineae subtribe. 'NAX Clade' stands for the Northern American Xerophyte Clade. Adapted from Douglas & Manos (2007).

The molecular information from the study made it possible to couple the phylogeny within Nyctagineae with the biogeographical pattern of the family. The most basal groups (Boldoeae, Leucastereae, Colignonia, Bougainvilleeae, and Pisonieae) are all prevalently South American, which is therefore most likely where Nyctaginaceae diverged from (Douglas & Manos, 2007). The only outlier, monospecific genus *Phaeoptilum* (Nyctagineae-Phaeoptilinae), which is endemic to arid Southwestern Africa is most closely related to *Belemia* Pires and *Bougainvillea* (Bougainvilleeae) from South America. The authors suggested wind dispersal as the cause for the disjunction.

The other geographically distinct phylogenetic group appeared to be the North American Xerotypic (NAX) clade (Fig. 3), which included the genera represented in the deserts of the southwestern United States and northwestern Mexico. For example *Commicarpus*, *Boerhavia,* and *Okenia* Schlecht. & Cham.*.* NAX incorporates all the gypsophilic, gypsum-tolerant, and cleistogamous genera described so far. The authors hypothesize that the ancestral state of the NAX clade lineage was most likely also self-compatible, which has thought to be unusual. The parsimony reconstruction of cleistogamous flowers indicated that three derivations from self-compatible to self-incompatible would have been enough to produce such a tree, whereas six transitions from self-incompatible to self-compatible would have be necessary if the ancestor was assumed to be self-incompatible (Douglas & Manos, 2007).

The implementation of molecular analysis in investigating the phylogeny within Nyctaginaceae has created a better understanding about the monophyletic relationships in the family and has proposed considerable changes in the tribal arrangement. Douglas and Spellenberg (2010) reviewed the existing classification based largely on morphology and suggested a rearrangement of the family at the supergeneric level. The following seven tribes were recognized: Leucastereae Benth. & Hook., Boldoeae Heimerl,  Colignonieae Standl., Bougainvilleeae Choisy (synonomous to Bougainvilleaceae J. Agardh), Pisonieae Meisn. (synonym to Pisoniaceae J. Agardh), Nyctagineae Horan. (synonym to: Allioniaceae Horaninow plus Mirabilidaceae W. Oliver), Caribeeae Douglas & Spellenb. (Table 2).

Table 2

*The historical classification schemes of Nyctaginaceae by Heimerl (1934), Bittrich and Kühn (1993) and the most up-to-date system by Douglas and Spellenberg (2010) with a phylogenetic tree.* Adapted from Douglas & Spellenberg (2010).

| Heimerl, 1934 | Bittrich and Kühn, 1993 | Douglas and Spellenberg, 2010 | |
|---|---|---|---|
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Boerhavia* |
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Okenia* |
| Mirabileae-Boerhaviinae | Nyctagineae-Nyctagininae | Nyctagineae | *Nyctaginia* |
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Anutocautis* |
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Cyphomeris* |
| Mirabileae-Boerhaviinae | Nyctagineae-Nyctagininae | Nyctagineae | *Allionia* |
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Commicarpus* |
| Mirabileae-Boerhaviinae | Nyctagineae-Nyctagininae | Nyctagineae | *Mirabilis* |
| Mirabileae-Abroniinae | Abronieae | **Nyctagineae** | *Tripterocalyx* |
| Mirabileae-Abroniinae | Abronieae | **Nyctagineae** | *Abronia* |
| Mirabileae-Boerhaviinae | Nyctagineae-Boerhaviinae | Nyctagineae | *Acleisanthes* |
| Pisonieae | Pisonieae | Pisonieae | *Pisonia* |
| Pisonieae | Pisonieae | Pisonieae | *Neea* |
| Pisonieae | Pisonieae | Pisonieae | *Guapira* |
| Pisonieae | Nyctagineae-Colignoniinae | **Pisonieae** | *Pisoniella* |
| Mirabileae-Bougainvilleinae | Bougainvilleeae | Bougainvilleeae | *Bougainvillea* |
| | Bougainvilleeae | Bougainvilleeae | *Belemia* |
| Mirabileae-Phaeoptilinae | Nyctagineae-Phaeoptilinae | **Bougainvilleeae** | *Phaeoptilum* |
| Colignonieae | Nyctagineae-Colignoniinae | **Colignonieae** | *Colignonia* |
| Boldoeae | Boldoeae | Boldoeae | *Salpianthus* |
| Leucastereae | Leucastereae | Leucastereae | *Leucaster* |
| Leucastereae | Leucastereae | Leucastereae | *Ramisia* |
| Leucastereae | Leucastereae | Leucastereae | *Andradea* |
| Leucastereae | Leucastereae | Leucastereae | *Reicheobachia* |
| | Nyctagineae-Boerhaviinae | **Caribeeae** | *Caribea* |
| Boldoeae | Boldoeae | Boldoeae | *Boldoa* |
| Boldoeae | Boldoeae | Boldoeae | *Cryptocarpus* |
| | Pisonieae | Pisonieae | *Grajalesia* |
| Pisonieae | Pisonieae | Pisonieae | *Cephalotomandra* |
| | Pisonieae | Pisonieae | *Neeopsis* |
| Mirabileae-Boerhaviinae | Nyctagineae-Nyctagininae | Nyctagineae | *Cuscatlania* |

*Notes.* Italicized names represent genera, hyphenated names correspond to tribe-subtribe, bold names indicate changes from Bittrich and Kühn (1993). Phylogenetic tree supported at least 70% in parsimony or likelihood bootstrap or at least 90% bayesian posterior probability.

Colignonieae was re-established, containing a single genus *Colignonia.* A separate monotypic Caribeeae was created for the genus *Caribea* Alain*,* which was removed from Nyctagineae, but was not placed in the phylogeny (thus the dashed line not connected to the phylogenetic tree next to Table 2). Abronieae was lost and both of the genera that it previously covered were placed into the Nyctagineae. However, no subtribes were recognized in Nyctagineae, which was based on the NAX clade of Douglas and Manos (2007). A number of rearrangements on generic level were made as well. However the placement of six genera to the phylogenetic tree is still unclear due to historical taxonomic confusion (*Boldoa*, *Cryptocarpus*) and exclusion from sampling (*Grajalesia*, *Cephalotomandra* H. Karts. & Triana, *Neeopsis* Lundell, and *Cuscatlania* Standl.) (Douglas & Spellenberg, 2010), therefore the tribal status of those genera was not changed compared to the system of Bittrich and Kühn (1993).

## 1.3 The position of Nyctaginaceae in Caryophyllales

The position of Nyctaginaceae in the core group of order Caryophyllales has been well established already by Braun (1864) and Eichler (1876) in terms of the free-central placentation and embryology (as cited in Brockington et al., 2009). The core group, Caryophyllales *sensu stricto* (*s.str.*) is also referred to as Centrospermae due to the central position of the seed. It is a group of families which were identified as closely related by morphological characteristics, however since the availability of genetic analysis, other families that appear to be phylogenetically close have been added to the group (Cuénoud et al., 2002). Caryophyllales *sensu lato* includes families that were recognized as more distantly related to the core group in the same order (Cuénoud et al., 2002).

The morphological features characteristic to Caryophyllales *s.str.* are free-central placentation, presence of perisperm, curved embryos, and p-type type plastids in sieve tube elements (contain globular protein crystals) (Douglas & Manos, 2007); in many families betalain pigments occur, whereas the rest of the flowering plants produce anthocyanins (Cuénoud et al., 2002). Bittrich (1993) indicates Nyctaginaceae as one of the families in the core group which does not have infallible synapomorphies, therefore the correct phylogenetic relationships and placement of the family before the availability

of molecular analysis was under question (as cited in Cuénoud et al., 2002). By now it is widely recognized that Nyctaginaceae is closely related to members of Phytolaccaceae R. Br. (Fig. 4) (Rodman et al., 1984), more specifically genus *Rivina* L. (Behnke, 1997; Brockington et al., 2009; Cuénoud et al., 2002; Downie et al., 1997; Downie & Palmer, 1994; Rettig et al., 1992;). Cuénoud et al. (2002) and Brockington et al. (2009) have even suggested that *Rivina* (or the whole subfamily Rivinoidea) is associated more closely with Nytaginaceae than Phytolaccaceae. Phytolaccaceae, like Nyctaginaceae, is common in tropical and warm temperate regions (Watson & Dallwitz, 1992 onwards). However Sarcobataceae Behnke, which is a holarctic-temperate family (Watson & Dallwitz, 1992 onwards), has also been found closely related to Nyctaginaceae and *Rivina* (Behnke, 1997; Brockington, et al, 2009; Downie et al., 1997).
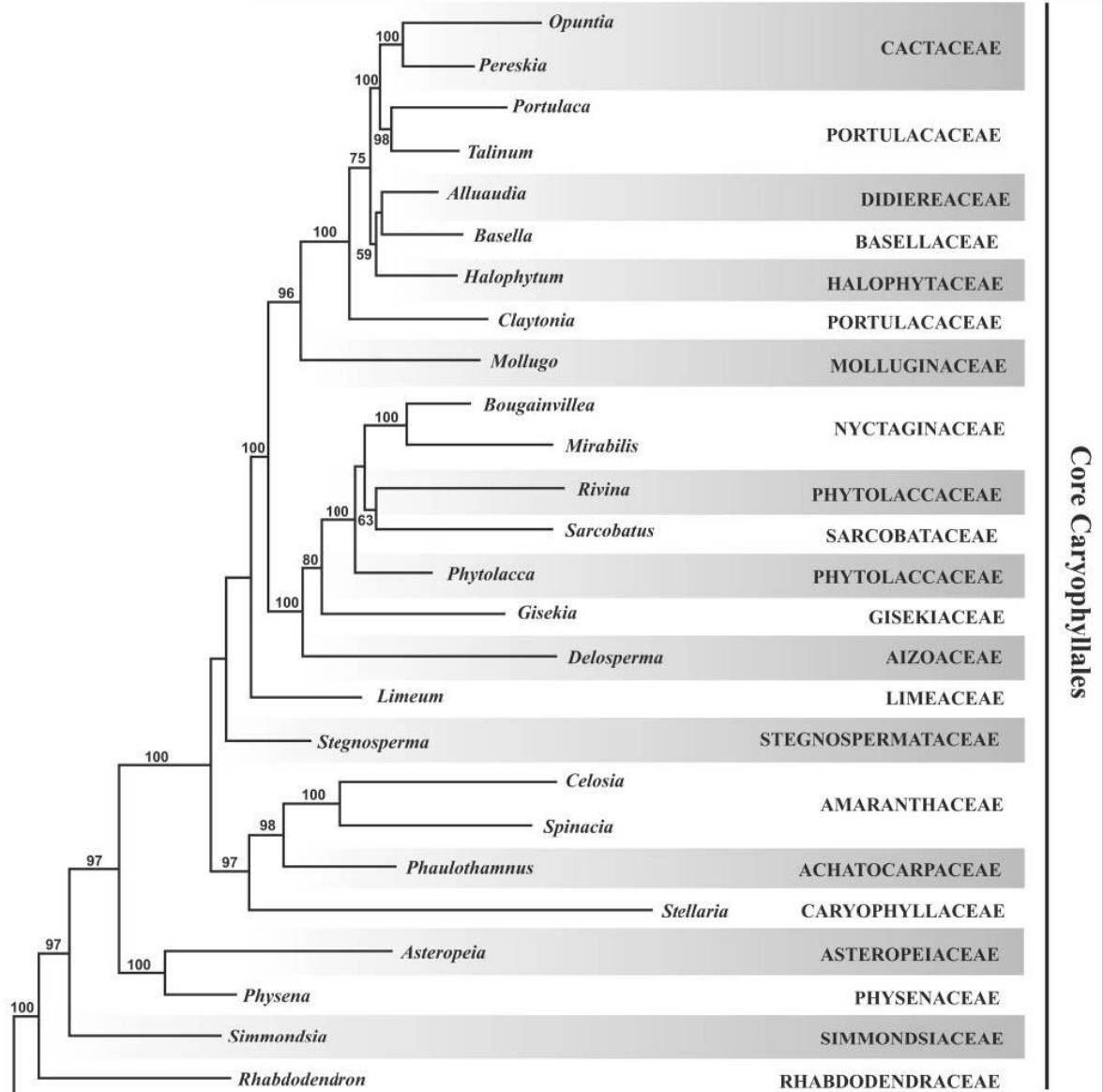
*Figure 4*. Phylogram of a single most parsimonious tree based on eight plastid genes from single copy regions, two nuclear genes, and the plastid inverted repeat for the Caryophyllales *s.str.* . Numbers above branches represent bootstrap values. Nyctaginaceae forms a highly supported monophyletic group, whereas *Rivina* (from a paraphyletic Phytolaccaceae) and *Sarcobatus* (Sarcobataceae) form its sister group. Adapted from Brockington et al. (2009).

# 2. Mycorrhizal associations

## 2.1 Overview

Most vascular plants are capable of associating with fungi to form mycorrhiza, which then becomes responsible for the nutrient uptake from the soil (Smith & Read, 1997). Most of the time, mycorrhiza is understood as a mutualistic symbiosis with a bidirectional transfer of nutrients (Smith & Read, 1997). The host plant supplies the fungus with carbon, whereas the mycobiont provides a number of benefits to the host including improved mineral and water uptake (therefore prevention from wilting and increase of seedling survival) (Janos, 1980) and under some conditions resistance to pathogenic microorganisms (Smith & Read, 1997). This results with the faster growth of the host plant, which is advantageous in relation to non-mycorrhizal plants. The formation of mycorrhiza, however, is dependent on factors such as the presence of the fungal component in the soil (Molina et al., 1992), availability of an exogenous carbon source in the soil (Duddridge, 1986a,b), successional stage of the plant and the ecosystem (Smith & Read, 1997), etc. Many taxa are able to grow with or without mycorrhiza.

Mycorrhizal associations are divided into two main categories: arbuscular mycorrhiza (endomycorrhiza in earlier literature) and ectomycorrhiza, based on the character of the hyphae to penetrate into the host's cells (Smith & Read, 1997). A number of other mycorrhizal types occur as well, but those are not of the interest of this work as they are only restricted to specific groups of vascular plants, and therefore will not be discussed in here.

Arbuscular mychorrizal relationships are established between plants and glomeromycetous fungi (phylum Glomeromycota C. Walker & A. Schuessler, previously included in Zygomycota C. Moreau), which acquire carbon from the host and are not able to grow without an association with a plant. The functional components of arbuscular mycorrhiza are the intracellular structures in the root cortex, such as arbuscules and/or vesicles, and the extending mycelium in the soil. The plant roots will not normally become covered with a mantle and therefore the determination of the

mycorrhizal status of the plant is complicated and only possible with staining methods (Smith & Read, 1997).

The arbuscular mycorrhizal fungal phylum Glomeromycota is established as a monophyletic lineage (Brundrett, 2002). The origin of arbuscular mycorrhiza has been hypothesized to have coincided with the emergence of vascular land plants (Tracheophyta) and the fossil evidence has confirmed that the ability of plants, which initially lacked true roots, to inhabit a terrestrial environment was facilitated by symbiotic hyphal fungi (Wang & Qiu, 2006). Arbuscular mycorrhiza might have been a solution for the extreme change of nutritional availability in terrestrial conditions (Wang & Qiu, 2006). There is fossil evidence of arbuscular mycorrhiza-like structures from as early as 400 million years ago (Remy et al., 1994; Taylor et al., 2005), whereas the evidence of the first land plants is only from 50 million years earlier. Given this information, arbuscular mycorrhiza is proposed as the ancestral type of plant-fungus partnership (Wang & Qiu, 2006) and it has been agreed upon as the basal condition of all main vascular plant groups (Brundrett, 2009). Therefore, the taxa which presently do not form arbuscular mycorrhiza must have lost the ability after the evolutionary divergence of Tracheophyta (Cairney, 2000). Presently, the number of families forming arbuscular mycorrhiza vastly outnumbers families with other types of mycorrhizal relationships (67.4%; Fig. 5) (Brundrett, 2009), whereas the number of arbuscular mycorrhizal fungi is comparably low: ca. 150 species (Cairney, 2000). There exists a low level of specificity between the host and the mycobiont (Smith & Read, 1997).
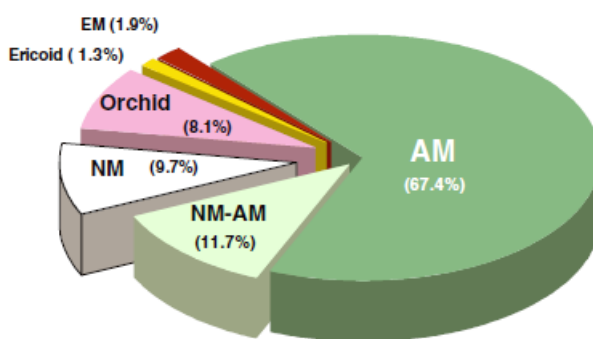


*Figure 5*. The relative diversity of mycorrhizal associations of extant vascular plant families. AM – arbuscular mycorrhiza; NM-AM – non-mycorrhiza, facultative arbuscular mycorrhiza; NM – non-mycorrhiza; Orchid – orchidoid mycorrhiza; Ericoid – ericoid mycorrhiza; EM – ectomycorrhiza. Adapted from Brundrett (2009).

Ectomycorrhiza, which occurs in a much smaller percentage of plant families, on the other hand, is facilitated by fungi from different lineages: Basidiomycota, Zygomycota and Ascomycota (Berk. 1857) Caval.-Sm. (Brundrett, 2002). The distinct characteristics of ectomycorrhiza are the fungal mantle or sheath on the host roots, the elaborate hyphal labyrinth between the host plant's epidermal and cortical cells called the Hartig net, and the hyphal structures radiating out from the root forming connections with fungus fruit bodies (Smith & Read, 1997). Intracellular penetrations are typically absent, however these can occur in the case of nutritional imbalance or root senescence (Smith & Read, 1997). There are quite significant structural variations in the formations that are still considered ectomycorrhiza: neither Hartig net nor the fungal mantle exclusively define ectomycorrhiza. For example, even though the mycorrhizal structures of *Pisonia grandis* (Pisonieae) lack a Hartig net, it is still classified as ectomycorrhiza.

The first evidence of ectomycorrhizal plant-fungus associations date back only 50 million years (Lepage et al., 1997). Due to the distribution of ectomycorrhizal representatives among basidiomycetous, zygomycetous and ascomycetous fungi it is proposed to have developed independently multiple times (Brundrett, 2009). LoBuglio et al. (1996) has shown that ascomycetes solely have four or more ectomycorrhizal origins, which evidently makes ectomycorrhizal fungi a polyphyletic group. It has been proposed that the evolution of ectomycorrhiza might have been a response to changes in the climate in Late Cretaceous (ca. 145-66 million years ago), which became more seasonal and arid (Malloch et al. 1980) and changed the soil composition (Smith & Read, 1997).

The relatively small proportion of ectomycorrhizal families (1.9%) among all vascular plants compared to arbuscular mycorrhizal or even non-mycorrhizal plant families is outweighed by their global economic importance. Ectomycorrhizal Pinaceae, Fagaceae, Dipterocarpaceae, and Myrtaceae, which take up a significant area of terrestrial land surface, are the main producers of timber (Smith & Read, 1997).

The total number of ectomycorrhizal mycobionts is estimated to be approximately 5,000-6,000 however this probably represents a conservative approach (Cairney, 2000). Surprisingly, the ectomycorrhizal fungal community richness is lower in tropics than in the temperate regions, which is opposite in the case with the host plant diversity

(Tedersoo & Nara, 2010). There exists some specificity between ectomycorrhizal fungi and host plants, however no evidence of sustained coevolution is present (Cairney, 2000). Most ectomycorrhizal mycobionts have broad host ranges and world-wide distributions (Smith & Read, 1997). However, molecular studies have showed that morphology based species often include cryptic taxa which haven't world-wide distribution (e.g. Carriconde et al., 2008). A single host might be infected by a significant number of fungi at the same time. For example, Bahram et al. (2010) identified 122 species of ectomycorrhizal fungi on a single European aspen (*Populus tremula* L.) in an old-growth boreal mixed forest ecosystem in Estonia and estimated the total species richness from 182 to 207 species. Some hosts (representatives of Salicaceae) are able to form associations with both ectomycorrhizal and arbuscular mycorrhizal fungi depending on the local soil conditions (Cairney, 2000).

## 2.2 Mycorrhizal taxa of Nyctaginaceae

Only a small number of mycorrhizal taxa have been identified in Nyctaginaceae (Ashford & Allaway 1982, 1985; Haug et al., 2005; Becerra et al., 2007; Sigüenza et al., 1996; Suvi et. al. 2010), therefore there is no strict consensus about its mycorrhizal status in the literature. Janos (1980) and Haug et al. (2005) among other authors have characterized it as a non-mycorrhizal family with a few isolated mycorrhizal taxa, whereas Brundrett (2009) and Wang and Qiu (2006) have considered the amount of arbuscular and ectomycorrhiza-forming taxa significant enough to classify the family as mycorrhizal. The notion of non-mycorrhizal lifestyle as prevalent within Nycatginaceae might be a misunderstanding caused by lack of informative sampling and should be considered with precaution.

Koske et al. (1992) identified *Boerhavia repens* L., *Bougainvillea spectahilis* Willd., *Mirabilis jalapa*, *Pisonia umbellifera* (G. Forster) Seem. as non-mycorrhizal in Hawaii. They also noted that vesicles and hyphae of arbuscular mycorrhiza forming fungi were found on *Boerhavia repens*, however did not consider it infected because no arbuscules were detected. *Boerhavia diffusa* L. and *Mirabilis jalapa* L. have been observed as not forming arbuscular mycorrhiza in Southern India (Muthukumar & Udaiyan, 2000). Nonetheless, there are still only a few comprehensive studies of the mycorrhizal status

of the majority of Nyctaginaceae species. It is very likely that if more research is performed on various species in different nutritional conditions, more taxa could be discovered to be able to form mycorrhizal associations.

Wang and Qiu (2006) give an overview of 263 land plant families, 21 of which are categorized as non-mycorrhizal and 242 as mycorrhizal, including Nyctaginaceae. They report the occurrence of arbuscular and ectomycorrhiza in three genera: *Guapira, Neea,* and *Pisonia* (Pisonieae). In addition, other authors have identified arbuscular mycorrhizal associations in two genera: *Abronia* (Nyctagineae) (Sigüenza et al., 1996) and *Colignonia* (Colignonieae) (Becerra et al., 2009).

### 2.2.1 Arbuscular mycorrhiza

Genus *Abronia*

Sigüenza et al. (1996) assessed the seasonality of arbuscular mycorrhiza populations in the coastal sand dunes in Baja California, Mexico. Their samples included *Abronia maritima* Nutt. Ex Wats. and *A. umbellata* Lam. Both are herb species from Nyctagineae. It was found that while *A. maritima*, a pioneer species, showed very low colonization throughout the growing season (one of the 50 plants sampled had a mycorrhizal root formation), *A. umbellata* was colonized by arbuscular mycorrhiza up to 70%, which was most extensive after flowering and fruit maturation. The habitat of *A. maritima* on the sand dunes is subject to burial due to strong winds on the coast and the resulting rapid growth rate could explain the low colonization rate (Black & Tinker, 1979). Therefore it is important to keep in mind that the successional stage of the plant is one factor that the level of mycorrhizal colonization is dependent on (Sigüenza et al., 1996). An additional factor could be the disability of *A. maritima* to reproduce vegetatively (Johnson, 1985), which means that each new plant needs to find a suitable fungal partner and get colonized independently (as cited in Sigüenza et al, 1996). The authors of the study did not identify the mycobionts associated with *Abronia maritima* or *A. umbellata.*

*Colignonia glomerata*

Becerra et al. (2007) investigated the extent of mycorrhizal colonization in the humid subtropical Yungas forests in Argentina. They found arbuscular mycorrhiza on all 42 sampled plant species' roots, including *Colignonia glomerata* Griseb. (Colignonieae). The extent of the *paris*-type arbuscular mycorrhiza in *C. glomerata* was 26-50%, which is fairly significant, however no mycobionts were identified (Becerra et al., 2007).

*Pisonia sechellarum*

Suvi et al. (2010) attempted to identify the mycobionts of two *Pisonia* species in Seychelles archipelago and found that endemic *P. sechellarum* F. Friedmann formed arbuscular mycorrhiza unlike *P. grandis,* which formed ectomycorrhiza.

### 2.2.2 Ectomycorrhiza

Several works have been published that discuss the ectomycorrhizal taxa of Pisonieae: genera *Neea, Guapira,* and *Pisonia* (Ashford & Allaway, 1982; Smith & Read, 1997; St John, 1980). Not all the species of these genera have been found to form ectomycorrhizal associations, however, as indicated previously, more sampling and information is needed about the mycorrhizal status in various conditions to get a more accurate picture of the situation.

Genus *Neea*

Few works of the mycorrhizal status of specific *Neea* species have been published however there are data about the ectomycorrhizal associations of this genus in general (St John, 1980; Alexander & Högberg, 1986; Tedersoo et al., 2010b). Molina et al. (1992) indicates that Janos (1985) has reported a white ectomycorrhiza on *Neea lactivirens* in Costa Rica. Alexander and Högberg observed normal Hartig net in 90% of the root sections of a *Neea* sp. No extraordinary structures were noted under light microscope. Tedersoo et al. (2010) found 11 fungal species associated with the roots of 15 species of *Neea* in Western Amazonia, whereas these belonged to four different evolutionary lineages, which does not support the proposed hypothesis of mycobiont

specificity among Nyctaginaceae (Haug et al., 2005; Suvi et al., 2010). The species-level identification of *Neea* was not carried out, though.

### Genus *Guapira*

Haug et al. (2005) refer to Moyersoen (1993) and Béreau et al. (1997) who have published data about arbuscular and ectomycorrhizal status of *Guapira sancarlosiana* Steyerm. However, it is also noted that they never found arbuscules nor vesicles, but only a low percentage of unpartitioned hyphae.

### Genus *Pisonia*

There are a number of ectomycorrhizal members of *Pisonia*: *P. fragrans* Dum.-Cours, *P. grandis* R. Br., *P. subcordata* Sw., and *P. zapallo* Griseb (Suvi et al., 2010). The best-studied mycorrhizal species of Nyctaginaceae is doubtlessly *P. grandis.*

## 2.2.3 Phylogeny of the mycorrhiza-forming taxa

Currently there are fewer genera where non-mycorrhizal representatives have been identified compared to genera with a mycorrhizal status in Nyctaginaceae. Completely non-mycorrhizal species have been recorded from *Boerhavia, Bougainvillea, Mirabilis,* and *Pisonia (*Koske et al., 1992), non-arbuscular mycorrhizal species from *Boerhavia* and *Mirabilis* (Muthukumar & Udaiyan, 2000). Arbuscular mycorrhizal associations have been identified in *Abronia*, *Colignonia*, and *Pisonia (*Becerra et al., 2007; Sigüenza et al., 1996; Suvi et al., 2010), which do not position close on the phylogenetic tree of Nyctaginaceae (Fig. 6). Ectomycorrhizal associations occur in *Guapira*, *Neea,* and *Pisonia* (Ashford & Allaway, 1982; Smith & Read, 1997; St John, 1980), which all belong to Pisonieae and thus position close on the phylogenetic tree*.*

The governing consensus is that the arbuscular mycorrhizal state is basal for all land plants (Brundrett, 2009). Therefore, the non-arbuscular and non-mycorrhizal genera of Nyctaginaceae must have lost the ability during their divergence. It is very likely that Nyctaginaceae diverged from its ancestor as an arbuscular mycorrhiza-forming group

however some of the emerged lineages have lost the ability whereas some have maintained it. It is highly unlikely that any of the currently arbuscular ectomycorrhiza-forming genera have regained the adaptation after losing it earlier.

The situation with ectomycorrhiza-forming ability is somewhat different. On the one hand, it is probable that the adaptation was obtained only once in Nyctaginaceae, more specifically Pisonieae lineage. On the other hand, no information about the mycorrhizal status of *Pisoniella* (appears as a sister taxon to the clade conjoining *Pisonia, Neea* and *Guapira,* where ectomycorrhiza has been recorded), *Grajalesia, Cephalotomandra,* and *Neeopsis* (which have not been excluded from Pisonieae *sensu* Douglas and Spellenberg due to lack of sampling) is available and therefore it is impossible to give any credible hypothesis about phylogenetic level where the ectomycorrhiza-forming adaptation was most likely to occur. Whether the ability to form arbuscular mycorrhiza was completely lost or not is also not clear, because *P. sechellarum* (and hypothetically other uninvestigated species) has been recorded with arbuscular mycorrhiza (Suvi et al., 2010).

The diversity of mycobionts associated with Pisonieae ectomycorrhizal species needs further investigation however the majority show little specification (Haug et al., 2005; Suvi et al., 2010; Tedersoo et al., 2010b). Exceptionally, *P. grandis* exhibits a remarkable specification and unique mycorrhizal features termed as pisonioid mycorrhiza (Imhof, 2009). Hayward and Horton (2012) indicate that it is not simple to deduce the level of specification of the most recent ancestor forming ectomycorrhizal relationships from this information. If the restricted range would be basal we should witness the feature in more current taxa. However, the ability to form mycorrhizal associations with a broad range of fungi might represent derived generalism because the selection pressure favors generalism in mutualistic systems (Hayward & Horton, 2012).
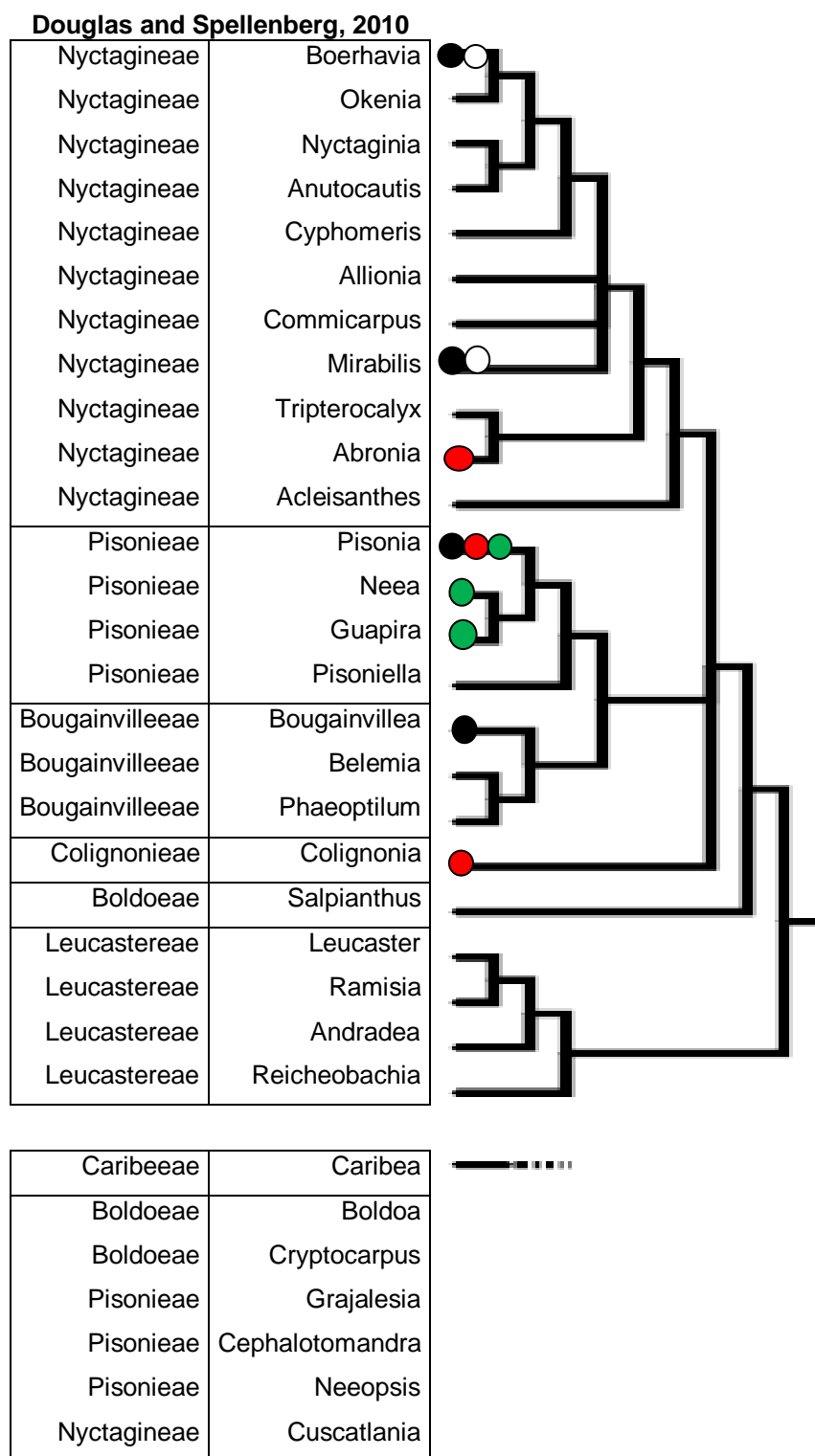
*Figure 6.* Nyctaginaceae *sensu* Douglas and Spellenberg (2010). Black circles - genera where non-mycorrhizal species have been identified; white circles - genera where non-arbuscular mycorrhizal species have been identified; red circles - genera where arbuscular mycorrhizal species have been identified; green circles - genera where ectomycorrhizal species have been identified. Adapted from Douglas and Spellenberg (2010).

# 3. Pisonia grandis - ecology and mycobionts

## 3.1 Ecology

*Pisonia grandis* is a flowering tree which has an untypical range of distribution compared to the majority of Nyctaginaceae genera. In addition to Southern America, it is common on tropical "bird-islands" in the Pacific, Atlantic, and Indian Ocean (Suvi et al., 2010), whereas its unusual dispersal has been correlated with multiple factors.

Firstly, the anthocarpic fruits of *P. grandis* are adhesive (Fig. 7a) and stick to seabirds' feathers and feet (Fig. 7b) (Burger, 2005; Suvi et al., 2010), which facilitates its dispersal to islands with highly nutritious coral soils where phosphorus and nitrogen are high due to bird guano, such as the Great Barrier Reef in Australia and Seychelles in the Indian Ocean (Ashford & Allaway, 1982, 1985). Even though birds do not feed on the fruits of *Pisonia,* they come into contact with them on the trees (e.g. Black Noddies (*Anous minutes* Boie) who nest and roost on the branches of *P. grandis* in Great Barrier Reef) or on the ground (e.g. Wedge-tailed Shearwaters (*Puffinus pacificus* Gmelin) who nest in burrows under *P. grandis* in the Great Barrier Reef) (Walker, 1991). It has been noted that during prolific years when *P. grandis* fruits are abundant, birds can get trapped in the tenacious clusters of fruit, which prevent them from flying and therefore kill them (Burger, 2005; Ogden, 1993; Walker, 1991). In April 1984 45 dead or dying Black Noddies were witnessed entangled in the area of 75 m² on Lady Musgrave Island in the Great Barrier Reef (Walker, 1991). Sixteen bird species in total were observed in contact with *P. grandis* anthocarps in Great Barrier Reef however the range of *P. grandis* was mostly correlated with the range of Black Noddies and/or Wedge-tailed Shearwaters (Walker, 1991). Due to the close associations with seabirds, *P. grandis* is sometimes called the Catchbirdtree.
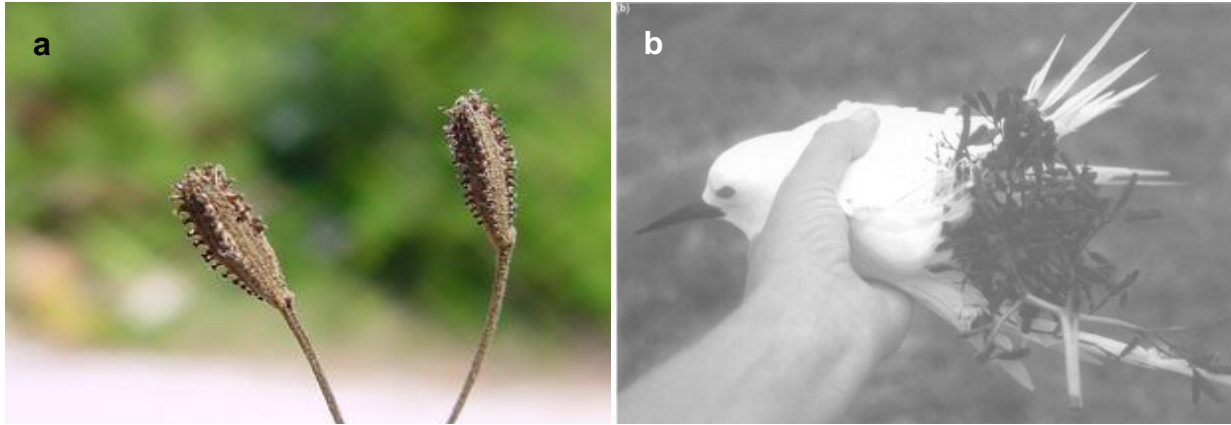
*Figure 7*. a.*Pisonia aculeata* L. mature anthocarps (by B. Hattaway, 2011). b. White tern *Gygis alba* Sparrman entangled in fruit cluster of *Pisonia grandis.* Adapted from Burger (2005).

It has been hypothesized (Burger, 2005; Walker, 1991) that dead animal matter could be necessary for the germination of *P. grandis* seeds and indirectly killing insects, birds and other animals, who get entangled in the fruit clusters, could be an adaptation to ensure the presence of the phosphorous and nitrogen rich nutrients, however it contradicts with the benefits of long-distance dispersal to guano habitats via viable bird vectors (Burger, 2005) and the ability of the seeds to germinate successfully without decaying animal matter or guano (Walker, 1991).

Secondly, in addition to untypical range of distribution, the majority of sampled *P. grandis* trees exhibit a unique type of ectomycorrhiza (Ashford & Allaway, 1982, 1985; Chambers et al., 1998, 2005; Suvi et al., 2010) with only a selected range of mycobionts (Hayward & Horton, 2012). The mycorrhizal associations might play a role in establishing a dominant population of the tree on guano soils. The characteristics of the ectomycorrhiza of *P. grandis* are transfer cells (Fig. 10) – epidermal and cortical cells with extensive wall-ingrowths in the host plant roots – which develop in the contact-sites with the fungal hyphae and are the sites of bidirectional nutrient transport between the myco- and phytobiont (Ashford & Allaway, 1982, 1985). Incomplete septa of fungal hyphae occur in close contact with transfer cells for more efficient nutrient exchange (Allaway et al., 1985). Hartig net, which is a characteristic of ectomycorrhiza (Smith & Read, 1997) and normally discharge the same function as the transfer cells, is absent or underdeveloped (Ashford & Allaway, 1982, 1985; Suvi et al., 2010). Granules high of

phosphorus and calcium occur occasionally in fungal mantle tissues (Ashford & Allaway, 1982; Cairney et al., 1994). This type of ectomycorrhiza has been termed as "pisonioid" due to its exceptional features (Imhof, 2009).
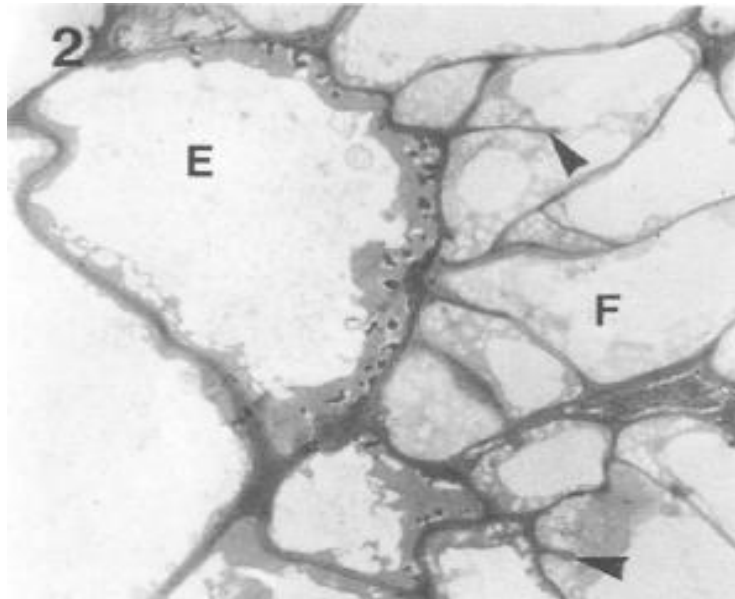


*Figure 8.* Electron micrograph of *P. grandis* mycorrhiza in a transverse section. The cell wall ingrowths are located only at the outer side of phytobiont epidermal cells facing fungal hyphae. 'E' – epidermal transfer cell; 'F' – fungal sheath hyphae; arrowheads indicate the incomplete septa which increase the surface area of the mycobiont. Adapted from Allaway et al. (1985).

Transfer cells are not unique to pisonioid mycorrhiza – these structures also occur among other plants and are associated with nutrient exchange. Transfer cell-like formations appear in monotropoid mycorrhiza, however the wall ingrowths develop around a fungal peg, a single hypha of the fungus that has penetrated through the plant cell wall (but not the plasma membrane). The peg typically originates from a well developed Hartig net. Like transfer cells of pisonioid mycorrhiza, these are the sites of bidirectional substance transfer, however no fungal pegs have been recorded in *P. grandis* root cells (Ashford & Allaway, 1982, 1985; Smith & Read, 1997).

The functional ectomycorrhizal structures are present only in the lateral root tips (Ashford & Allaway, 1982, 1985; Cairney et al., 1994). The ectomycorrhizal relationships between a specific *P. grandis* root and the mycobiont seem to be relatively short-lived and the fungal mantle does not provide protection against other soil

organisms (Ashford & Allaway, 1982, 1985). The nutrient exchange surface area created by transfer cells is less than in the case of a typical ectomycorrhizal Hartig net (Allaway et al., 1985).

The conventional importance of ectomycorrhiza in obtaining phosphorous and nitrogen in nutrient poor soils is paradoxical in the case with *P. grandis* (Chambers et al., 1998). Ectomycorrhizal associations are untypical for the nutrient rich tropical soils (Walker, 1991) and no assistance of nutrient pick-up should be necessary in coral and guano soils where phosphorous and nitrogen are abundant (Ashford & Allaway, 1985; Allaway et. al., 1985; Hayward & Horton, 2012). Even though nonmycorrhizal specimens of *P. grandis* have also been recorded in nature (Walker, 1991) and successfully cultivated in artificial conditions (Hayward & Horton, 2012), pisonioid mycorrhiza occurs with a significant frequency both on guano-rich and guano-poor soils (Ashord & Allaway, 1985; Hayward & Horton, 2012).

Walker (1991) proposed that the formation of pisonioid mycorrhiza could provide an advantage in gaining dominance in the competition for light. Mature *P. grandis* forests are shady and lack any substantial undergrowth and other trees (Walker, 1991). Chambers et al. (1998) hypothesized that the mycobiont might be necessary to maintain more or less constant nitrogen supply to the host during the transient availability of guano, which is variable due to bird migration and rainfall. However, none of these explain the short-lifetime of the mycorrhizal associations of *P. grandis* roots.

Ashford and Allaway (1985) found that those *P. grandis* trees which grew on soil derived from granite instead of coral and guano on Cousin Island in Seychelles formed transfer cells substantially less frequently and did not develop pisonioid mycorrhiza, but were still abundant. At the same time, there was only one site (Cousin Island) where mycorrhizal trees outnumbered non-mycorrhizal trees in the sample. In contrast, Hayward and Horton (2012) examined *P. grandis* trees for ectomycorrhiza on guano-rich and guano-poor soils on Rota, in The Commonwealth of the Northern Mariana Islands, and found that all sampled trees had ectomycorrhizal root tips. This information suggests that the soil conditions might affect the formation of pisonioid mycorrhiza. The ectomycorrhizal formations on *P. grandis* roots are probably most significantly affected by the distribution and survival of its symbiotic fungi in typical guano habitats (Walker,

1991). Seabirds are likely to be a dispersal vector for *P. grandis* mycobionts either by carrying the already infected seeds or fungal spores (Walker 1991). The limited range of mycobionts could result from restricted survival of fungi in phosphorous and nitrogen rich soils (Suvi et al., 2010).

There are only six fungal associates, which have been recorded to be able to facilitate the formation of pisonioid mycorrhiza on *P. grandis*, whereas the range of mycobionts does not expand in non-guano environments where edaphic factors should not limit the range of mycobionts. This indicates that the relationship between *P. grandis* and its mycobionts is highly specific. Therefore, genetic factors rather than the soil conditions limit the range of fungi capable of associating with *P. grandis* (Hayward & Horton, 2012).

The narrow range of mycobionts and unique features of the pisonioid mycorrhiza of *P. grandis* raise doubts about the classification of the relationship under ectomycorrhiza. It could be the case that it is a different type of mycorrhizal association. Moreover, the fact that *P. grandis* can grow as successfully without an association with a fungus (the majority of the sampled trees were non-mycorrhizal in Seychelles (Ashford & Allaway, 1985)) and the absence of Hartig net could indicate that the relationship could be more beneficial for the fungus than for *P. grandis* and therefore it could incline towards parasitism. Kõljalg (1992) described mycorrhizal association between *Pinus sylvestris* L. and *Tomentella crinalis* Fr. synthesized under sterile conditions. Based on descriptions the Hartig net was also not well-developed or missing. This demonstrates that some *Tomentella* species may form relationships with plant roots, which are not equally beneficial for both participants. However, this hypothesis should be verified in future studies.

## 3.2 Mycobionts

When Ashford and Allaway (1982, 1985) first characterized the distinct mycorrhiza of *P. grandis* in Australia and on the Seychelles, they found two differently pigmented fungal sheaths, brown and black, on different individuals of *P. grandis.* These had only slight differences in structure. The brown mycorrhiza had well-differentiated layers of

hyphae in the fungal mantle, which penetrated in between the epidermal cells (not into the cortex). In contrast, the black mycorrhiza exhibited empty and damaged hyphae and gradually lighter pigmentation of the layers (the outermost layer the darkest). There were more fungal hyphae radiating from the black sheath surface, the brown mantle was relatively smooth. The authors suggested that the coloration and morphological difference could be caused by consecutive stages of mycorrhizal development of the same fungal species rather than different mycobionts. The darker pigmentation and hairier appearance was attributed to an older plant-fungus association. The degenerated black mantle was supposedly an indicator that the plant-fungus relationship was relatively short-lived (Ashford & Allaway, 1982, 1985).

The first successful isolate of any mycobionts of *P. grandis* was not obtained before 1998 (Chambers et al.) due to difficulties of purifying the fungus from the mycorrhizal associations to an axenic culture. Cuttings of infected roots were collected from Heron Island of the Great Barrier Reef in Australia, but only one attempt yielded a positive sample in the experiment. Therefore it was certain that a single fungal strain (UNSW 014) was extracted. The isolate was used to re-infect *P. grandis* seedlings in sterilized soil however it resulted with a black rather than an expectedly brown mycorrhiza, which was previously considered as a preceding stage of pisonioid mycorrhizal infection. Otherwise it exhibited typical pisonioid mycorrhizal features. Therefore it raised doubts about one mycobint exhibiting both brown and black morphotypes (Chambers et al., 1998).

The same isolate was used to sequence the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genes for taxonomic purposes (Chambers et al., 1998). The nuclear rRNA sequences are the most commonly used molecular markers used for fungal taxonomy and phylogeny (Schoch et al., 2011). The structure of the eukaryotic rRNA cistron consists of 18S, ITS1, 5.8S, ITS2, 28S genes, whereas the posttranslationally removed fragment of ITS1, 5.8S, and ITS2 is referred as the ITS region (Schoch et al., 2011). It has been widely used as near-species-level identification marker because it has multiple advantages, such as highly conserved sites (necessary for primers) next to highly variable regions and multiple copies in the genome (Peay et al., 2008). In addition, there have accumulated many sequences available for

comparisons and it has been determined that approximately 97% sequence similarity correlates with morphologically defined fungal species (Peay et al., 2008). Schoch et al. have shown that compared to the 28S nuclear large subunit rRNA gene (LSU; used for yeasts), 18S nuclear ribosomal small subunit rRNA gene (SSU; commonly used in phylogenetics), and the largest subunit of RNA polymerase II (*RPBI*; a protein coding gene also used in fungal taxonomy studies) ITS shows the broadest reliability of species identification among fungal taxa and high amplification and sequencing success.

The complete ITS sequence of the UNSW 014 isolate, 673 nucleotides long, was submitted to GenBank Nucleotide Database (accession number AF020770). GenBank is a database housed by the National Center for Biotechnology Information (NCBI), which is turn in a part of a public sequence library between the European Nucleotide Archive (ENA) and the DNA Data Bank of Japan (DDBJ). The three sequence libraries are comprised into the overarching International Nucleotide Sequence Database (INSD). The comparison of the ITS of UNSW 014 with database sequences showed greatest homology (87%) with an unidentified Thelephoraceae strain (U83467) and an unidentified *Tomentella* sp. (Thelephoraceae) (U83482), which indicate belonging to that family and potentially being a representative of *Tomentella,* however the exact same species had not been sequenced before*.* The next closest similarity occurred with other Thelephoraceae fungi (Chambers et al., 1998).

Thelephoraceae (Basidiomycota) has gained more importance as forming ectomycorrhizal associations with many tree species in temperate, boreal and tropical regions (Chambers et al., 2005; Suvi et al., 2010). Specialized associations have also been recorded between the /tomentella-thelephora lineage and *Guapira* and *Neea* (Pisonieae) species (Haug et al., 2005).

The coral cays of the Great Barrier Reef were investigated for the diversity of *P. grandis* mycobionts by Chambers et al. (2005). Similarly to Ashford and Allaway (1982, 1985) they noted two different morphotypes of mycorrhiza, brown and black, which were not represented evenly among all examined sites. The ITS regions of the mycobionts forming the brown (AY955494) and the black morphotype (AF020770) were sequenced and the data revealed only 80% homology between them. The ITS-RFLP analysis affirmed the discrepancy, however RFLP of the black morphotype matched with the

previous *P. grandis* mycobiont isolate (UNSW 014; hence the same accession number). According to the GenBank database the closest matches for both types were sequences of various Thelephoraceae strains. The brown morphotype aligned closest to uncultured ectomycorrhizal fungi from *Guapira* and *Neea* and the black morphotype showed the greatest homology with *Tomentella* strains. In a neighbor-joining analysis of *P. grandis* mycobionts and Thelephoraceae taxa from GenBank the brown morphotype grouped together with *Tomentella ellisii* Jülich & Stalpers (AF272913) and *Thelephoraceae* sp. from *Guapira* (AY667422) and *Neea* (AY667424), whereas the black morphotype placed as a sister taxa to the /tomentella-thelephora group (Chambers et al., 2005).

Suvi et al. (2010) investigated the mycobionts of 10 *P. grandis* root samples and sporocarps from Seychelles and identified three additional species of its ectomycorrhizal fungi based on the ITS sequence analysis: *Tomentella pisoniae* Suvi & Kõljalg (accession number in ENA: FM244908), *Tomentella* sp. (FN396394), and *Tomentella tedersooi* Suvi & Kõljalg (FM244909). *T. pisoniae* aligned closest with the mycobiont from the Great Barrier Reef forming the brown morphotype (AY955494) and *Tomentella* sp. was found to be a sister taxa of the black mycorrhiza-forming isolate UNSW 014 (AF020770) (Chambers et al., 2005). However the third species, *T. tedersooi*, was found to be close to taxa not associated with Nyctaginaceae (UDB004242). The three mycobionts identified in this work did not indicate sister relations in between each other (Suvi et al., 2010).

Hayward and Horton (2012) characterized ectomycorrhizal associates of *P. grandis* from Rota, Commonwealth of the Northern Mariana Islands. In total they detected three distinct species of mycobionts, two of which (JQ405658; JQ405660) showed 97% and 98% similarity to *T. pisoniae* and *Tomentella* sp. respectively identified from the Seychelles (Suvi et al., 2010). However, the most abundant mycorrhizal associate of *P. grandis* on Rota (JQ405659) had not been previously described, but showed 90% similarity to a Thelephoraceae species.

Currently six species of *P. grandis* mycobionts have been identified, all of which belong to the /tomentella-thelephora lineage. The lineage comprises the paraphyletic *Tomentella* and *Thelephora* as a well-supported group based on 28S rDNA (Tedersoo

et al., 2010a). Tedersoo and Nara (2010) have found that this group is the most species-rich ectomycorrhizal fungal lineages in tropical and temperate regions.

## 3.3 UNITE based mycobiont identifications

UNITE is a database for the molecular identification of fungi which comprises the ITS sequences used for taxonomy. It was initially founded in 2001 (Kõljalg et al., 2005) to create a library of reliable DNA sequences from well defined Northern European ectomycorrhizal herbarium sources, however further developments in response to the needs of the scientific community have led to the acceptance of well annotated samples of all fungal groups from anywhere in the world (Abarenkov et al., 2010a). In addition to the ITS sequences, information about the identification, collector/source, and ecological data is available in UNITE and accessible for all the registered users (Kõljalg, et al., 2005). Recently, other genetic markers relevant for fungi (e.g. nuclear large subunit nLSU/28S gene) have started to be accepted (Abarenkov et al., 2010a).

The UNITE database is maintained in a PlutoF cloud. PlutoF system includes a web based workbench, which allows data submission, retrieval, and analysis of various databases (Abarenkov et al., 2010b). The aim of this service is to create synergy between the data input among the scientific community and therefore make the data univocal. I used PlutoF to acquire information about one of the *Tomentella* species (JQ405658) that Hayward and Horton (2012) identified on *P. grandis* on the Northern Mariana Islands (Fig. 11). The figure indicates 99% ITS sequence similarity with *Tomentella pisonieae* (Suvi et al., 2010) and other samples from Seychelles and Mauritius.

**DSH version 5** | date: 2012-12-18 | DSH cluster code: **SH5_154069**

Total number of sequences in cluster: 14

• chimeric • low quality • UNITE core sequence • Cluster centroid sequence

| Sequence ID | UNITE taxon name | INSD terminal taxon | Country | DNA source | DSH 99% | Clust |
|---|---|---|---|---|---|---|
| JQ405658 | | Thelephoraceae (uncultured Tomentella) | Northern Mariana Islands | | | ---- |
| JX548238 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548234 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548236 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548242 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548235 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548240 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| JX548237 | | Thelephoraceae (uncultured Thelephoraceae) | Northern Mariana Islands | | | ---- |
| UDB002643\|FM244908 Link out | **Tomentella pisoniae** | | Seychelles | Fruitbody | unset ref | ---- |
| UDB002642\|FM244911 Link out | Fungi | | Seychelles | Ectomycorrhiza | | ---- |
| FN396392 | | Fungi (uncultured ectomycorrhizal fungus) | Seychelles | Plant root | | ---- |
| FN185986 | | Tomentella (Tomentella sp TU 103655) | Seychelles | Ectomycorrhiza | | ---- |
| UDB007875 Link out | | | Mauritius | Ectomycorrhiza | | ---- |
| UDB002641\|FM244910 Link out | Fungi | | Seychelles | Ectomycorrhiza | | ---- |

*Figure 9.* PlutoF excerpt of the species cluster based on 99% similarity of the ITS sequences, which includes *Tomentella* species (JQ405658) that Hayward and Horton (2012) identified on *P. grandis* on the Northern Mariana Islands, sequences from the Seychelles and Mauritius. "Sequence ID" – INSD (red) or UNITE (yellow) sequence accession code; "UNITE taxon name" – taxon name in UNITE database; "INSD terminal taxon" – taxon name in INSD database; "Country" – country of origin; "DNA source" – fungal material where the DNA was isolated; "DSH 99%" – species hypothesis with threshold similarity value in percentages.

# Kokkuvõte

Käesolev uurimustöö annab ülevaate sugukonna Nyctaginaceae (e.k. imelillelised) taksonoomiast, fülogeneesist ning mükoriissetest suhetest. Sellele lisaks sisaldab töö liigi *Pisonia grandis* (Nyctaginaceae) ökoloogia ja mükobiontide täpsemat iseloomustust. Töö esimene osa käsitleb sugukonna üldisi tunnuseid, sugukonnasisest klassifikatsiooni, muutusi selles süsteemis pärast molekulaarsete meetodite kasutuselevõttu ja Nyctaginaceae positsiooni taimeriigi klassifikatsioonis. Kuna tegemist on morfoloogiliste tunnuste poolest väga mitmekesise sugukonnaga, oli enne geneetilise analüüsi meetodite rakendamist keeruline usaldusväärset ja laialt toetatud klassifikatsiooni koostada.

Töö teine osa annab üldise ülevaate mükoriisa tüüpidest, levikust ja kujunemisest, imelilleliste mükoriissetest liikidest, mis käesoleva ajani on identifitseeritud, ning mükoriisa moodustamise kohastumuse evolutsioonist imelilleliste sugukonnas. Ebapiisav imelilleliste mükoriisa uurimine on viinud lahkarvamusteni sugukonna iseloomustamisel ning enne põhjapanevate järelduste tegemist on vaja läbi viia laiapõhjalisemaid uuringuid.

Töö viimane osa käsitleb *Pisonia grandis* ökoloogiat, eripärast pisonioidset mükoriisat ning mükobiontide ringi. *Pisonia grandise* ja /tomentella-thelephora grupi seeneliikide vahelist suhet on kirjeldatud ektomükoriisana, kuid nendevahelise mükoriisa väga eripärased tunnused ja imelillelistele ebatavaliselt kitsas mükobiontide ring annavad põhjust arvata, et tegu võib olla kas omaette mükoriisa tüübiga või *Tomentella* liikide parasiitlusega.

# Summary

# Phylogeny and mycorrhizal associations of Nyctaginaceae

**Laura Suur**

This literature review gives an overview of the taxonomy, phylogeny, and mycorrhizal associations of Nyctaginaceae and additionally provides a synopsis of the ecology and mycorrhizal associations of *P. grandis*. The first part covers the general features, intrafamilial classification, changes in the classification system with the implementation of molecular markers, and the position of Nyctaginaceae in the plant kingdom. The high morphological diversity in the family has made it difficult to create a well-supported and reliable classification system. The availability of genetic testing has opened up the possibility to create phylogenies and upgrade the existing classification.

Secondly, features of mycorrhizal associations, the mycorrhizal species of Nyctaginaceae identified so far, and hypotheses about the phylogeny of the mycorrhiza-forming taxa are discussed. The mycorrhizal status of Nyctaginaceae has been determined based on only a small number of informative samplings which elicit that more testing needs to be carried out before any conclusions can be made.

The final part of this study concentrates on the ecology, characteristics of the "pisonioid" mycorrhiza and the mycobionts of *Pisonia grandis*. Even though the plant-fungus relationship of *P. grandis* and its /tomentella-thelephora mycobionts has been determined as ectomycorrhiza, the unusual features and narrow range of fungal associates indicate that it could be another type of mycorrhiza or even an adaptation of the *Tomentella* species to form parasitic associations.

# Acknowledgements

# References

**Articles**

Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010a). The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist*, *186*(2), 281 - 285.

Abarenkov, K., Tedersoo, L., Nilsson, R. H., Vellak, K., Saar, I., Veldre, V., Parmasto, E., Prous, M., Aan, A., Ots, M., Kurina, O., Ostonen, I., Jõgeva, J., Halapuu, S., Põldmaa, K., Toots, M., Truu, J., Larsson, K.-H., & Kõljalg, U. (2010b). PlutoF – a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics*, *6,* 189 - 196.

Alexander, I., J. & Högberg, P. (1986). Ectomycorrhizas ot tropical angiospermous trees. *New Phytologist, 102*, 541-549.

Allaway, W. G., Carpenter, J. L., & Ashford, A. E. (1985). Amplification of Inter-Symbiont Surface by Root Epidermal Transfer Cells in the *Pisonia* Mycorrhiza. *Protoplasma, 128*, 227-231.

Ashford, A. E., & Allaway, W. G. (1982). A sheathing mycorrhiza on Pisonia grandis R. Br. (Nycataginaceae) with development of transfer cells rather than a Hartig net. *New Phytologist, 90*, 511–519.

Ashford, A. E., & Allaway, W. G. (1985). Transfer Cells and Hartig Net in the Root Epidermis of the Sheathing Mycorrhiza of Pisonia grandis R. Br. from Seychelles. *New Phytologist, 100*, 595-612.

Bahram, M., Põlme, S., Kõljalg, U., & Tedersoo, L. (2010). A single European aspen (*Populus tremula*) tree individual may potentially harbor dozens of Cenococcum geophilum ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiol Ecol 75*, 313-320.

Becerra, A., Cabello, M., & Chiarini, F. (2007). Arbuscular mycorrhizal colonization of vascular plants from the Yungas forests, Argentina. *Annals of Forest Science, 64*, 765-772.

Behnke, H. D. (1997). Sarcobataceae—a new family of Caryophyllales. *Taxon, 46*, 495–507.

Black, R., & Tinker, P. B. (1979). The development of endomycorrhizal root systems. II. Effect of agronomic factors and soil conditions on the development of vesicular-arbuscular  mycorrhizal infection in barley and on the endophyte spore density. *New Phytologist, 83*, 401–413.

Brockington, S., Alexandre, R., Ramdial, J., Moore, M., Crawley, S., Dhingra, A., Hilu, K., Soltis, P., & Soltis, D. (2009). Phylogeny of the Caryophyllales *sensu lato*: revisiting hypotheses on pollination biology and perianth differentiation in the core Caryophyllales. *International Journal of Plant Science, 170*, 627–643

Brundrett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil, 320*(1-2), 37-77.

Burger, A. E. (2005). Dispersal and germination of seeds of *Pisonia grandis*, an Indo-Pacific tropical tree associated with insular seabird colonies. *Journal of Tropical Ecology, 21*(3), 263-271.

Cairney, J. W. G. (2000).Evolution of mycorrhiza systems. *Naturwissenschaften, 87*, 467-475.

Carriconde, F., Gardes, M., Jargeat, P., Heilmann-Clausen, J., Mouhamadou, B., & Gryta, H. (2008). Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, *Tricholoma scalpturatum. Microbial Ecology, 56*(3), 513-524.

Chambers, S. M., Sharples, J. M., & Cairney, J. W. G. (1998). Towards a molecular identification of the *Pisonia* mycobiont. *Mycorrhiza, 7*, 319-321.

Chambers, S. M., Hitchcock, C. J., & Cairney, J. W. (2005). Ectomycorrhizal mycobionts of *Pisonia grandis* on coral cays in the Capricorn-Bunker group, Great Barrier Reef, Australia. *Mycological Research, 109*, 1105–1111.

Cuénoud, P., Savolainen, V., Chatrou, L. W.,  Powell, M., Grayer, R. J., & Chase, M. W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *American Journal of Botany, 89,* 132–144.

Douglas, N. A., & Manos, P. S. (2007). Molecular phylogeny of Nyctaginaceae: taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America. *American Journal of Botany, 94*(5), 856-872.

Douglas, N. A., & Spellenberg, R. (2010). A new tribal classification of Nyctaginaceae. *Taxon, 59*, 905–910.

Downie, S. R., Katz-Downie, D. S., & Cho, K. J. (1997). Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. *American Journal of Botany, 84*, 253–273.

Downie, S. R., & Palmer, J. D. (1994). A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. *Systematic Botany, 19*, 236–252.

Duddridge, J. A. (1986a). The development and ultrastructure of ectomycorrhizas. III. Compatible and incompatible interactions between *Suillus grevillei* (Klotzsch) Sing. and 11 species of  ectomycorrhizal hosts *in vitro* in the absence of exogenous carbohydrate. *New Phytologist, 103*(3), 457-464.

Duddridge, J. A. (1986b). The development and ultrastructure of ectomycorrhizas. IV. Compatible and incompatible interactions between *Suillus grevillei* (Klotzsch) Sing. and a number of  ectomycorrhizal hosts *in vitro* in the presence of exogenous carbohydrate. *New Phytologist, 103*(3), 465-471.

Haug, I., Weiß, M., Homeier, J., Oberwinkler, F., &  Kottke, I. (2005). Russulaceae and Thelephoraceae form mycorrhizas with members of the Nyctaginaceae (Caryophyllales) in the tropical mountain rainforest of southern Ecuador. *New Phytologost, 165*, 923–936.

Hayward, J. A., & Horton, T. R. (2012). Edaphic factors do not govern the ectomycorrhizal specificity of *Pisonia grandis* (Nyctaginaceae). *Mycorrhiza, 22*(8), 647-652

Imhof, S. (2009). Arbuscular, ecto-related, orchid mycorrhizas—three independent structural lineages towards mycoheterotrophy: Implications for classification? *Mycorrhiza, 19*, 357–363.

Janos, D. P. (1980). Mycorrhizae influence tropical succession. *Biotropica, 12*(2), 56-64.

Johnson, A. F. (1985). The ecology of *Abronia maritima*. *Biotica, 10*, 19–34.

Koske, R. E., Gemma, J. N., & Flynn, T. (1992). Mycorrhizae in Haiwaiian angiosperms: a survey with implications for the origin of the native flora. *American Journal of Botany, 79*, 853—862.

Kõljalg, U. (1992). Mycorrhiza formed by basidiospores of *Tomentella crinalis* on *Pinus sylvestris*. *Mycological Research, 96*, 215 - 220.

Kõljalg, U., Larsson, K. H., Abarenkov, K., Nilsson, R. H., Alexander, I. J., Eberhardt, U., Erland, S., Hoiland, K., Kjoller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Vralstad, T., & Ursing, B. M. (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist, 166*(3), 1063 - 1068.

LePage, B. A., Currah, R. S., Stockey, R. A., & Rothwell, G. W. (1997). Fossil ectomycorrhizae from the Middle Eocene. *American Journal of Botany, 84*, 410–412.

Levin, R., A. (2000). Phylogenetic relationships within Nyctaginaceae tribe Nyctagineae: Evidence from nuclear and chloroplast genomes. *Systematic Botany,25*, 738-750.

Levin, R., A. (2002). Taxonomic status of *Acleisanthes, Selinocarpus,* and *Ammocodon* (Nyctaginaceae). *Novon, 12*, 58-63.

LoBuglio, K. F., Berbee, M. L., & Taylor, J. W. (1996). Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among Ascomycetes. *Molecular Phylogenetics and Evolution, 6*, 287–294.

Malloch, D. W., Pirozynski, K. A., & Raven, P. H. (1980). Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A review). *Proceedings of the National Academy of Sciences, 77*, 2113–2118.

Muthukumar, T. & Udaiyan, K. (2000). Arbuscular mycorrhizas of plants growing in the Western Ghats region, southern India. *Mycorrhiza, 9,* 297-313.

Ogden, J. (1993). On cyclones, *Pisonia grandis* and the mortality of Black Noddy *Anous minutus* on Heron Island. *EMU – Australian Ornithology, 93*, 281-283.

Peay, K. G., Kennedy, P. G., & Bruns, T. D. (2008). Fungal community ecology: a hybrid beast with a molecular master. *Bioscience, 58*, 799-810.

Remy, W., Taylor, T. N., Hass, H., & Kerp, H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *PNAS,* 91, 11841–11843.

Rettig, J. H., Wilson, H. D., & Manhart, J. R. (1992). Phylogeny of the Caryophyllales— gene sequence data. *Taxon, 41*, 201–209.

Rodman, J. E., Oliver, M. K., Nakamura, R. R., McClammer Jr., J. U., & Bledsoe, A. H. (1984). A taxonomic analysis and revised classification of Centrospermae. *Systematic Botany, 9*, 297–323.

Schoch, C. L., Seifert, K. A., Hunhdorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., & Fungal Barcoding Consortium. (2011). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi. PNAS Early Edition*, 1-6.

Sigüenza, C., Espjel, I., & Allen, E. B. (1996). Seasonality of mycorrhizae in coastal sand dunes of Baja California. *Mycorrhiza, 6*, 151-157.

St John, T. V. (1980). A survey of mycorrhizal infection in an Amazonian rainforest. *Acta Amazon, 10*, 527–533.

Suvi, T., Tedersoo, L., Abarenkov, K., Beaver, K., Gerlach, J., & Kõljalg, U. (2010). Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia, 102*, 522-533.

Tedersoo, L., May, T. W., & Smith, M. E. (2010a). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza, 20*, 217-263.

Tedersoo, L., & Nara, K. (2010). General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. *New Phytologist, 185*, 351-354.

Tedersoo, L., Sadam, A., Zambrano, M., Valencia, R., & Bahram, M. (2010b). Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. *ISME J,* 4, 465–471.

Taylor, T. N., Kerp, H., & Hass, H. (2005). Life history biology of early land plants: deciphering the gametophyte phase. *PNAS, 102*, 5892–5897.

Walker, T. A. (1991). The distribution, abundance and dispersal by seabirds of *Pisonia grandis. Pisonia islands of the Great Barrier Reef* (pp. 1-23). Washington D.C., US: National Museum of Natural History, Smithson Institution.

Wang, B., & Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza, 16*(5), 299-363.


**Books**

Mabberley, D. J. (2008). *Mabberley's plant-book: A portable dictionary of plants* (3rd ed.). Avon, UK: Cambridge University Press.

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* (2$^{nd}$ ed.). ???? Academic Press.


**Internet sources**

Jago, B. (Photographer). *Flower* [Photograph]. Retrieved Dec 17, 2012, from: http://keys.trin.org.au/key-server/data/0e0f0504-0103-430d-8004-060d07080d04/media/Html/taxon/Pisonia_umbellifera.htm .

Jago, B. (Photographer). *Flower and Buds* [Photograph]. Retrieved Dec 17, 2012, from: http://keys.trin.org.au/key-server/data/0e0f0504-0103-430d-8004-060d07080d04/media/Html/taxon/Pisonia_umbellifera.htm .

Hattaway, B. (Photographer). *Pisonia aculeata* [Photograph]. Retrieved Dec 25, 2012, from: http://www.discoverlife.org/mp/20q?search=Pisonia .

Missouri Botanical Garden. [Untitled figure of the distribution of Nyctaginaceae]. Retrieved Dec 17, 2012, from: http://www.mobot.org/MOBOT/research/APweb/orders/caryophyllalesweb.htm .

Molina, R., Massicotte, H., & Trappe, J. M. (1992). Specificity phenomena in mycorrhizal symbioses: community–ecological consequences and practical implications. In M. Allen (Ed.), *Mycorrhizal Functioning: an Integrative Plant-*

*Fungal Process,* 357–423. Retrieved from Dec 21, 2012:
http://books.google.ee/books?id=Sf8bA9JRBWMC&printsec=frontcover&dq=Myc
orrhizal+Functioning:+An+Integrative+Plant-
Fungal+Process&hl=en&sa=X&ei=crjUUOGjM8en0QWqhIHQCg&ved=0CDQQ6
AEwAA#v=onepage&q=Mycorrhizal%20Functioning%3A%20An%20Integrative%
20Plant-Fungal%20Process&f=false .

Nickels, I. (Photographer). (2011). *Abronia villosa. Anthocarp (fruit) in longitudinal
section* [Photograph]. Retrieved Dec 17, 2012, from:
http://www.phytoimages.siu.edu/imgs/paraman1/r/Nyctaginaceae_Abronia_villos
a_27767.html .

Nickrent, D. L. (2009). *Mirabilis nyctagineae fruits (anthocarps), some in section*
[Photograph]. Retrieved Dec 17, 2012, from:
http://www.phytoimages.siu.edu/imgs/paraman1/r/Nyctaginaceae_Mirabilis_nycta
ginea_17927.html .

Spellenberg, R. W. (2004). Nyctaginaceae Jussieu. Four-o'clock Family. *Flora of North
America, 4.* Retrieved from
http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=10617 .

Watson, L., and Dallwitz, M.J. (1992 onwards).*The families of flowering plants:
descriptions, illustrations, identification, and information retrieval.* Version: 18th
May 2012. Retrieved Dec 12, 2012 from: http://delta-
intkey.com/angio/www/phytolac.htm .

Watson, L., and Dallwitz, M.J. (1992 onwards).*The families of flowering plants:
descriptions, illustrations, identification, and information retrieval.* Version: 19th
December 2012.  Retrieved Dec 20, 2012 from: http://delta-
intkey.com/angio/www/sarcobat.htm

**Doctoral dissertations**

Douglas, N. A. (2007). *Molecular phylogenetic studies in Nyctaginaceae: patterns of
diversification in arid North America.* (Doctoral dissertation). Retrieved Jan 5,

2013 from:

http://dukespace.lib.duke.edu/dspace/bitstream/handle/10161/188/D_Douglas_N
orman_a_052007.pdf?sequence=1

Suvi, T. (2010). *Mycorrhizal fungi of native and introduced trees in the Seychelles Islands.* (Doctoral dissertation). Retrieved Dec 20, 2012 from:
http://dspace.utlib.ee/dspace/handle/10062/14622?show=full .