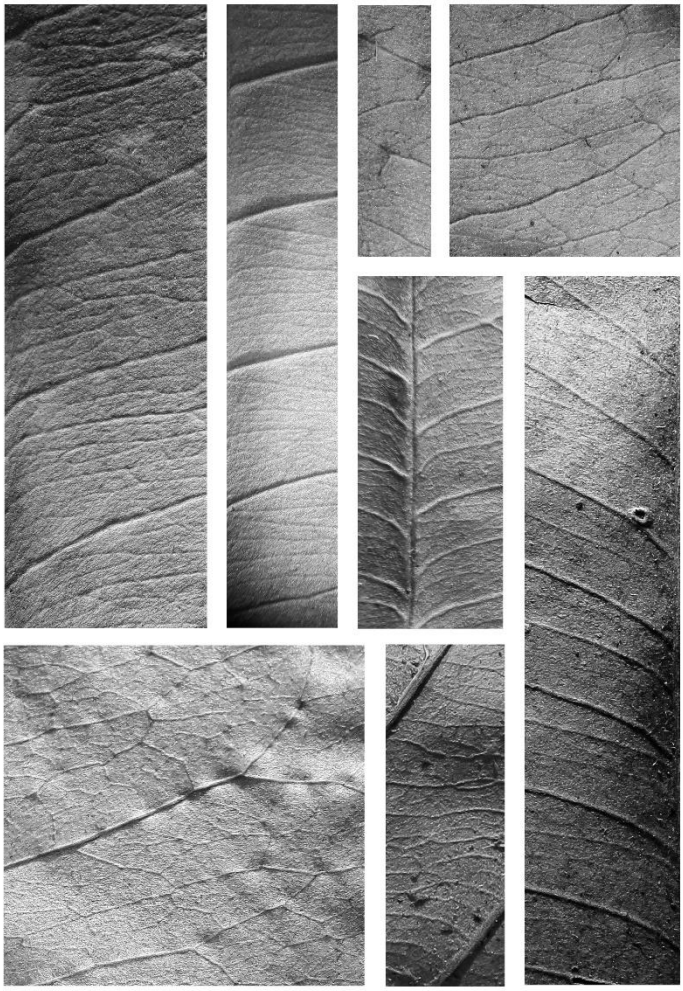
The Circumscription of the Historically and Economically Important Species *Palaquium gutta* (Hook.) Baill. (Sapotaceae)



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Declaration

I hereby declare that the work contained in this thesis is my own. Unless otherwise acknowledged and cited. This thesis has not in whole or part been previously presented for any degree.

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Abstract

An overview of the milky latex of Sapotaceae, termed gutta-percha is presented, as is a taxonomic history of species *Palaquium gutta* and its forms. The species is highly variable in morphology, and hence confusing in its determination. A morphological character assessment of specimens provisionally identified as *Palaquium gutta* is given as is a molecular phylogenetic estimate using the nuclear region ITS. The taxonomically informative vegetative characters of the species are presented. Seventeen new sequences are incorporated into an existing larger Isonandreae matrix and the results of both a maximum parsimony and Bayesian analysis are shown to support the monophyly of the species, including the placement of easily confused species with *Palaquium gutta* in the phylogenetic tree. Using the morphological and molecular data a discussion is presented on the circumscription of *Palaquium gutta* and its forms, the putative placement of specimens provisionally identified as *Palaquium gutta* into taxonomic groups, and the challenges with identification of sterile specimens in Sapotaceae.

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1. INTRODUCTION

1. 1 Aims of Research Project

The main aims of this research are:

1. To review the taxonomic history of *Palaquium gutta* (Hook.) Baill.
2. To investigate the current circumscription of *Palaquium gutta* using traditional herbarium methods to reassess morphological variation within the species and to investigate if micro-morphological characters can be used to support or refute the current circumscription and published forma.
3. To incorporate new sequence data of species provisionally identified as *Palaquium gutta* into an existing phylogeny of the Isonandreae to help confirm the current circumscription of the species, clarify synonomy and check identifications.
4. To investigate the key vegetative characters that define *Palaquium gutta* (Hook.) Baill.

1. 2 Introduction to *Palaquium gutta* & Gutta-Percha

Palaquium gutta is a 25 – 45 m tree species which is distributed in lowland forest up to 1600 m a.s.l. in Malay Penninsular, Sumatra, and Borneo, and surrounding islands (Heyne, 1927; Ng, 1972; van Royen, 1960). Many fruits of Sapotaceae are ecologically important as a common diet of mammals and birds. It is considered Near Threatened A2c according to the IUCN Red List and there is a suspected population decline due to continuing decline in the extent and quality of habitat (Olander & Wilkie, 2018). The wood is not of high quality, the seed oil is reported as a cooking oil in Borneo and it is popular due to its coagulated milky latex termed gutta-percha (Heyne, 1927).

Gutta-percha is the term given to the milky white latex of Sapotaceae species. The name is derived from the Malay word “getah” meaning latex or gum, and “percha” a local name for the tree producing the latex (Collins, 1878; Green, 1851; Tully, 2009; Prakash *et al.*, 2005). The latex is produced by several different species of Sapotaceae growing throughout Southeast Asia, particularly the Malesia region. Despite the same name being given to the latex produced by different species, each species produce a diverse quality of latex and resin content. Gutta-percha producing species are found in the genera *Palaquium* (*Dichopsis*), *Payena*, *Planchonella,* and *Madhuca* (Collins, 1878; Heyne, 1927) However, very few species produce a high quality gutta-

percha. The genus *Palaquium* however is considered to produce the finest latex and *Palaquium gutta* to be "true" gutta-percha.

Gutta-percha is chemically similar to rubber, a polyisoprene with a different atomic structure and physical characters (Collins, 1878; Prakash *et al.*, 2005; Tully, 2009). Instead of having a cis-isomer like natural rubber, gutta-percha has a trans-isomer that allow the properties to behave more like crystalline polymers (Prakash *et al.*, 2005; Tully, 2009). Unlike rubber which remain elastic in room temperature, gutta-percha softens and is pliable at high temperature (>65°C) and becomes hard, tough, but not brittle, as it cools. It is purified using the Obach's technique before it is moulded into blocks for shipping (Prakash *et al.*, 2005)

John Tradescant first brought gutta-percha, called "pliable mazer wood", to London when he returned from his travels in the Far East in 1656. However, it was regarded as only a curiosity for long time. The honour of introducing gutta-percha to science goes to Dr. W. Montgomerie in 1843 when he referred his medical work using the material to the Medical Board of Calcutta and The Royal Society of Art in London. He had observed gutta-percha from a Malay labour who was using a "parang", a machete, with a handmade handle made from the latex and recognized the unique pliable character of the material (Collins, 1878; Felter & Lloyd, 1898; Green, 1851).

The first gutta-percha company was established in London, 1845 and the demand for the raw material grew rapidly for a variety of ornamental and utilitarian purposes, including boats, soles of boots and shoes, furniture, upholstery, and surgical appliances (Green, 1851; Prakash *et al.*, 2005; Tully, 2009). In 1845, the first gutta-percha golf ball was introduced in Scotland by J. Patterson and became popular and was in use until 1900. In 1847, gutta-percha was first used for dental filling by Edwin Treuman and is still used in orthodontics today. It was also in demand by hatters, fuse makers, chemical factories, photographic and other laboratories, and roof tile companies (Tully, 2009). In 1887, the potential of gutta-percha was seen by Werner von Siemens, a German engineer, for use as an insulator on electric telegraph cables along railway lines (Tully, 2009).

The most important use of this natural plastic however was in the insulation of submarine telegraph cables. Gutta-percha proved to be an ideal insulator and remained the superior material for submarine cable insulation for over 80 years. The first cross-channel cables linked

England and France in early 1850, and by the early twentieth century there were about 370,000 km of submarine cable around the world (Tully, 2009). The establishment of world-wide telegraph cables improved the speed and the dependability in communication at that time, before it was replaced by synthetic plastics and invention of wireless technology in the early twentieth century. Although the demand for gutta-percha is relatively small nowadays, it is still commonly used in dentistry, medical, toys, and the automotive industry.

1. 3 Taxonomic History of *Palaquium* gutta (Hook.) Baill.

At the time gutta-percha was being exploited, the tree it came from had no scientific name. To address this Sir William Hooker of Kew Gardens wrote to Dr Oxley at Singapore requesting flowering specimens. Dr Oxley duly sent material collected by Thomas Lobb and this allowed Hooker (Hooker, 1847) to scientifically name the species *Isonandra gutta* Hook. in the family Sapotaceae. The species was distinguished by the hexamerous flower compared to the tetramerous flower of other Isonandra species, a new genus in Sapotaceae at that time.

Miquel (1856) reported a specimen of *Isonandra gutta* Hook. found in West Sumatra, with longer, more oblong and more acuminated leaves and proposed *Isonandra gutta* var. *sumatranum*. In 1860, another new species was described from the same area, *Isonandra acuminata* Miq., which was similar to *Isonandra gutta* Hook but differed in having smaller petiole, oblong-lanceolate leaves with golden sericeous hairs on the underside.

(Bentham & Hooker, 1876) later placed *Isonandra gutta* Hook. together with *I. argentea* Teijm., *I. calophylla* Teijm., *I. caloneura* Kurz., *I. rostrata* Miq., *Bassia elliptical* Dalz., *Bassia polyantha* Wall, and the genus *Palaquium* Blanco into *Dichopsis* Thw., a new genus in Sapotaceae proposed by Thwaites (1864). They distinguished this genus from *Isonandra* by the strictly 6-merous flower, axillar or subsessile inflorescence and absence of indumentum on leaves and exalbuminous seed. However, it took them almost three decades to say with confidence that *Isonandra gutta* Hook. did not belong to the genus of *Isonandra*, and proposed a new name, *Dichopsis gutta* (Hook.) Benth.

Baillon (1883) who made a list of some species producing gutta-percha, revised the genus *Dichopsis* Thw. and recognised it as a synonym of *Palaquium* Blanco (Blanco, 1837) in the process *Dichopsis gutta* (Hook.) Benth became *Palaquium gutta* (Hook) Baill.

Pierre (1885) added four new species to *Palaquium* which he considered as producing the best gutta-percha (*P. malaccense*, *P. formosum*, *P. princeps* and *P. borneense*). His species circumscription was based mostly on leaf characters such as the length of the petiole, the shape of leaves and the number of secondary nerves. However, he admitted that these species had overlapping characters and were difficult to distinguish. In this publication he determined that *P. malaccense* Pierre was the species that produced the best quality gutta-percha.

At the same time, Burck (1886) published the first of monograph of Sapotaceae of the Dutch East Indies along with a botanical history of gutta-percha producing plants. He added many new species and divided the genus *Palaquium* into two groups. The first was composed of species with a golden indumentum on their leaves and the second those without a golden indumentum or glabrous. Burck concluded that *Palaquium gutta* (Hook.) Baill. was found only in Singapore, and was different from *P. oblongifolium* Burck, and the other new species he described in his publication, *P. borneense* Burck, *P. treubii* Burck, *P. vrieseanum* Burck, *P. acuminatum* Burck, *P. gloegoerense* Burck, *P. pisang* Burck, *P. selendit* Burck, and *P. obscurum* Burck. The recognition of these separate species was based on leaf characters and the regions where they were found. Like Pierre (1885), Burck also dealt with some sterile specimens which led to incomplete descriptions of new species and weakness in their circumscription. However, he was aware that *P. gutta* (Hook.) Baill., *P. oblongifolium* Burck, *P. borneense* Burck, *P. treubii* Burck (including var. parvifolium), and *Payena lerri* Teijsm. All produced equal high quality gutta-percha latex.

After several new gutta-percha producing species were described by Burck (1886) some were subsequent found to show some related characters to *Palaquium gutta* and other already described gutta-percha producing species. Engler (1890) added a new species collected by Beccari in Borneo, namely *Palaquium fulvosericeum* Engler. This species shared many characters with *Palaquium calophyllum* Becc., but differing by its more oblong and thicker leaves, and shorter pedicel which approaches *P. oblongifolium* Burck. *Palaquium gutta* (Hook.) Baill. subsp. *sessiliflora* Boerl. was proposed by Boerlage (1900) to separate this smaller-leaved species from *P. borneensis* Burck. Based on herbarium specimen and field observations of the

cultivated specimen, he reported transitional forms between *P. gutta* Burck and *P. oblongifolium* Burck, as well as between *P. borneense* Burck, *P. selendit* Burck and *P. treubii* Burck. Fox (1902), assistant superintendent of Singapore Botanic Gardens maintained the old genus name, *Dischopsis*, and applied to at least three of Burck's species, *Dischopsis oblongifolium* (Burck) Fox, *D. borneesis* (Burck) Fox, and *D. treubii* (Burck) Fox. Unfortunately the circumscription of these species cannot be confirmed as the protologues do not provide any description.

In *Nelle Foreste di Borneo*, Beccari (1902) discussed gutta-percha producing species, particularly in Sarawak, Borneo. After comparing a specimen, which was claimed to produce the highest quality gutta-percha in Sarawak, with both *P. gutta* Hook and *P. oblongifolium* Burck., Beccari described *Palaquium optimum* Becc. Based on its clearly obovate leaves, fewer lateral nerves, longer petiole, and longer pedicel (up to 1 cm). In his publication he also described four new gutta-percha producing species, *Palaquium tammadek* [not *P. tammrdak* as in WCSP], *P. magnoliaefolium* nom. nud., *P. retusum* nom.nud., and *P. ellipsoideum*. He noted that *Palaquium oblongifolium* Burck., the most common gutta-percha producing species, occurred widely in the Malay Peninsular, Sumatra and Borneo, and was possibly a synonym of *P. formosum* Pierre, while King & Gamble (1905) believed that *P. malaccense* Pierre, and *P. formosoum* Pierre are belong to forma of *P. gutta* (Hook.) Baill. However, he realised his new species could overlap with Burck's new species, and indicated a complete revision for all described species was needed.

Between 1907 and 1915 Dubard gathered a lot of herbarium material (deposited in Paris) and improved the Sapotaceae classification of Baillon's and Pierre's. In Dubard (1909) for the first time, he split *Palaquium* into two sections: *EuPalaquium* and *Palaquioides* based on androecium characters, and divided the section *EuPalaquium* into two groups based on venation characters: transverse and descendent venation. He also highlighted some characters that were not taxonomically useful due to high variation such as leaf size, number of seed per fruit, indumentum density on anthers. He also considered *P. borneense* Burck to be a synonym or a variant of *P. treubii* Burck that *P. malaccense* Pierre is a synonym of *P. gutta* (Hook.) Baill. since both of them produced the best quality gutta-percha, and that some differences in flower characters were just variations. He agreed with Beccari, that *P. formosum* Pierre was a synonym of *P. oblongifolium* Burck. After observations of both herbarium and living trees, he strongly suggested that *P. oblongifolium* Burck is actually a synonym of *P. gutta* (Hook.) Baill. as the

leaf shape in both species varies from obovate to oblong and is not constant, that the ratio of leaf length-width are always similar and that the larger flower size in *P. oblongifolium* Burck could also be found in *P. gutta* (Hook.) Baill. in the wild. In summary Dubard (1909) put *P. formosum* Pierre, *P. oblongifolium* Burck, *P. gloegloerense* Burck, *P. princeps* Pierre, *P. borneense* Pierre, *P. obscurum* Burck, P. *vrieseanum* Burck as synonyms of *P. gutta* (Hook.) Baill..

Inspired by Dubard who grouped *P. borneense* Burck and *P. treubii* Burck together due to their transverse venation and *P. gutta* (Hook.) Baill. and its synonyms due to the parallel venations, Lam (1925; 1927) proposed to put them all into one species, *P. gutta* (Hook.) Baill with four subdivisions of forma. This subdivision was based mainly on leaf shape, leaf apex, leaf size and the secondary and tertiary venation. Forma I (Borneensis) consists of *P. borneense* Burck, *P. treubii* Burck and *P. fulvosericeum Engler*, forma II (Selendit) consists of *P. selendit* Burck and partly of *P. pisang* Burck, forma III (Genuinum) subforma Gutta consists of *P. gutta* (Hook.) Baill. s.s. and subforma Oblongifolium consists *P. oblongifolium* Burck, *P. gloegoerense* Burck, *P. obscurum* Burck, *P. acuminatum* Burck, *P. leiocarpum* var. *longe-acuminatum* Boerl., and forma IV (Vrieseanum) consists of *P. vrieseanum* Burck. In his later publication (Lam, 1927), he dropped the subforma gutta and oblongifolium since he considered them practically inseparable.

Lam (1925; 1927) followed Boerlage (1900), Beccari (1902), van Romburgh (1903), King & Gamble (1905), Schlechter (1903), and Dubard (1909) in considering many species as synonyms of *Palaquium gutta*. Van Romburgh (1903) reported variation in leaves shape of seedling produced by parents from cultivation (botanic gardens) while Schlechter (1903) thought the distinguished characters recognized by Burck were actually a small “deviations” that could be found in individual trees. Lam, after dealing with a great number of specimens, realised that there is no single character that can divide clearly those species and considered *P. gutta* (Hook.) Baill. to be an extremely polymorphic species with several transitional characters among forms and subforms. He did however in 1925 describe a new species, *Palaquium tjipetirense* Lam, which was growing in the gutta-percha plantation or experiment garden in Cipetir village, Sukabumi Regency, West Java. He distinguished this species from *P. gutta* (Hook.) Baill. by possessing longer pedicels and petioles, larger acutely lobes of sepals, larger and pubescent corolla tube, pubescent anthers and obovoid and glabrous fruits. Since the specimen of the species is from cultivation in Java only, some hypothesis were made towards

the species origin: either from Sumatra (Burck's or Van Romburgh's) or Borneo (Van Romburgh's). Since it is closely related to *P. gutta* (Hook.) Baill., it could be a mutation of the species or a hybrid *P. gutta* s.l. complex between species that were growing in the plantation (Heyne, 1927; Lam, 1925; 1927). In other notes Lam (1925) suggests that *P. tjipetirense* Lam could be related or identical to *P. optimum* Becc. from Borneo.

K. Heyne (1927), who was the Chief of the Museum and Inquiry Office for Economic Botany at Bogor, refused to follow Lam's *Palaquium gutta* circumscription and kept *P. acuminatum* Burck, *P. treubii* Burck and *P. selendit* Burck as separate considering that the characters of latex, particularly the low quality gutta-percha they produced. From an economic view point, it was important to separate these species from *P. gutta* (Hook.) Baill. to differentiate them from the many gutta-percha producing species. It is clear that this did not always correspond to herbarium based taxonomic research.

Three and a half decades later, van Royen (1960) published a revision of *Palaquium* of the Malesia region. He dropped the four forma described by Lam (1925; 1927) since the delimitation of each forma had to be expanded in many new specimens he had examined. It showed many in-between characters, and obscured the distinct line between forma. Van Royen (1960) noted that both f. gutta (genuinum) and f. borneensis possessed both parallel and transverse tertiary venation in some specimens regardless of the leaf shape. He added that f. vrieseanum is regarded as a small-leaved form of f. gutta (genuinum) by having parallel tertiary veins and asimilar number of secondary veins. Like f. vrieseanum and f. gutta, f. selendit was also regarded as a small–leaved forms of f. borneensis with the transverse tertiary veins. However, van Royen (1960) stated the difference in size, number of secondary veins, type of tertiary venation, and their combinations cannot be regarded as character sufficient to define the four forma.

Van Royen (1960) distinguished seven main groups in *Palaquium* based on the leaf shape, leaf size, and tertiary venation, and indumentum presence. *P. gutta* (Hook.) Baill. belonged to *Group 6* (elliptic/elliptic-obovate leaves) – *series 2*, by having golden sericeous pubescence on the underside of leaves, together with *P. calophyllum* Teijm. & Binnendjik, *P. ferrugianum* Pierre ex Dubard, *P. quercifolium* (de Vriese) Burck, and *P. tjipetirense* Lam. He noted that two species of series 1, *P. lobbianum* Burck and *P. pseudocalophyllum* Lam can look similar except the indumentum is more tomentose than sericeous. Van Royen (1960) also mentioned

many species in the other groups and series related to Group 6. For example, *P. decurrenns* Lam., *P. stipulare* Pierre ex Dubard, *P. maingayi* (Clarke) King & Gamble, and other species in *Group 2 series d* are distinguished by having obovate leaves, acute leaf base, and pubescent underside of the leaves. Group 5, with the character of spathulate leaves, might be related to the Group 6 due to the tertiary venation, such as, *P. burckii* Lam, *P. firmum* White, *P. leiocarpum* Boerlage, *P. obstusifolium* Burck, and *P. oxleyanum* Pierre.

Ng (1972), a Malaysian botanist, published a Sapotaceae monograph for Malaya (Malay Peninsular) and recognized 22 species of *Palaquium* native to that area. Ng (1972) accepted *P. gutta* (Hook.) Baill. revised by Royen, but transferred all forma borneensis to *P. oxleyanum* Pierre due to the transverse tertiary venation being distinguishable from the other forma. Pennington (1991) mentioned *P. gutta* (Hook.) Baill. as an accepted species, however he did not discussed any previous confusion within the species.

1. 4 Forma concept

According to WCSP, at least 3 homotypic names and 28 heterotypic names are recorded as synonyms of *Palaquium gutta* (Hook.) Baill. indicating that that circumscription of the species in the past has been problematic (Tab F.1). The forma concept of Lam (1925) is not recognised in the current classification of *Palaquium gutta*, which follows van Royen (1960).

As more specimens were collected and examined after the description of Oxley's specimen more new species similar to *Palaquium gutta* were published. Most of the authors before Lam (1925) published some new species from sterile specimens. Burck (1885), Pierre (1885), and Beccari (1902) were the most important botanist before Lam (1925) and published many of the new species that have contributed to the problems relating to the circumscription of *Palaquium gutta*.

Van Romburgh (1903) and Schlechter (1903) recognized that often the same tree or its descendants could have several different leaf shapes. Boerlage (1900) and King & Gamble (1905) noted that *P. gutta, P. oblongifolium, P. borneense, P. treubii* and *P. selendit* were possibly conspecific. Dubard (1909) criticised the confusion among many gutta-percha producing species as reflection of the limitation of western botanist in considering the variations in morphology within the same species. He proposed to unite *P. treubii* and *P. borneense*, and highlighted that *P. gutta, P. malaccense*, and *P. oblongifolium* did not have a truly distinct

characters. Lam (1925), who looked at a large number of specimens in BO, put many species into synonym and recognised forma to accommodate the morphological character variation within species of *Palaquium gutta*, particularly the vegetative characters.

Lam (1925; 1927) recognised four forma of *P. gutta* (Hook.) Baill.; Forma I (Borneensis), Forma II (Selendit), Forma III (Genuinum), consists Subforma Gutta and Subforma Oblongifolium and Forma IV (Vrieseanum) (Tab. 1.1). This subdivision was based mainly on leaf shape, leaf apex shape, leaf size and the secondary and tertiary venation. It should be noted that Lam (1927) dropped the subforma gutta and oblongifolium and revised the leaf shape of forma Borneensis from *ovate* to *obovate*. At the end, Lam (1925) reported that *Palaquium gutta* is such a polymorphic species with many transitional characters within species, and he mentioned that the "genetic methods" might be able to address the problem.

Table 1.1 Summary of *Palaquium gutta*'s forma and their defining characters (Lam, 1925; 1927).

Forma	Synonyms	Leaf shape	Apex	Base	Sec. veins	Ter. veins	Angle	Leaf size L x W (cm)
(I) Borneensis	*P. borneense* Burck, *P. treubii* Burck P. *fulvosericeum* Englar	Broadly obovate	apex rounded, sometimes acuminate	decurrent	14-20	transverse	85-90	16 x 7; 9 x4.8; 26 x 10 (large)
(II) Selendit	*P. selendit* Burck *P. pisang* Burck	Obovate	apex blunt or more or less acute	decurrent	14-16	Mostly transverse	110 - 120	10.5 x 4.5; 7.5-8.5 x 3.2 (small)
(III) Genuinum (Subforma gutta & subforma Oblongifolium)	*P. gutta* (Hook.) Baill. s.s. *P. oblongifolium* Burck *P. gloeg*oerense Burck *P. obscurum* Burck *P. acuminatum* Burck *P. leiocarpum* var. *longe-acuminatum* Boerl	obovate to oblong to very narrowly lanceolate	apex rounded to long tapering	decurrent	20-30	transverse, parallel	85 - 90	16 x 4.6; 22 x 8; 11.5 x 3.5; 9.5 x 4; 13.5 x 3.7; 20 x 5; 27 x 7 (small to large)
(IV) Vrieseanum	P. *vrieseanum Burck.*	ovate or ovate-oblong	acuminate	acuminate	20	parallel	-	10.5 – 17 x 4.5 – 7 (small)

1. 5 *Palaquium gutta* (Hook.) Baill. and *Palaquium gutta* (Hook.) Burck

There are two authorities related to the combination of *Isonandra gutta* Hook. into *Palaquium*. Baillon combined this in Traite Bot. Méd. Phan. 2: addenda & errata (1500, 1313-1314) (1883) (*Palaquium gutta* (Hook.) Baill.,) while Burck made the same combination (*Palaquium gutta* (Hook.) Burck) in Ann. Jard. Bot. Buitenzorg 5: 24 (1886). Both have the same basionym. Baillon mentioned his new combination only in the appendix of the publication instead of in the body of the text which might have cause him to be unaware of the name change. Lam (1925) used Burck's combination until he made correction in his next publication (1927) by using Baillon's combination after reading Pierre's (1885) who used Baillon's combination. Burck combination is a later homonym and is a superfluous combination.

2. MATERIALS & METHODS

2. 1 Morphological Character Assessment

Herbarium material from the RBGE (E) and RBG Kew herbaria (K) were examined as were images of herbarium material from L, BO, P, SAR, SAN, KEP, KRB herbaria. From a total of 83 physical specimens and at least 213 images examined in this study 35 herbarium specimen from K and E were determined as *Palaquium gutta* (Hook.) Baill (Appendix A). All material was sorted into taxon piles and checked against type specimens. To understand the forma concept of Lam (1925, 1927), specimens were first sorted into the forma of *Palaquium gutta*. These were then reassessed once material in these taxon piles had been sequenced.

A morphological character matrix was produced from these specimens. The terminology of Ellis *et al.* (2009) and Hickey (1973) was followed for most of the categories and terminology of leaf characters. Morphological characters were observed using a standard binocular dissecting microscope. Photos were produced using a Leica MZ75 microscope, regular pocket camera, or smartphone camera.

Morphological assessment of specimens focused on the vegetative characters as they had been found to be taxonomically informative (Lam, 1925, 1927; Ng, 1972; van Royen, 1960) and because many herbarium specimens were sterile.

From the morphological matrix the most taxonomically informative characters, leaf shape, leaf apex, pattern and number of secondary veins, intersecondary veins characters, tertiary venation pattern, hairs or indumentum type, were selected for further observation to help differentiate between specimens or groups. Leaf shape followed Hickey (1973): oblong, elliptic, ovate, and obovate. To determine the exact whole leaf shape or leaf form, the length, width and the length/width (l/w) ratio of the leaves at the widest portion was measured. The ratio l/w was used to determine the forms: Narrow oblong (3.0 - 5.9/1), Oblong (2.0 - 2.9/1), Elliptic (2.0 - 2.9/1), Narrow elliptic (3.0 - 5.9/1), Oblanceolate (3.0 - 5.9/1), Narrow Obovate (2.0 - 2.9/1), or Wide Obovate (1.2 - 1.9/1).

Leaf apex shape followed Ellis *et al.*, (2009). Ellis *et al.*, (2009) recognised mucronate as an apex feature instead of apex shape and allowed the grouping the leaf apex regardless of the presence of a mucronate tip or mucro.

As the number of secondary veins can vary in each lamina in the same branch, sampling of the number of veins was taken from the smallest, a moderate-sized, and the largest leaf and the range noted. These were categorised into four groups based on range; group 1 (≤ 15), group 2 (16 – 20), group 3 (21 – 25), and group 4 (≥ 26). The angle of divergence of the secondary vein was measured to determine the gradient of the secondary veins: acute – narrow (< 45^{o}), acute – moderate (45-65^{o}), acute – wide (65-80^{o}), right angle (80 – 100^{o}) or obtuse (>100^{o}).

The observation of the secondary vein pattern was carried out under a microscope by adjusting the light angle. However, using this method it was sometimes difficult to see all the detail of the veins running parallel to light direction. The term intersecondary vein is used here to describe veins with courses similar to major secondary veins, but usually shorter in length and the thickness is intermediate between secondary veins and tertiary veins (Hickey, 1973; Ellis *et al.*, 2009).

Classification of tertiary vein patterns followed Ellis *et al.*, (2009): percurrent, ramified and reticulate. The same method used to determine the secondary vein pattern (see paragraph above) was used to also determine tertiary vein patterns.

Hairs and indumentum on leaves, petioles, buds and twig were observed under a dissecting microscope. The hairs on young abaxial leaves were observed because of their consistency. These were grouped based on length of the hairs, hair colour and any unique features observed, Group 1 or short hairs (10 – 20 µm), Group 2 or intermediate hairs (21 – 30 µm), Group 3 or long hairs (31 – 40 µm), and Group 4 or very long hair (> 41 µm).

2. 2 Databasing and Geo-referencing

Information from herbarium specimens was entered into the PADME Sapotaceae database, this included collector, collector number, collection date, locality information and when possible latitude and longitude data from the label. If no latitude and longitude information was available on the label then it was sourced, when possible, using web geography and gazetteer sites. If an image of the specimen was available then this was attached to the record. If a record was already in the database this information was checked and cleaned.

2. 3 Phylogenetic Assessment

2. 3. 1 Taxon sampling & selected genomic regions

Molecular data for 17 samples of *Palaquium gutta* (Hook.) Bail. were newly acquired in this study and from collections from Peninsular Malaysia, Sumatra, Borneo and Java. All samples were legally acquired from expeditions either by RBGE or collaborating scientists and institution (Tab. 2A.1).

In order to properly place these samples, two analyses were conducted, one using a larger Isonandreae-focussed matrix and one using a more restricted, smaller, *Palaquium*-focussed matrix. The large matrix comprised the 17 new samples from this study and an additional 261 selected samples of Sapotaceae obtained from RBGE Sapotaceae DNA Bank (EDNA) (Appendix B) and GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) (Clark, K. *et al.*, 2016). This analysis was rooted on four outgroups selected based on the publication of Swenson & Anderberg (2005), and Richardson *et al.*, (2014). These included two species of *Sarcosperma*, one *Eberhardtia* and one *Omphalocarpum* sample. *Sarcosperma* has been proposed to be in the new subfamily Sarcospermatoideae (Swenson & Anderberg, 2005) which is sister to two large subfamilies in Sapotaceae, Sapotoideae and Chrysophylloideae. *Eberhardtia* was predicted to belong to Sapotoideae, however it is placed as sister to Sarcospermatoideae (Richardson *et al.*, 2014), while *Omphalocarpum* belongs to Tribe Omphalocarpeae in Chrysophylloideae (Swenson & Anderberg, 2005).

The smaller *Palaquium* analysis comprised all samples residing in the *Palaquium* clade of the larger Isonandreae matrix (59 samples, including *Aulandra*) and the 11 newly acquired sequences from this study. The outgroup was selected based on the larger matrix analysis and consisted of four samples of *Burckella*, four samples of *Isonandra*, four samples of Tribe Sideroxyleae (*Lecomtedoxa, Neolemonierra, Capurodendron,* and *Northia*). *Burckella* and *Isonandra* are sisters to *Palaquium* in the Tribe of Isonandreae (Pennington, 1991, Richardson *et al.*, 2014). In total the *Palaquium* matrix included 82 samples.

The complete nuclear ribosomal internal transcribed spacer (ITS) region was used in the present study that included part of the 18S, ITS1, 5.8S, ITS2 and part of 26S. Previous molecular phylogenetic publications on Sapotaceae showed that the ITS region can be successfully applied in producing a phylogenetic tree with an expanded species sampling (Armstrong *et al.*, 2014; Richardson *et al.*, 2014). ITS is a relatively fast evolving region (Baldwin *et al.*, 1995)

and more variable than plastid genes (Richardson *et al.*, 2014). Therefore, it is expected to be useful at resolving relationships between closely related species and at the infraspecific level.

2. 3. 2 DNA Extraction and Amplification

DNA Extraction

Total DNA was extracted from silica gel-dried leaf samples using the Qiagen Plant DNeasy Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. A sample of about 20 mg of leaf material was ground using a grinding bead (3 mm Retsch cone ball) in two cycles of TissueLyser II (Mixer Mill) at 20 Hz frequency for 2 minutes each until the material was turned into a fine powder. 400 µl of Buffer AP1 was added and the mixture subsequently incubated in a Thermomixer for an hour at 65° C, set at 8000 rpm. The DNA was then extracted according to the Qiagen manual. The quality and amount of genomic DNA was assessed by gel electrophoresis (see below).

Amplification of Targeted Region

The targeted region, the ITS region was amplified using the Polymerase Chain Reaction (PCR) with the ITS5p and ITS4 primer pair. PCR was carried out in 20 µl volume reactions containing 6.1 µl distilled water, 2 µl dNTPs, 2 µl 10x NH4 reaction buffer, 0.6 µl $MgCl_2$, 2 µl forward primer (ITS5P), 2 µl reverse primer (ITS4), 0.3 µl BioTaq DNA polymerase buffer, 4 µl CES, and 1 µl genomic DNA template. The details of the primers are given in Table 2.1 The thermal cycling profile consisted of 4 min initial denaturation at 95ºC, followed by 30 cycles of 1 min at 94ºC for denaturation, 55ºC for 1 min for annealing and 72ºC for 45 seconds for strand extension, with a final extension period of 5 min at 72ºC.

Table 2.1 Details of primers used for amplifying target DNA regions

Primer	Direction	Prime Sequence (5'-3')	Authors
ITS 4	Reverse	TCCTCCGCTTATTGATATGC	(White *et al.*, 1990)
ITS 5p	Forward	GGAAGGAGAAGTCGTAACAAG	(Möller & Cronk, 1997)

Gel Electrophoresis

Gel electrophoresis was conducted to determine if the PCR amplification was successful. A 1% agarose gel was prepared by heating 1 g of agarose powder and 100 ml of 1xTBE (Tri-Borate-EDTA) buffer. Before pouring it to the tray with a comb, 5 µl of Sybrsafe DNA gel stain was added to the mixture.

A mixture of 5 µl of PCR product and 3 µl of gel loading dye was prepared for each sample, including the negative control PCR reaction. Once the gel had solidified, it was transferred to a larger tray containing 1xTBE buffer solution. Each mixture was then loaded into the well. A 1 kb ladder, and the negative control was also included on the gel. The gel was run at 80V, 400 A for 35 – 45 minutes which allows the negatively charged DNA molecules to migrate to the positive end.

2. 3. 3 DNA Sequencing

ExoSAP IT (GE Healthcare) was used to purify the PCR products to remove unwanted dNTPs and primers that could interfere with the sequencing process. For each sample, 2 µl of ExoSAP IT was mixed with 5 µl of PCR product. The total of 7 µl solution was then centrifuged and an incubation programme run in a thermocycler at 37^{o}C for 15 minutes, followed by heating at 80^{o}C for 15 minutes to inactive the enzyme.

BigDye (Applied Biosystems, UK) was used for cycle (Sanger) sequencing the purified PCR products. Each cycle sequencing PCR reaction contained 6.18 µl of dH2O, 2 µl of 5x BigDye Buffer, 0.32 of 10 µM primer, 0.5 Bigdye and 1 µl of PCR template. The ITS region was sequenced in both directions using the PCR primers, ITS5P and ITS4. The sequencing profile used involved 25 cycles of an initial denaturation at 95^{o}C for 30 seconds, primer annealing at 50^{o}C for 20 seconds, and primer extension at 60^{o}C for 4 minutes. The products of the sequencing PCR were sent to the University of Edinburgh GenePool facility for sequencing.

2. 3. 4 Phylogenetic Analyses

Sequence editing & Alignment

The newly acquired raw sequences were edited and assembled using Sequencher 5.4.6 (Gene Codes Corporation, 2019) including removing primer sequences, correcting some errors, and checking the chromatograms of each base pair to assess sequence quality. The quality of

contiguous sequences (contigs) was noted as *good, fair,* and *poor*. A batch of sequences was exported in concatenated FASTA format and aligned automatically online using MAFFT (https://mafft.cbrc.jp/alignment/server/) (Katoh *et al.*, 2017). The resulting alignment was then inspected and manually adjusted using Mesquite 3.6 (Maddison & Maddison, 2018) and exported as a simplified nexus file for phylogenetic analysis.

Maximum Parsimony analyses of the Isonandreae and Palaquium matrices

The Isonandreae and *Palaquium* matrices were analysed using maximum parsimony (MP) in PAUP*4.0a165 (Swofford, 2003). A heuristic search was carried out using 10,000 random addition replicates, with TBR (Tree-Bisection Reconnection) branch swapping, MulTrees and SteepestDescent activated, with no more than 10 trees of score (length) greater than or equal to 1 saved. The trees obtained were then filtered by the 'best score' filter, saved, and a phylogram (depicting branches proportionately to the number of nucleotide changes), strict and majority-rule consensus tree generated. To evaluate node support, a parsimony bootstrap analyses was performed using 10,000 replicates with TBR activated, but MulTrees deactivated (Möller *et al.* 2016). Due to the time limitations and focus of the study, a Bayesian analysis was not performed on the Isonandreae matrix. Following Richardson *et al.*, (2000) bootstrap values between 50-74% were considered weak support, 75-84% moderate support and 85-100% strong support.

Bayesian Inference analysis of the Palaquium matrix

In addition to an MP analysis, the *Palaquium* matrix was also analysed using Bayesian inference (BI) with MrBayes 3.2.7 (Ronquist *et al.,* 2012). The additional BI analysis was performed in order to obtain an alternative phylogenetic hypothesis of the target group, *Palaquium,* which was the focus of the present study. Congruence in tree topology between the MP and BI analyses would strengthen support for the hypothesis of evolutionary relationships.

The BI analysis was performed with MrBayes 3.2.7. Before running the BI analysis, MrModelTest version 2.3 (Nylander, 2004) was used to select the optimum models of evolution for the combined spacers (ITS1 and ITS2) and separately for the 5.8S gene under the Akaike's Information Criterion (Akaike, 1974). The analysis in MrBayes run a Markov Chain Monte Carlo (MCMC) chain of 2,000,000 generations in two independent runs with four chains in each, and sampled every 1,000th generation, discarding the first 25% of the trees as burn-in. A Bayesian consensus tree was generated from the remaining trees, and visualized and the posterior probabilities extracted using FigTree 1.4.0 (Rambaut, 2012). Clade support was given

by posterior probability (PP) values (following Swenson *et al.*, 2008). PP values between 50 and 95% were considered weak support for nodes and values greater than 95% indicated strong support.

3. RESULTS

3. 1 Morphological Character Assessment

3. 1. 1 Specimens Grouping into Forma (Lam, 1925; 1927)

Of the 35 herbarium specimen from K and E that were determined as *Palaquium gutta* (Hook.) Baill., 15 were fertile, with six specimens having fruits only.

A preliminary grouping of the 35 specimens was based on Lam's forma and using gross morphology- mostly leaf shape, leaf size and tertiary venation patterns (Lam, 1925, 1927). This resulted in 16 specimens placed in forma genuinum-subforma gutta, 11 specimens placed in forma genuinum – subforma oblongifolium, two specimens placed in forma borneensis, one specimens placed doubtfully in forma selendit, and none in forma vrieseanum. Five specimens could not be placed in any of the forma and are potentially not *Palaquium gutta* (Fig. 3.1.1).

3. 1. 2 Herbarium sampling and assessment

Of the 62 vegetative characters assessed, ten were found to be potentially taxonomically informative as they had clear variation within specimens putatively named *Palaquium gutta*. These characters were leaf shape, leaf apex shape, number, pattern, angle of divergence and pattern of secondary veins, the presence/absence of intersecondary veins and pattern, tertiary venation pattern, and hair groups (Tab. 3.1.1).

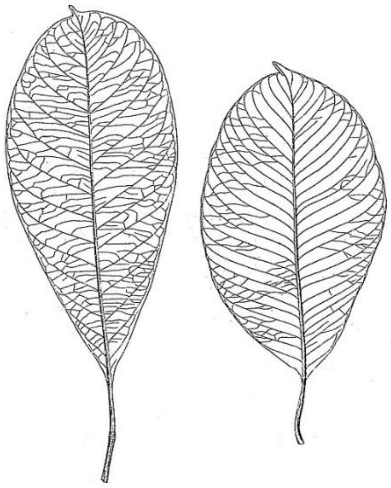
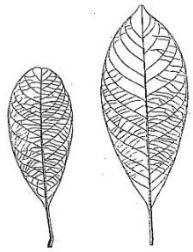
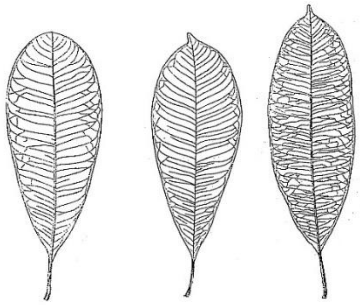
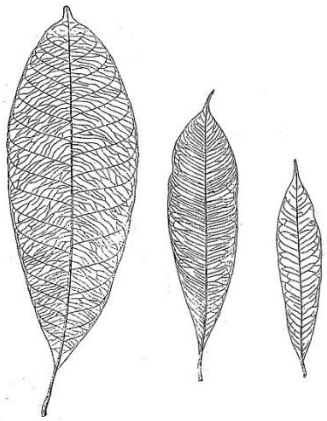
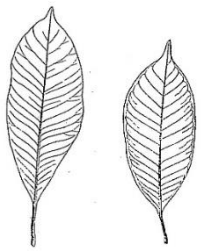
 **Forma Borneensis (I)**: *Wilkie* PW822 (E); *Raintai Jawa et al.* S 71048 (E)	 **Forma Selendit (II)**: *Cockburn* FRI7682 (K)?
 **Forma Genuinum (III) – subforma gutta**: *Oxley* s.n. (K); *Lobb* 290 (K); *Lobb* 290 (E) & (E); *G. Wray* 1100 (E); Rec-B:1887 (K); *Lobb* s.n. (K) & (K), *Wilkie* FRI 52919 (E); FRI 72142 (E); *Whitmore* FRI 12527 (K); *Maingay* 983 (K); *Cleghorn* s.n. (E); s.n. (K); *Lobb* s.n. (K); *Curtis* 780 (K); *Curtis* B5282 (K),	 **Forma Genuinum (III) – subforma oblongifolium**: *Sinclair* 5474 (E); *Wilkie* PW862 (E); PW1001 (E); PW504 (E); PW536 (E); PW842 (E); FRI 52855 (E); PW1010 (E); FRI 75020 (E); PW1024 (E); *T&P* 362 (K).
 **Forma Vrieseanum (IV)**: -	**unplaced specimens**: *Markus* SAN 151019 (E); *Wilkie* PW1031 (E); SFC 07419 (E), SFC 07432 (E), SFC 07443 (E).

Figure 3.1.1 Grouping of the 35 specimens into *Palaquium gutta* forma (Lam, 1925) based on leaf shape, leaf size and the tertiary venation patterns of the leaves.

Table 3.1.1 Informative vegetative characters of 35 specimens provisionally determined as *Palaquium* gutta (Hook.) Baill., including leaf shape, leaf apex, numbers, patterns, angle of divergence, and framework of secondary veins, intersecondary veins presence and characters, tertiary venation pattern, and hairs type. ***Form ratio***: Narrow oblong (3.0 - 5.9/1), Oblong (2.0 - 2.9/1), Elliptic (2.0 - 2.9/1), Narrow elliptic (3.0 - 5.9/1), Oblanceolate (3.0 - 5.9/1), Narrow Obovate (2.0 - 2.9/1), Wide Obovate (1.2 - 1.9/1); ***Score range***: group 1 (≤ 15), group 2 (16 – 20), group 3 (21 – 25), group 4 (≥26). ***Angle of divergence***: acute – narrow (<45^{o}), acute – moderate (45-65^{o}), acute – wide (65-80^{o}), right angle (80 – 100^{o}), obtuse (>100^{o}); ***Hair Group***: *Group* 1: short hairs (10 – 20 µm), *Group* 2: intermediate hairs (21 – 30 µm), *Group* 3: long hairs (31 – 40 µm), *Group* 4: very long hair (>41 µm).

				Leaf					Leaf Apex		Secondary veins					Intersecondary veins					
No.	**Barcode**	**Collector**	**No. Coll.**	**Length (cm)**	**Width (cm)**	**Ratio L/W**	**Shape**	**Form**	**Shape**	**Mucro**	**Number**	**SCORE**	**Angle**	**Angle of divergence**	**Pattern**	**Presence**	**course**	**distal course**	**Freq.**	**Tertiary Vein**	**Hairs Group**
1	E00 013587	J. Sinclair	5474	9.7 - 14.6	3.9 - 5.3	2.7/1	obovate	narrow obovate	convex	YES	26 - 30	4	65 - 75	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1 or > 1	ramifying - admedial	1
2	E00 230060 (isotype)	Lobb	290	12.5 - 15.4	5.2 - 6.2	2.4/1	obovate	narrow obovate	convex - rounded, convex	YES	24	3	70	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	< 1	ramifying - admedial	1
3	E00 230061 (isotype)	Lobb	290	10.3 - 12.9	5 - 5.3	2.2/1	obovate	narrow obovate	convex	YES	n/a	n/a	70 - 80	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
4	E00 277821	G. Wray	1100	7.0 - 12	3.2 - 5.9	2.0/1	obovate	narrow obovate	convex - rounded, convex	YES	20 - 28	3	70	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
5	E00 290117	Wilkie, P. & Gwee, A. T.	PW504	10.7 - 15.5	4.1 - 6.1	2.5/1	oblong & elliptic	oblong; elliptic	acuminate, convex, retuse	YES	25 - 26	4	75 - 80	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
6	E00 290128	Wilkie, P. Khoo, MS,	PW536	10 - 13.1	3.4 - 5.0	2.7/1	oblong & elliptic	oblong; elliptic	convex, retuse	YES	26 - 28	4	75	acute - wide	Brachidodromous;	YES	perpendicular	Basiflexed	1	ramifying - admedial	1

No.	Barcode	Collector	No. Coll.	Leaf					Leaf Apex		Secondary veins					Intersecondary veins				Tertiary Vein	Hairs Group
				Length (cm)	Width (cm)	Ratio L/W	Shape	Form	Shape	Mucro	Number	SCORE	Angle	Angle of divergence	Pattern	Presence	course	distal course	Freq.		
		Leong, PKF & Ibrahim A.													festooned & simple						
7	E00304060	Wilkie, P. & Imin, K, Kueh, HL.	FRI 52855	12.5 - 18.4	4.9 - 6.3	2.9/1	oblong & obovate	oblong; narrow obovate	convex	YES	26 - 32	4	75	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
8	E00304198	Wilkie, P.	FRI 52919	9.7 - 13	3.9 - 4.8	2.6/1	obovate	narrow obovate	convex	YES	23 - 25	3	70 - 75	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	>1	ramifying - admedial	1
9	E00331748	s.n.	-	14 - 16	5 - 5.5	2.8/1	obovate	narrow obovate	convex	YES	27 - 32	4	80	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
10	E00416389	Wilkie, P.	FRI 72142	8.5 - 11	3.9 - 5.1	2.2/1	obovate	narrow obovate	convex - rounded	YES	19 - 20	2	70 - 75	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
11	E00646660	Wilkie, P. & Imin	PW842	11.2 - 17.2	3.2 - 4.9	3.5/1	oblong & obovate	narrow oblong; oblanceolate	acuminate, convex	YES	27 - 30	4	75	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
12	E00664277	Wilkie P. , Anak Kapi, A.	PW862	9.7 - 16.3	3.1 - 4.9	3.2/1	obovate	oblanceolate	convex, acuminate	YES	24 - 25	4	70 - 75	acute - wide	Brachidodromous: festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
13	E00898190	Wilkie, P, , Hutabarat P.	PW1001	13.2 - 23	4.1 - 7.7	3.1/1	obovate	oblanceolate	convex	YES	20 - 26	4	70	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
14	H2012/01101 12	Curtis	780	8.5 - 13.2	3.0 - 4.7	2.8/1	obovate	narrow obovate	convex	YES	16 - 19	2	55 - 60	acute - moderate	Brachidodromous; simple	YES	perpendicular	Basiflexed	>1	ramifying - admedial	1
15	H2012/01104 11	Curtis	B 3582	11.2 - 13.2	5.8 - 6.8	2.0/1	obovate	narrow oboavte	convex - rounded	YES	17 - 18	2	65 - 70	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1

No.	Barcode	Collector	No. Coll.	Leaf					Leaf Apex		Secondary veins					Intersecondary veins				Tertiary Vein	Hairs Group
				Length (cm)	Width (cm)	Ratio L/W	Shape	Form	Shape	Mucro	Number	SCORE	Angle	Angle of divergence	Pattern	Presence	course	distal course	Freq.		
16	H20 12/0 110414	s.n.	Rec-B:1887	8.4 - 13	3.8 - 4.9	2.2/1	obovate	narrow obovate	convex - rounded	YES	18 - 20	2	70	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
17	H20 12/0 110415	Lobb	-	7.2 - 11.2	3.3 - 4.3	2.3/1	obovate	narrow obovate	convex	YES	21 - 24	3	60 - 65	acute - moderate	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
18	H20 12/0 110416	Lobb	-	8.3 - 12.5	3.5 - 4.6	2.5/1	obovate	narrow obovate	convex - rounded	YES	21 - 24	3	70 - 75	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1 or >1	ramifying - admedial	1
19	H20 12/0 110417	A. C. Maingay, M.D.	983	7.5 - 12.5	3.3 - 5.1	2.3/1	obovate	narrow obovate	convex	YES	18 - 23	3	70 - 75	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
20	H20 12/0 110418	s.n.	-	10.5 - 11.5	4.4 - 5	2.4/1	obovate	narrow obovate			18-20	2	65	acute - moderate	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
21	H20 12/0 110420	T. C. Whitmore	FRI 12527	10 - 13.6	4.7 - 5.8	2.2/1	obovate & elliptic	narrow obovate; elliptic	convex, acuminate	YES	14 - 18	2	60 - 75	acute - wide	Brachidodromous: festooned & simple	YES	perpendicular	Basiflexed	>1	ramifying - admedial	1
22	H20 12/0 110419	P. F. Cockburn	FRI 7682	6.0 - 7.7	2.4 - 3.2	2.5/1	Obovate	narrow obovate	convex - rounded, convex	YES	15 - 21	3	60	acute - moderate	Brachidodromous; simple	YES	perpendicular	Basiflexed	1 or >1	ramifying - admedial	1
23	H20 12/0 110421	T & P	T&P362	10.5 - 17.2	3.3 - 5.1	3.3/1	oblong & elliptic	narrow oblong; oblanceolate	acuminate	YES	21 - 26	4	70	acute - wide	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1
24	K000639125 (type)	Lobb	290	8.5 - 12.9	3.8 - 6.0	2.2/1	Obovate	narrow obovate	convex	YES	25 - 26	4	72 - 75	acute - wide	Brachidodromous; simple	YES	perpendicular	Basiflexed	1 or >1	ramifying - admedial	1
25	K000776800	Oxley	s.n.	9.6 - 11.4	3.6 - 4.5	2.5/1	obovate	narrow obovate	convex	YES	27 - 30	4	60	acute -	Brachidodromous; simple	YES	perpendicular	Basiflexed	1	ramifying - admedial	1

No.	Barcode	Collector	No. Coll.	Leaf					Leaf Apex		Secondary veins					Intersecondary veins				Tertiary Vein	Hairs Group
				Length (cm)	Width (cm)	Ratio L/W	Shape	Form	Shape	Mucro	Number	SCORE	Angle	Angle of divergence	Pattern	Presence	course	distal course	Freq.		
	(type)													moderate							
26	E00 903606	Wilkie, P. *et al.*	SFC 07419	14 - 16.8	5.5 - 7.2	2.5/1	elliptic & obovate	elliptic, narrow obovate	acuminate	NO	10 - 13	1	40	acute - narrow	Eucamptodromous	NO	N/A	N/A	N/A	percurrent - sinous, perpendicular	3
27	E00 913534	Rantai Jawa *et al.*	S 71048	12.6 - 15	8.8 - 9.2	1.5/1	Obovate	wide obovate	convex - rounded, retuse	YES	18 - 21	3	70	acute - wide	Brachidodromous; festooned & simple	NO	N/A	N/A	N/A	percurrent - sinous, perpendicular & obtuse	1
28	E00 639765	Wilkie, P.	PW 822	10 - 14.2	4.8 - 7.1	1.8/1	Obovate	wide obovate	convex - rounded, retuse	YES	13 - 15	1	60 - 65	acute - moderate	Brachidodromous; festooned & simple	YES	perpendicular	Basiflexed	<1	percurrent - sinous; perpendicular	1
29	E00 903604	Wilkie, P. *et al.*	SFC 07443	14.3 - 28	5.2 - 8	3.1/1	oblong	narrow oblong	acuminate, retuse	NO	13 - 15	1	50	acute - narrow	Eucamptodromous	NO	N/A	N/A	N/A	percurrent - sinous; perpendicular	3, 4
30	E00 903605	Wilkie, P. *et al.*	SFC 07432	10.5 - 19	4 - 5.5	3.0/1	oblong	narrow oblong	acuminate, retuse	NO	13 - 17	2	50	acute - narrow	Eucamptodromous	NO	N/A	N/A	N/A	percurrent - sinous; perpendicular	3, 4
31	E00 898191	Wilkie, P., Hutabarat P.	PW1010	25 - 27	8.4 - 11	2.8/1	oblong	oblong	acuminate	NO	23 - 28	4	65	acute - wide	Brachidodromous; simple	YES	parallel	parallel	1	ramifying - admedial	2, 3
32	E00 898554	Wilkie, P. & Hutabarat	PW1024	13.5 - 21	4.3 - 7.2	3.0/1	obovate & elliptic	oblanceolate, narrow elliptic	convex, acuminate	NO	20 - 27	4	75	acute - wide	Brachidodromous: simple	YES	parallel	parallel	1	ramifying - admedial	2, 3
33	E00 416341	Wilkie, P.	FRI75020	27.8 - 30	7.9 - 9	3.5/1	oblong	narrow oblong	acuminate	NO	22 - 23	3	70 - 75	acute - wide	Brachidodromous: simple	YES	parallel	parallel	1	ramifying - admedial	4
34	E00 834389	Markus, G.	SAN 151019	9.8 - 18.8	3.4 - 5.5	3.1/1	elliptic & obovate	narrow elliptic, oblanceolate	acuminate	NO	17 - 23	3	60	acute - moderate	Brachidodromous; festooned & simple	NO	N/A	N/A	N/A	percurrent - sinous; perpendic	2

No.	Barcode	Collector	No. Coll.	Leaf					Leaf Apex		Secondary veins					Intersecondary veins				Tertiary Vein	Hairs Group
				Length (cm)	Width (cm)	Ratio L/W	Shape	Form	Shape	Mucro	Number	SCORE	Angle	Angle of divergence	Pattern	Presence	course	distal course	Freq.		
																				ular & obtuse	
35	E00898547	Wilkie, P., Hutabarat, P., Lancar & Idel	PW1031	11.5 - 14.7	5.2 - 6.4	2.2/1	obovate & oblong	narrow obovate, oblong	Convex, convex-rounded	YES	12.0 - 14.0	1	70	acute - wide	Brachidodromous: festooned	NO	N/A	N/A	N/A	percurrent - sinous; Perpendicular & obtuse	2

Leaf shape

From the specimens examined three basic leaf shapes (oblong, elliptic, and obovate) were observed and seven forms, including narrow oblong, oblong, narrow elliptic, elliptic, oblanceolate, narrow obovate, and wide obovate (Fig. 3.1.2). Ovate leaf shape was not found in any specimens. Oblanceolate and narrow obovate forms were most commonly found in the specimens. It was also common to find more than one leaf form or a transitions between forms in one specimen.

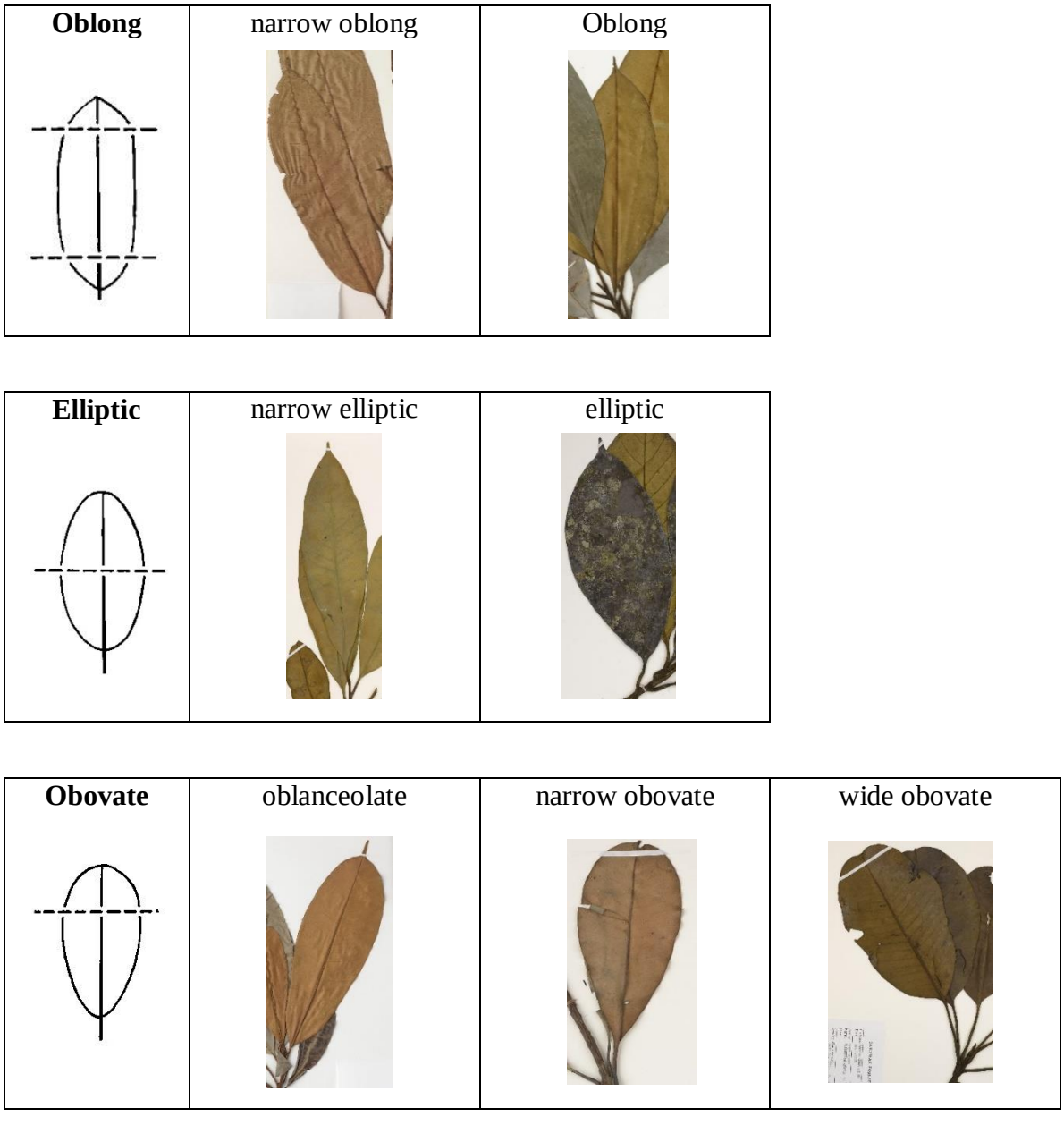


Figure 3.1.2 Three leaf shapes (oblong, elliptic, and obovate) and seven forms of leaf shape found in the specimens provisionally determined as *Palaquium gutta*.

Leaf Base and Apex

Both leaf apex and leaf base were examined. The leaf base observation showed three leaf base shapes; deccurent, cuneate, and cordate however, in general, leaf base shape was uninformative with cuneate and deccurent leaf base shapes being quite similar and difficult to define in specimens. A cordate base was only found in two specimens (*Wilkie* SFC 07443 & SFC 07432) and these were potentially not *P. gutta*.

Four groups of leaf apex are recognized from the specimens examined: convex, convex-rounded, acuminate, and retuse, (Fig. 3.1.3.). Convex, convex-rounded and retuse shapes were observed to occur on leaves on the same branch in some specimens. A mucro (the extension of midvein) was found in convex and convex-rounded apices only and of various lengths. Both convex and convex rounded leaf apices with a mucro are the most common apex shape.

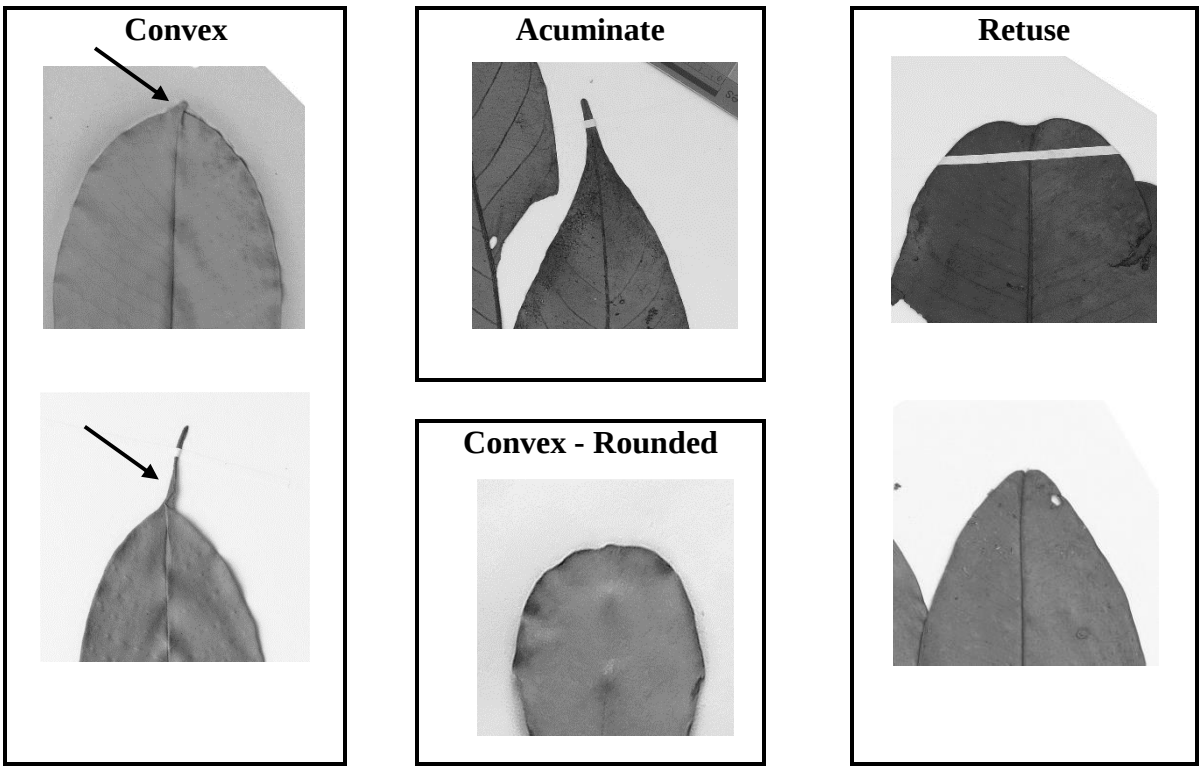


Figure 3.1.3. Four apex shapes: convex, convex – rounded, acuminate and retuse found in the specimens provisionally determined as *Palaquium gutta*. A mucro is usually found in convex or convex – rounded apices (see arrows).

Number & pattern of secondary veins

Specimens were placed in four groups based on the number of secondary veins. Four specimen are in group 1 (≤ 15 veins), seven specimen in group 2 (16 – 20 veins), 10 specimens in group 3 (21 – 25 veins) and 13 specimens in group 4 (≥ 26 veins). One specimen (*Lobb* 290) which only provided adaxial side, could not be counted due to the lack of visibility of venation (Tab. 3.1.1).

All specimens had major secondary veins that did not terminate at the margin. Two *Group*s of secondary vein patterns were observed. Four specimens belong to the eucamptodromous *Group*, and 31 specimens belong to the brachidodromous *Group* (Hickey, 1973, Ellis *et al.*, 2009). The Eucamptodromous *Group* is when the major secondary veins fades in thickness near the margin (Fig. 3.1.4.), while the major secondary veins in the brachidodromous *Group* form loops near the margin. They can be simple, festooned or mixed (Fig. 3.1.5.).

Among the brachidodromous group, 16 specimens were simple brachidrodomous (the major secondary veins joined together directly in a prominent loops) (Fig. 3.1.5. a, b), and 15 specimens were a mix of simple and festooned brachidodromous (secondary veins branched into multiple sets of loops or the major of secondary vein joining only to an external branch of an adjacent major secondary vein) (Fig. 3.1.5. c, d). Brachidodromous venation could be difficult to determine due to the complexity of secondary and tertiary vein branches and the limitation of lighting.

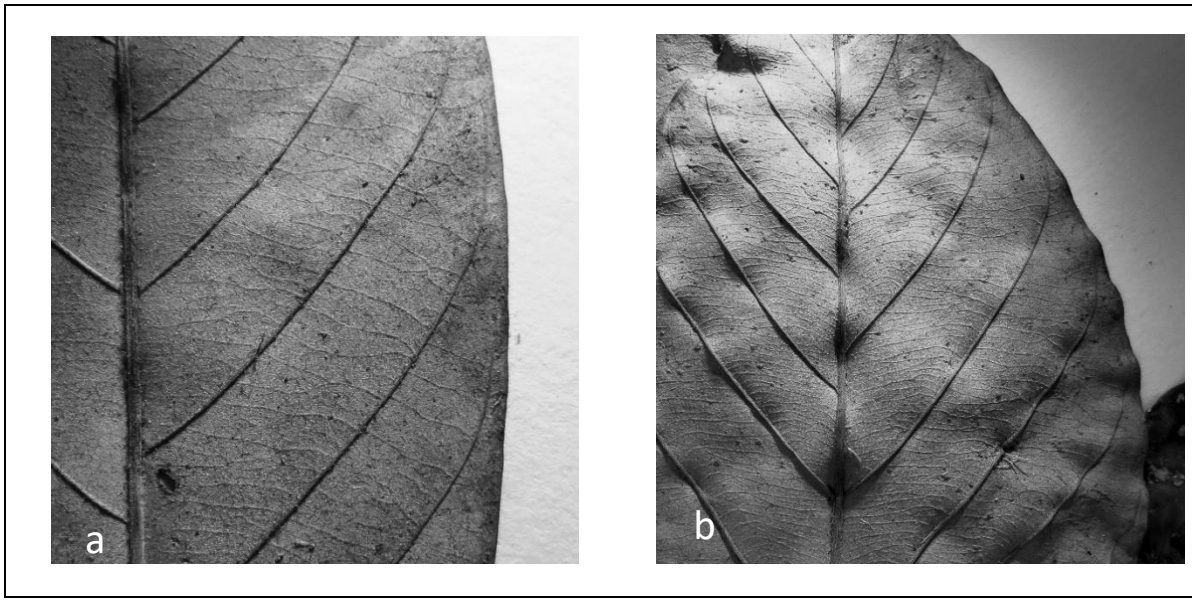


Figure 3.1.4 Eucamptododromous patterns observed from specimen.

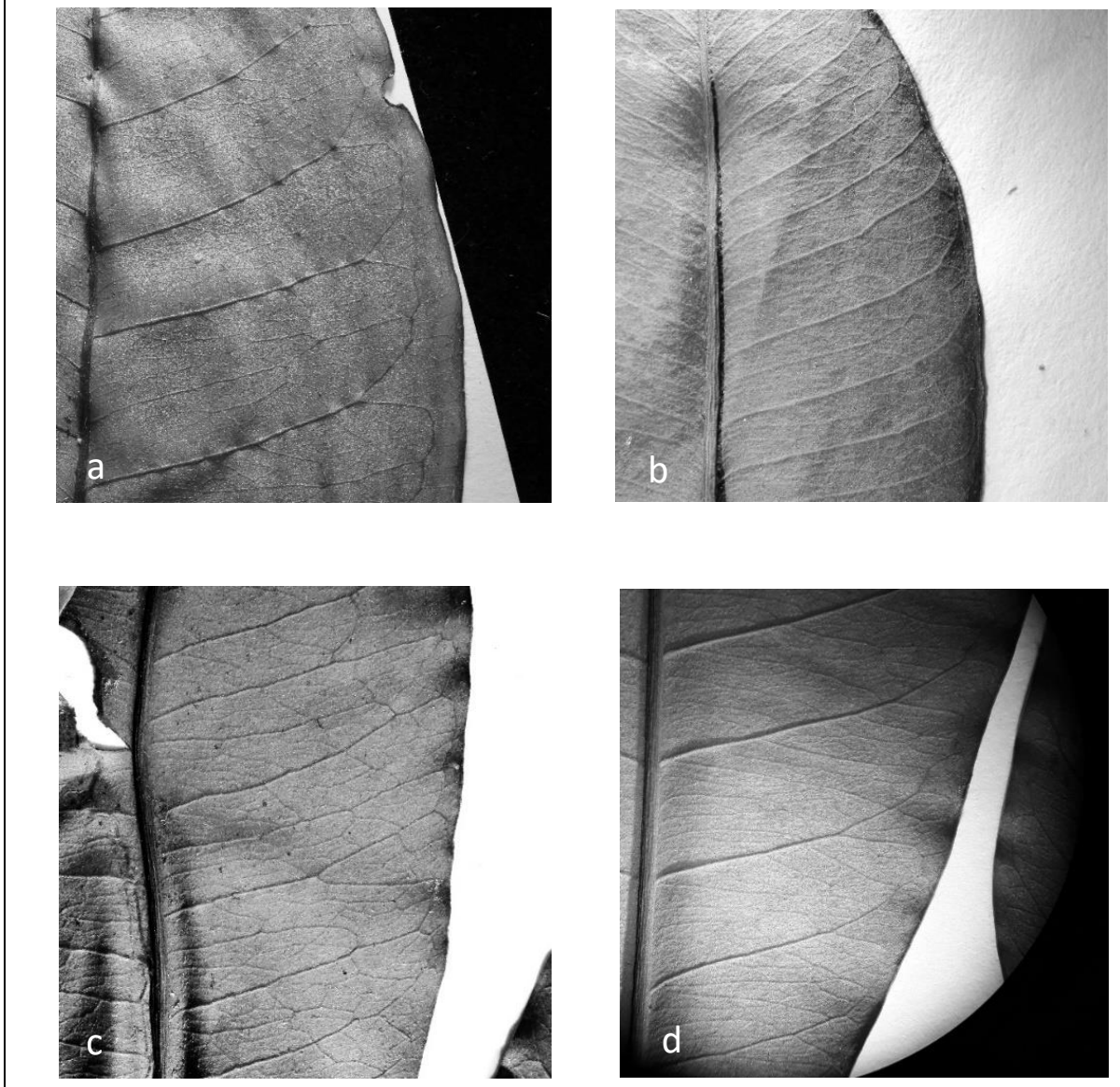


Figure. 3.1.5 Brachidodromous patterns observed from specimens. Simple brachidodromous (a, b), and mixed (simple & festooned) brachidodromous (c, d).

Angle of divergence

The angle at which secondary veins emerge from the midrib was found to be informative. Three distinct angles of divergence of secondary veins were recognized in the specimens. They are acute-narrow (<45^{o}), acute-moderate (45-65^{o}), and acute-wide (65-80^{o}) (Hickey, 1973) (Fig. 3.1.6). Acute-wide was the most common (25 specimens), followed by acute-moderate and acute-narrow(seven and three specimens respectively). The angle in specimens with the acute-

narrow divergence were usually uniform from the base to the apex, while the specimens with acute-moderate and acute-wide divergences were generally more acute towards the apex.

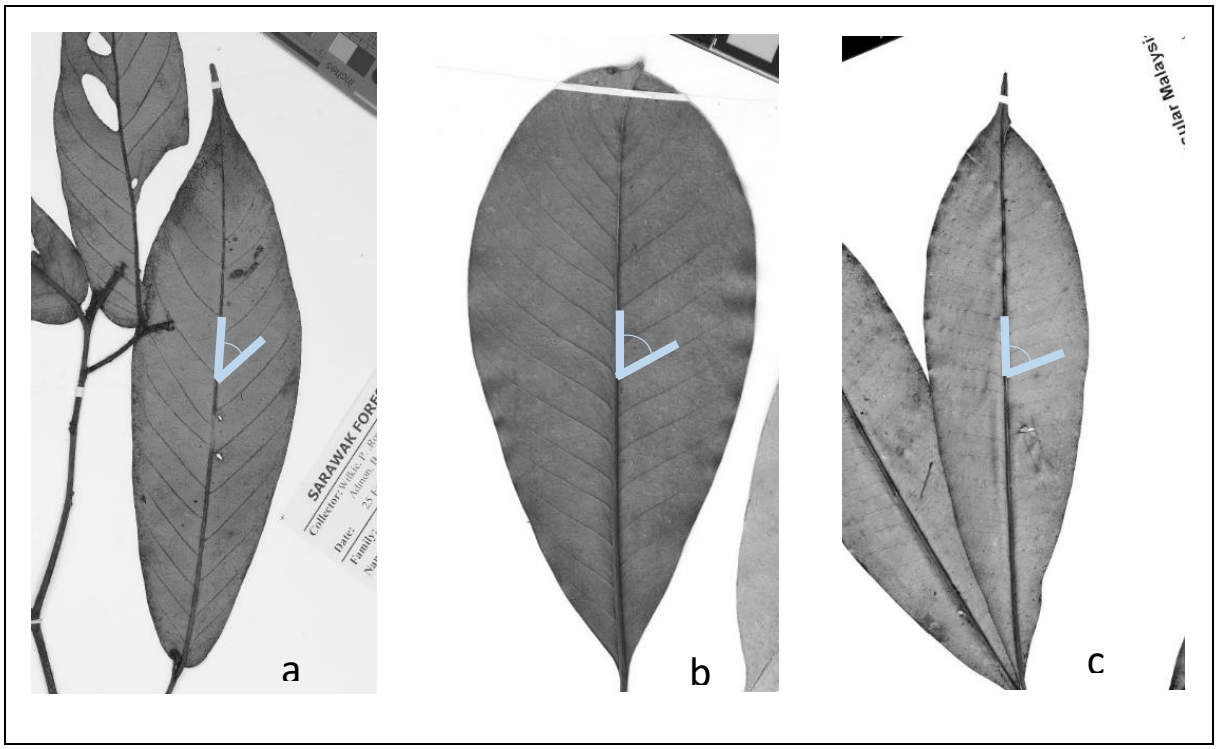


Figure 3.1.6 Three angle of divergence: acute – narrow (<45º) (a), acute – moderate (45-65º) (b), and acute – wide (65-80º) (c).

Pattern of Tertiary Veins

Two tertiary venation patterns were found, percurrent and ramified. Within the sampling in this study six were percurrent and 29 ramified. All percurrent vein courses were categorised as sinous (i.e. the majority of tertiary venation crossed between adjacent secondaries with random or changing direction curvatures). The angle of percurrent veins toward the midvein was obtuse (>90º), perpendicular (~90º), or both obtuse and perpendicular, but never acute (<90º) (Fig. 3.1.7).

Ramified tertiary veins were most common. These were admedially ramified (i.e. the multiple tertiary veins branch toward the midvein) (Fig. 3.1.8.). It was sometimes not possible to see the tertiary venation, particularly in young leaves or mature leaves with thick hairs covering the abaxial part.

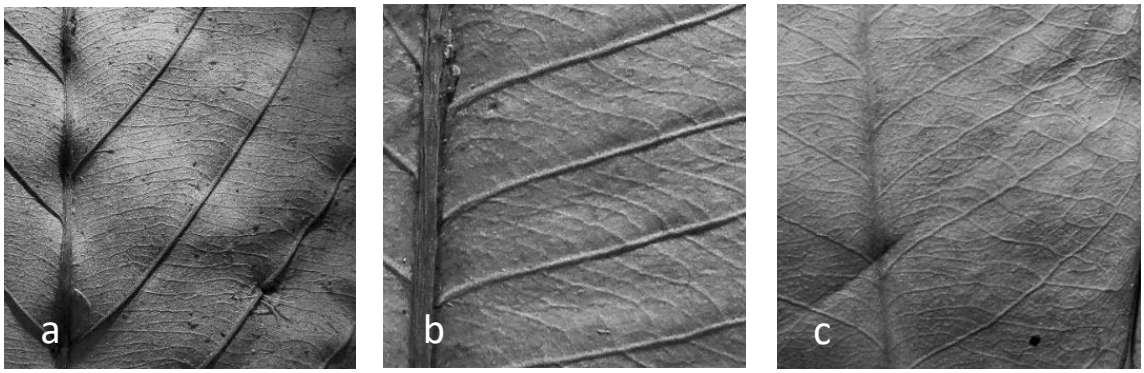


Figure. 3.17 The three different angle of percurent tertiary veins: perpendicular (a), obtuse (b) and mixed (perpendicular & obtuse) (c)

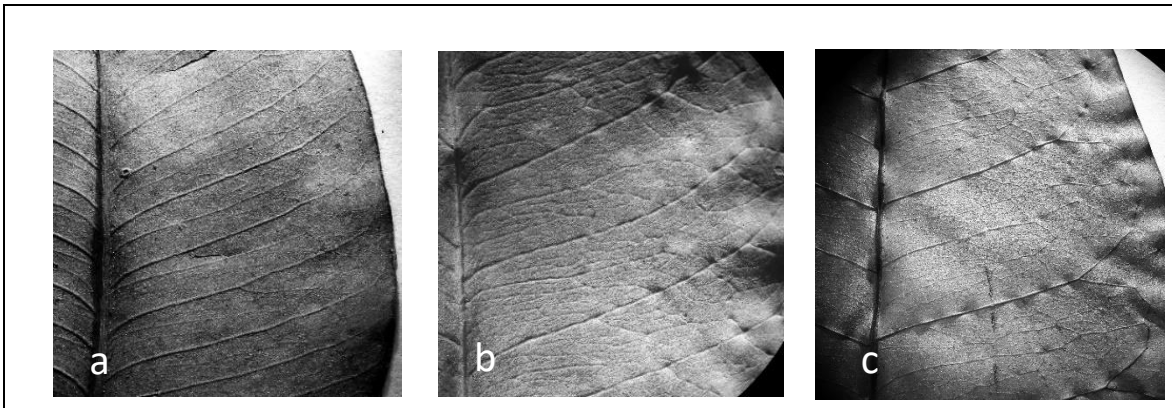


Figure 3.1.8 Some variation of admedially ramified tertiary veins found in specimens.

Intersecondary veins

Intersecondary veins were recorded from 29 specimens. They are recognized by their intermediate thickness between secondary and tertiary veins and usually occurred in the intercostal area (the area between two major secondary veins). The length of intersecondary veins in the specimens were always more than 50% that of the adjacent secondary veins and could be as long as them. The distal course of the intersecondary veins could be parallel to the major secondary veins or basiflexed (joining the adjacent secondary veins but not at a right angle). Only three specimens (*Wilkie* PW1010, PW1024, FRI 75020) had intersecondary veins running parallel to major secondary veins with a parallel distal courses (Fig. 3.1.9 b). Most

specimens had intersecondary veins perpendicular to the midvein with a basiflexed distal course (Fig. 3.1.9 a). In perpendicular specimens the intersecondary veins were slightly more parallel toward the apex.

The number of intersecondary veins varied and were categorised into three groups: absent in some or most of intercostal area (seldom occuring in each intercostal area), usually 1 per costal area (80 - 90% occur in intercostal area), and more than 1 per intercostal area. Young leaves usually did not have intersecondary veins, or they were not yet visible.

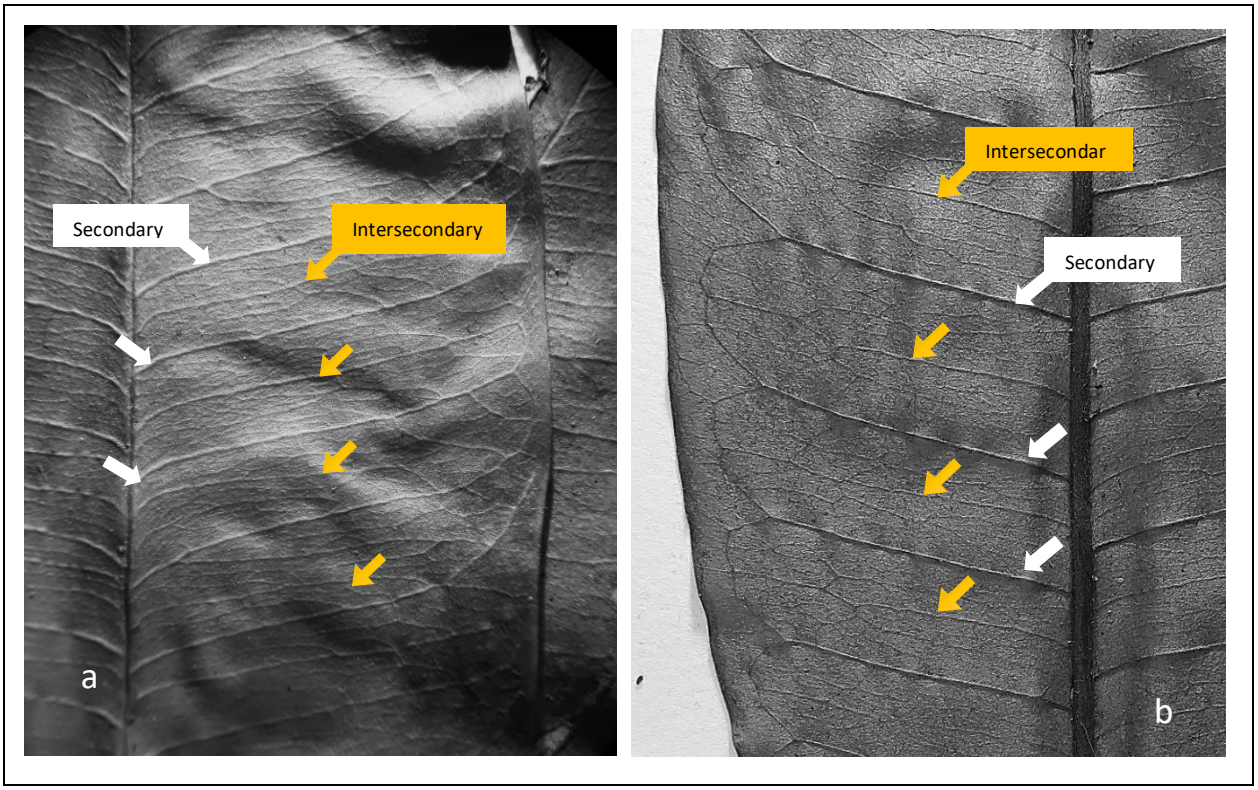


Figure. 3.1.9 Intersecondary veins (yellow) in the intercostal area (a,b). Proximal course of intersecondary veins perpendicular to midvein, with distal course: basiflexed (a.). Proximal course of intersecondary parallel to major secondaries (white), with distal course: parallel (b).

Hairs

Hair or indumentum was found on twigs, leaves (adaxial and abaxial sides), petioles, and leaves buds. However, the abaxial hairs on opened young leaves were emphasized in observations since they were more consistent in presence and density, and easy to observe using a dissecting microscope. Hairs on the twig and old leaves were mostly less than the younger leaves. The hairs progressively disappeared during development and aging. The remaining hairs turned into

a joined adpressed hairs texture, “pellicle”, which is difficult to define and the remining strands are likely to change colour making the leaf surface paler, or greyish. The remaining hair strands are often found along the midrib.

The hair texture on all specimens was very fine, adpressed and silky to touch. The term “sericeous” (Stearn, 1966; Beentje, 2016) was used in this study and is identical to “velvety” used by Ng (1972). Hairs were found to be taxonomically informative and were categorised based on hair length, hair colour and hair pattern. Four groups were recognised (Fig. 3.1.10). Group 1 (composed of 27 specimens) was recognized by short curved or sinuous hairs (10 – 20 µm) with more less a single orientation (without significant random direction) (Fig. 3.1.10 a,b). Group 2 (Fig. 3.1.10 c) had an intermediate size of curved hairs (21 - 30 µm) and was found in four specimen (*Wilkie* PW1010, PW1024, PW1031, *Markus* SAN 151019). Group 3 (Fig. 3.1.10 d) had long hairs (31 – 40 µm) with darker hairs scattered above the regular hairs. This occurred on four specimens (*Wilkie* SFC 07443, SFC 07432, PW1010, PW1024). Group 4 (Fig. 3.1.10 e, f) had the longest straight hairs (>41 µm) and had darker hairs scattered above and occurred in three specimens (*Wilkie* FRI 75020, SFC 07443, SFC 07432). Two specimens, PW1010 and PW1024, had both Group 2 and 3 type hairs, and the other two specimens, SFC 07443 and SFC 07432 had a both Group 3 and 4 type hairs. The darker hairs above the regular hairs were obvious in Group 3 and 4.

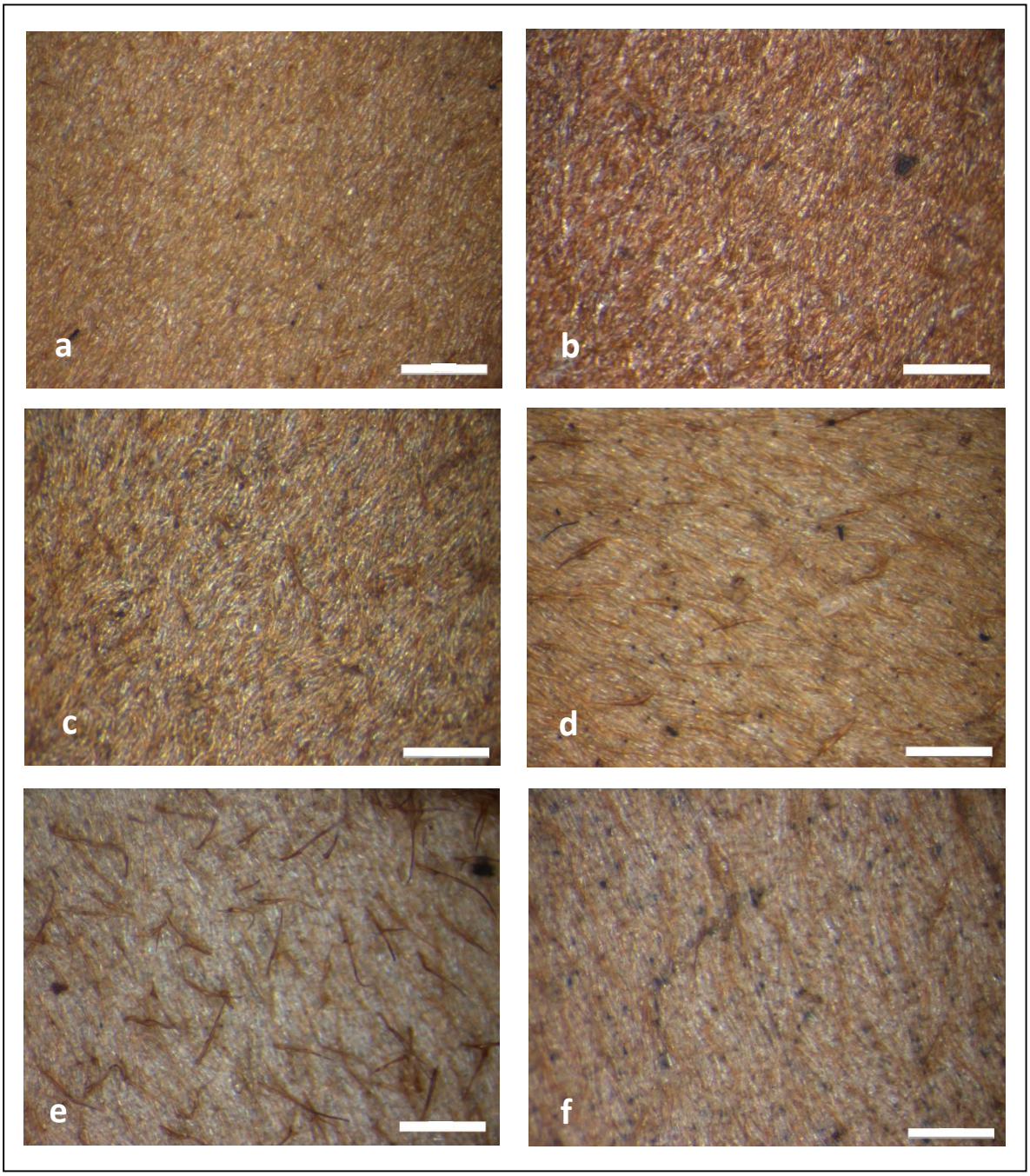


Figure 3.1.10 The four hair groups found on specimens. Group 1: short curved hairs (a, b), Group 2: intermediate curved hairs with with scattered darker hairs (c), Group 3: long hairs, with scattered darker hairs (d), Group 4: very long straight hairs with scattered darker hairs (e & f). White scale bar length = 40 µm

Grouping Taxa

Based on taxonomically informative characters, a matrix was built to group specimens into taxa (Tab. 3.1.11). In total eight taxa were recognized from the specimens examined.

Table 3.1.11 The eight taxa produced from vegetative characters observation with summary of taxonomically informative characters defined them.

Taxon	Narrow Oblong (3.0 - 5.9 : 1)	Oblong (2.0 - 2.9 : 1)	Narrow Elliptic (3.0 - 5.9 : 1)	Elliptic (2.0 - 2.9 : 1)	Oblanceolate (3.0 - 5.9 : 1)	Narrow Obovate (2.0 - 2.9 : 1)	Wide Obovate (1.2 - 1.9 : 1)	Apex: Convex	Apex: Convex - rounded	Apex: Acuminate	Apex: Retuse	Mucro feature	Number secondary veins : ≤ 15	Number secondary veins : 16 - 20	Number secondary veins : 21 - 25	Number secondary veins : ≥ 26	Angle of div. Acute narrow	Angle of div. Acute moderate	Angle of div. Acute Wide	Eucamptodromous	Brachidodromous	Intersecondary veins presence	Proximal course: perpendicular	Proximal course: parallel	Intersecondary distal course: Basiflexed	Intersecondary distal course: Parallel	Intersec. freq. : absent on some	Intersec. freq.: usually 1	Intersec. freq.: more than 1	Tertiary Pattern: Ramifyling admedial	Tertiary Pattern: Percurrent - sinous	Hairs Group 1 (10 – 20 µm)	Hairs Group 2 (21 - 30 µm)	Hairs Group 3 (31 – 40 µm)	Hairs Group 4 (>41 µm)
1	■	■		■	■	■		■	■	■	■	■		■	■	■		■	■		■	■	■		■		■	■	■	■		■			
2				■		■				■			■				■			■											■			■		
3							■		■		■	■			■				■		■									■	■				
4						■			■		■	■	■					■			■	■	■		■		■				■	■			
5	■									■	■		■	■			■			■											■			■	
6 (a,b)	■	■	■		■			■		■						■			■		■	■		■		■		■		■			■	■	■
7			■		■					■				■				■			■										■		■		
8		■				■		■	■			■	■						■		■										■		■		

3. 2 Phylogenetic assessment

3. 2. 1 Data acquisition and sequence quality

The amplified ITS region of the seventeen samples was successfully obtained through PCR, visualised as flourescent bands on agarose gels under a UV lightbox (Figure 3.2.1).

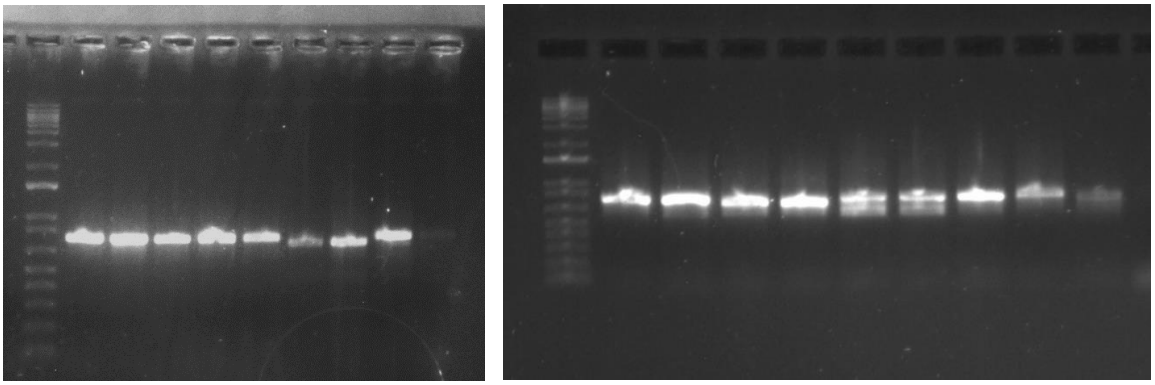


Figure 3.2.1 The 17 fluorescent bands showing the ITS region from 17 samples of *Palaquium gutta* that had been amplified successfully through the PCR procedure.

The assembly and assessment of quality of the seventeen new sequences highlighted sequences of two samples (Pal._ sp._002 and Pal._ sp._013) that could not be assembled automatically and that showed a poor quality. These were assembled by reducing the minimum match percentage. In total out of the 17 samples which were provisionally identified as *Palaquium gutta,* 6 produced good quality sequence data, 7 satisfactory data and 4 poor quality sequence data (Table 3.2.1).

Table 3.2.1 Table of specimen information, EDNA numbers and indication of quality of sequence data.

Sp code	EDNA No.	Species	Collector	Coll. Num.	Country	Quality
Pal._ sp._001	EDNA19_0053646	*Palaquium gutta*	Wilkie, P & A.T. Gwee	PW504	SG	good
Pal._ sp._002	EDNA19_0053647	*Palaquium gutta*	Wilkie, P & A. Anak Kapi	PW862	MY	poor
Pal._ sp._003	EDNA19_0053648	*Palaquium gutta*	Wilkie, P	PW822	ID	good
Pal._ sp._004	EDNA19_0053649	*Palaquium gutta*	Wilkie, P & K. Imin	PW842	MY	good
Pal._ sp._005	EDNA19_0053650	*Palaquium gutta*	Wilkie, P, M.S. Khoo, P.K.F. Leong & A. Ibrahim	PW536	MY	fair
Pal._ sp._006	EDNA19_0053651	*Palaquium gutta*	Wilkie, P, P. Hutabarat, Lancar & idel	PW1010	ID	fair
Pal._ sp._007	EDNA19_0053652	*Palaquium gutta*	Wilkie, P, P. Hutabarat, Lancar & Basuki	PW1024	ID	good
Pal._ sp._008	EDNA19_0053653	*Palaquium gutta*	Wilkie, P, P. Hutabarat, Lancar & idel	PW1031	ID	fair
Pal._ sp._009	EDNA19_0053682	*Palaquium gutta*	Hutabarat, P & Wilkie P	PWH413	ID	fair
Pal._ sp._010	EDNA19_0053683	*Palaquium gutta*	Wilkie, P & P. Hutabarat	PW1001	ID	good
Pal._ sp._011	EDNA19_0053684	*Palaquium gutta*	Wilkie, P, P.W.K. Hutabarat, Firmansyah, Hendra, Mansyah & Asgar	PW-E1102	ID	fair
Pal._ sp._012	EDNA19_0053685	*Palaquium gutta*	Wilkie, P, P.W.K. Hutabarat, Firmansyah, Hendra, Mansyah & Asgar	PW-E1098	ID	fair
Pal._ sp._013	EDNA19_0053686	*Palaquium gutta*	Wilkie, P, Y.C. Chan, Mohd. Hairul, M.A. & A. Norazmi	FRI72142	MY	poor
Pal._ sp._014	EDNA19_0053687	*Palaquium gutta*	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07419	MY	poor
Pal._ sp._015	EDNA19_0053688	*Palaquium gutta*	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07432	MY	good
Pal._ sp._016	EDNA19_0053689	*Palaquium gutta*	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07443	MY	fair
Pal._ sp._017	EDNA19_0053690	*Palaquium gutta*	Wilkie, P, Siti Munirah, Mohd. Hairul, M.A. & Nazre	FRI75020	MY	poor

* ID= Indonesia, MY= Malaysia, SG= Singapore

3. 2. 2 Maximum Parsimony Analyses of the Isonandreae and *Palaquium* Matrices

A summary of the statistical information for each of the parsimony analysis carried out on the Isonandreae and *Palaquium* matrices are shown in Table 3.2.2 below.

Table. 3.2.2 Parsimony analysis statistic of Isonandreae matrix and *Palaquium* matrix

	Isonandreae Matrix	***Palaquium* Matrix**
Total Samples	277	82
Total Characters (aligned length)	885	723
Constant Characters (excluded)	234 (26.4%)	427 (59.1%)
Included characters	651 (73.6%)	296 (40.9%)
Uninformative characters	172 (26.4%)	99 (33.4%)
Parsimony Informative	479 (73.6%)	197 (66.6%)
Number of most parsimony trees	17	139
Tree length (steps)	3036	546
Steps per character	3.43	0.76
CI (Consistency Index)	0.385	0.685
RI (Retention Index)	0.8175	0.8680

The maximum parsimony analysis of the Isonandreae matrix included 277 samples, 17 of which are new to this study, and consisted of a data matrix of 885 characters. Of these 234 characters were constant and 479 potentially parsimony informative. The maximum parsimony analysis produced 17 equally parsimonious trees with a tree length of 3036 steps. The consistency index (CI) and retention index (RI) values give an idea of amount of homoplasy and proportion of synapomorphy respectively. The trees produced from the Isonandreae matrix had a consistency index (CI) of 0.385 and retention index (RI) of 0.8175.

A phylogram of one of the trees from the maximum parsimony analysis showing branch lengths is shown in Fig. 3.2.2. Samples with unusually long branches appear to be caused by the poor quality of their sequences. Bootstrap value on branches generated from the parsimony bootstrap analysis are placed in the majority rule consensus tree to show the support for each branch (Appendix E).

A majority rule consensus trees using the 17 most parsimonious trees is shown in Fig. 3.2.3, with frequencies above the branches and bootstrap values pertaining to the newly added sequences below the branches. This tree shows that five newly added sterile samples were erroneously identified as *Palaquium* sp. and in fact belong to the genus *Payena* since they fall within a *Payena* clade (EDNA19_0053685, EDNA19_0053684, EDNA19_0053690,

EDNA19_053652, EDNA19_0053651). All other newly added samples fell within the *Palaquium* clade (Fig 3.2.3). One previously sequenced sample of *Payena obscura* (EDNA12_0025088) fell within the *Palaquium* clade (*P. impressionervium* sub-clade). This, however, had been erroneously identified and has subsequently been afterwards identified as *P. impressionervium*. In the analysis of the *Palaquium* matrix this sample had been eliminated.

Isonandreae Matrix Tree

The phylogenetic tree produced by maximum parsimony of the ITS data set of tribe Isonandreae, is well resolved at the tribe and genus level. The tree corresponds to the Pennington classification (1991) which supports the monophyly of Tribe Isonandreae and, it is sister relationship to Mimusopeae. It was represented by the genera *Palaquium, Aulandra, Isonandra, Burckella, Madhuca, Diploknema,* and *Payena,* which were included in the analysis.

At least five major clades were generated from the MP analysis, *Payena* clade, *Madhuca* clade, *Burckella* clade, *Isonandra* clade and *Palaquium* clade. The first three clades are statistically well supported, while the *Palaquium* clade and *Isonandra* clade are less strongly supported (74% BS and 78% BS respectively). Other than *Burckella,* most genera showed polyphyly within the Isonandreae clade, however most sample of each genera are placed in the same clade. Likewise, most of *Palaquium* sample are placed in the *Palaquium* clade, and the polyphyly of *Palaqium* was believed to be caused by low quality sequences or simply misdetermination due to polymorphic characters in many member of Sapotaceae and that the specimens were sterile.This was not investigated further as it was outside the scope of this project.

In the Isonandreae tree, *Palaquium* was sister to other genera within Isonadreae. Even though the large clade of *Palaquium* was not strongly supported, the sub-clade consisting of *P. impressionervium* and the large sub-clade consisting of the rest of the *Palaquium* was strong supported (100% BS and 90% BS respectively).

The genus *Aulandra* was nested in the *Palaquium* clade. *Aulandra* which comprises only three species, is morphologically sister to *Palaquium* by sharing most of the characters except a cauliflorous characters and partially united filaments (Pennington, 1991). Further study into this genus with molecular data is suggested to elucidate the relationship between *Palaquium* and *Aulandra*.

Palaquium Matrix Tree

In total 12 newly added samples were placed within the *Palaquium* clade. However, sample P._sp._13 (EDNA19_0053686) was eliminated from the *Palaquium* analysis due to its poor sequence quality highlighted by its long branch in the phylogram (Fig. 3.2.2).

The maximum parsimony analysis of the realigned *Palaquium* matrix, including 11 new sequences placed within the *Palaquium* clade in the Isonandreae analysis, produced 139 most parsimonious trees. A phylogram of one of these trees showing branch lengths is shown in Fig. 3.2.4. Two of the newly added samples in the *Palaquium* matrix with poor quality sequences (Tab. 3.2.1) both showed longer branch lengths (EDNA19_0053687, EDNA190053647). The samples Pal._dasyphyllum_EDNA07_01936 and the Genbank accession of *Aulandra_longifolia*_KF597536 had particularly long branches which is also likely due to their low quality sequence.

The strict consensus tree and the 50% majority rule consensus trees using the 139 most parsimonious trees is shown in Fig. 3.2.6 and 3.2.5 respectively., with frequencies above and bootstrap values below the branches. This tree shows that new accessions provisionally identified as *Palaquium gutta* fell into 5 different sub-clades. Most (six) samples were placed with a specimen previously identified as *Palaquium gutta* in a weakly supported (55% bootstrap, BS) "GUTTA" clade, one is placed with *P. oxleyanum* and two, as yet unidentified, species in a weakly supported (60% BS) "OXLE" clade, one with *P. eriocalyx* in an unsupported (<50% BS) "ERIO" clade, one with two accessions of *P. calophyllum* in a weakly supported (63% BS) "CALO" clade and two within an unsupported clade (<50% BS) containing two *P. serecium* accessions, one *P. dasyphyllum* accession and one *P. formosanum* accession in a "SERI" clade.

The level of character changes in the Isonandreae matrix trees was highly saturated compared to character changes in the *Palaquium* matrix. This is indicated by the high value of 3.43 average steps for each character, while it was 0.76 for the *Palaquium* matrix (Table 3.2.2.). The Isonandreaea matrix (CI=0.385) had more homoplasy compared to the *Palaquium* matrix (CI=0.685). This is expected since the Isonandreaea matrix comprised a taxonomically and genetically wider sampling which allowed more number of character changes in parallel to occur. However, the proportion of synapomorphy (not homoplastic) of both matrices were similarly high, with an RI of above 0.8.

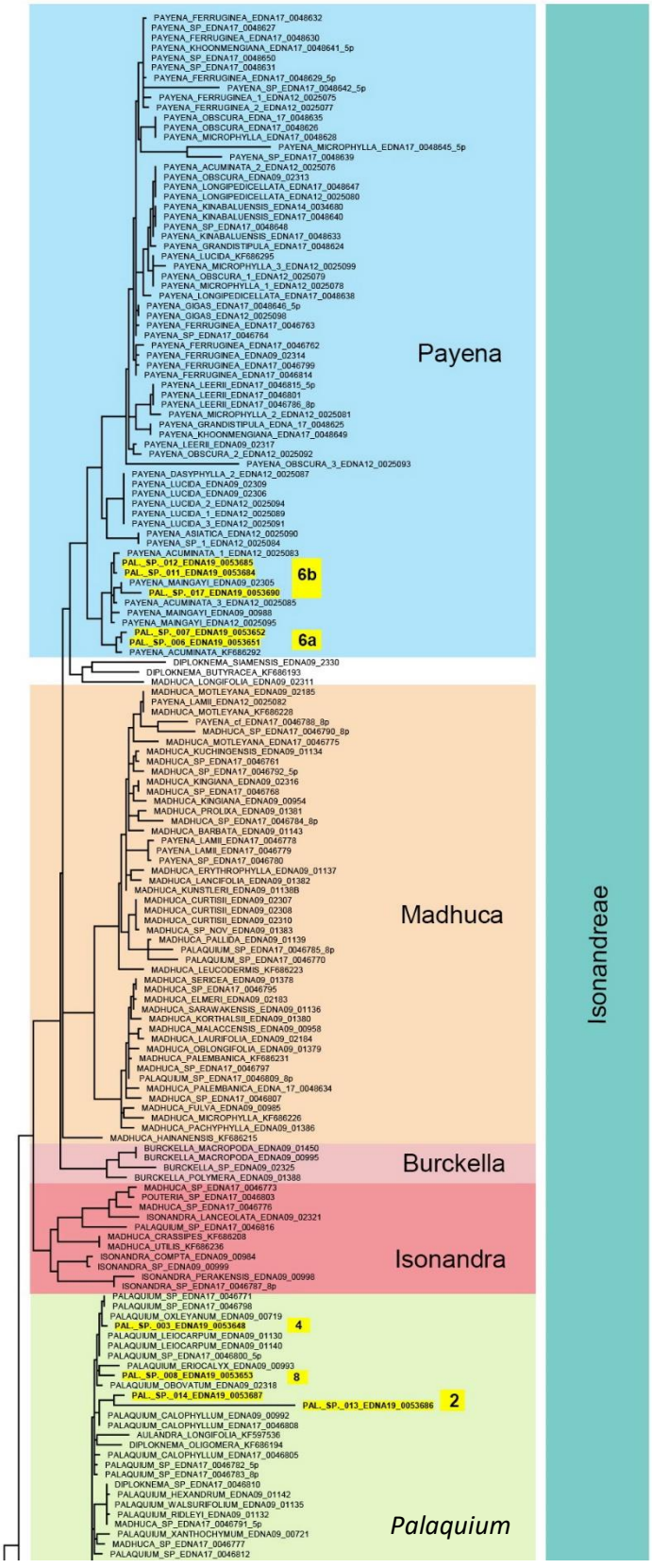


Figure 3.2.2 (***part 1***). Phylogram of one of the 17 equally most parsimonious trees (3036 steps, CI= 0.385, and RI =0.8175) of the Isonandreae matrix of 277 taxa. Branch length shows the number of character change (divergence) along the branches. New accessions and the taxon group are marked in yellow.

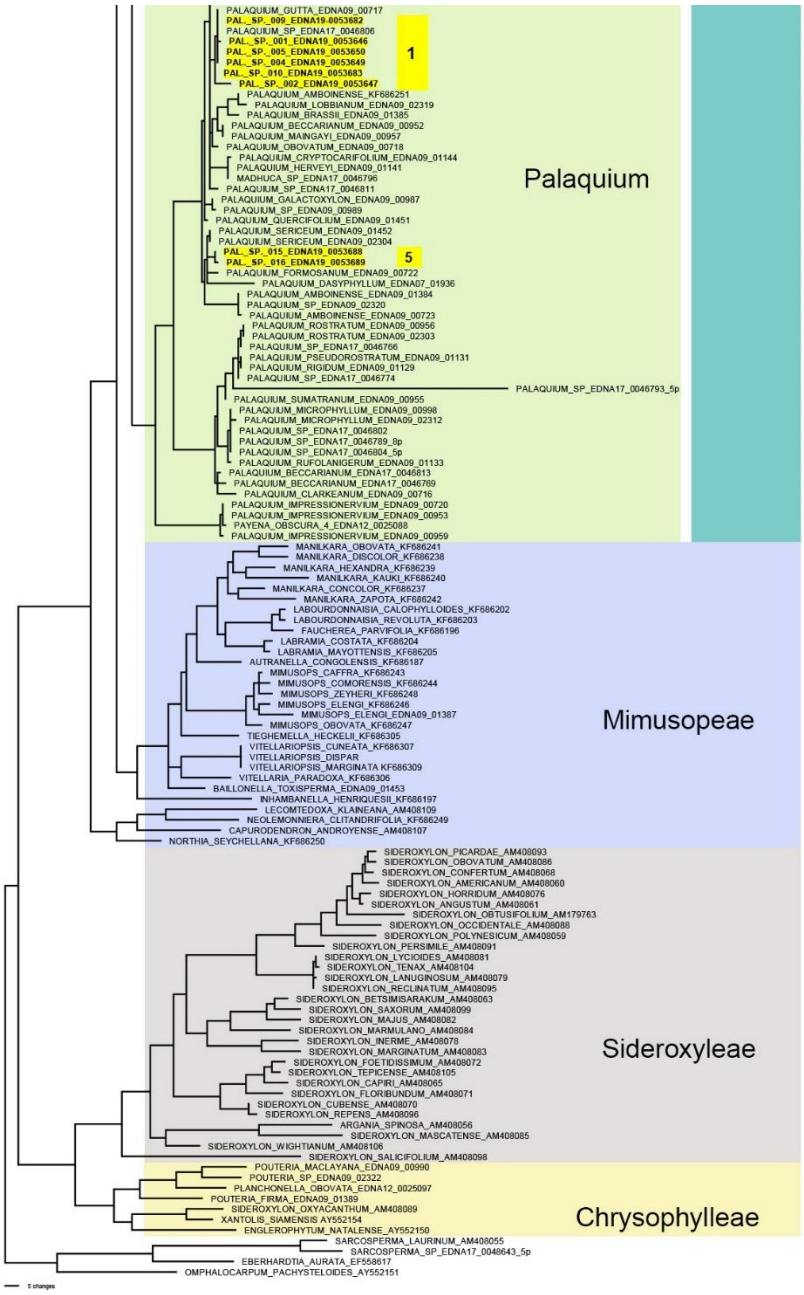


Figure 3.2.2 (part 2). Phylogram of one of the 17 equally most parsimonious trees (3036 steps, CI= 0.385, and RI =0.8175) of the Isonandreae matrix of 277 taxa. Branch length shows the number of character change (divergence) along the branches. New accessions and the taxon group are marked in yellow.

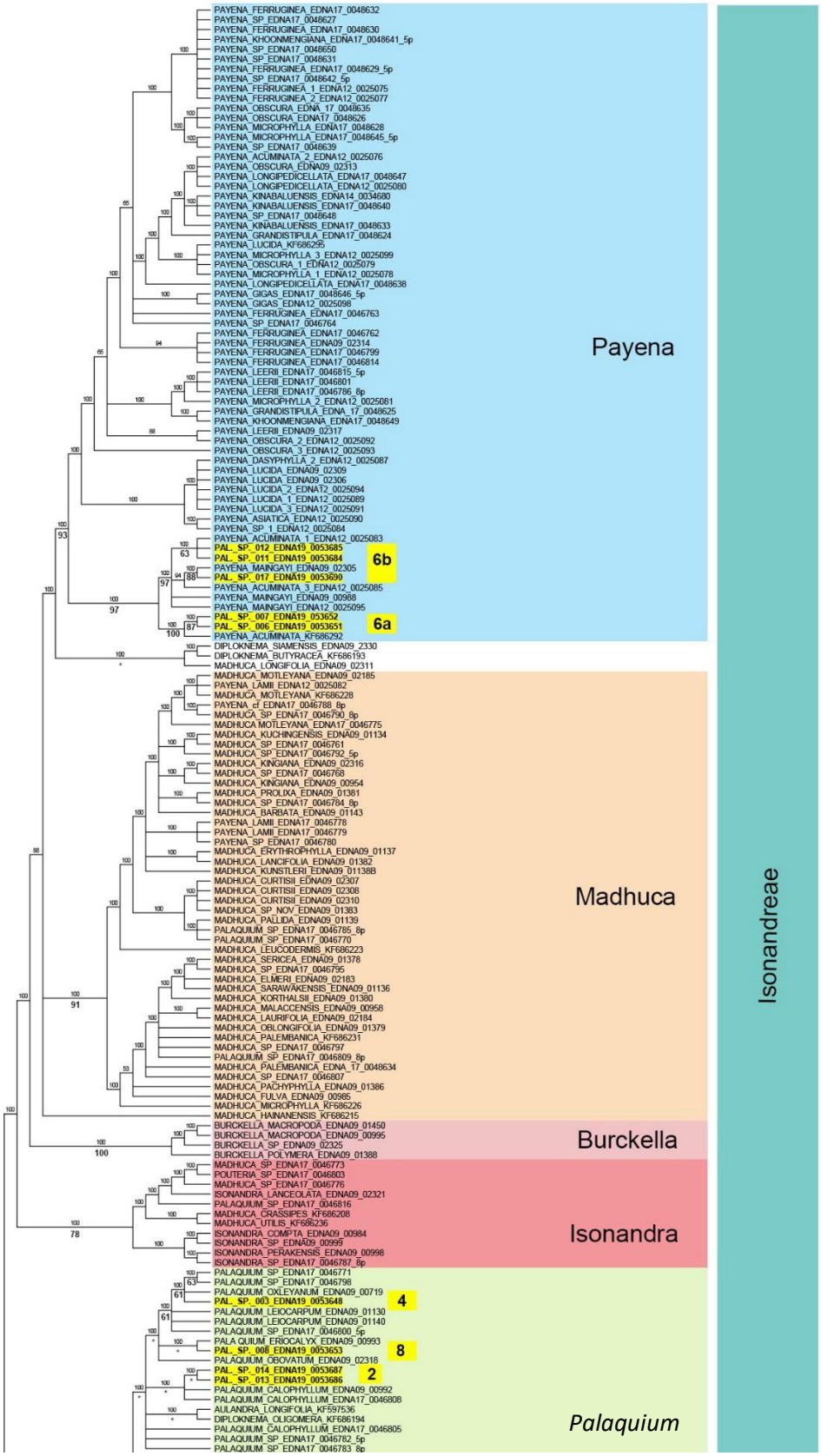


Figure 3.2.3 (part 1) 50% majority rule consensus tree produced from 17 equally most parsimonious trees (3036 steps, CI= 0.385, and RI =0.8175) for the Isonandreae matrix of 277 taxa. Branch consensus frequencies are shown above the branches and bootstrap support (BS) values are shown below for branches relevant for the newly added samples. New accessions and the taxon group are marked in yellow.

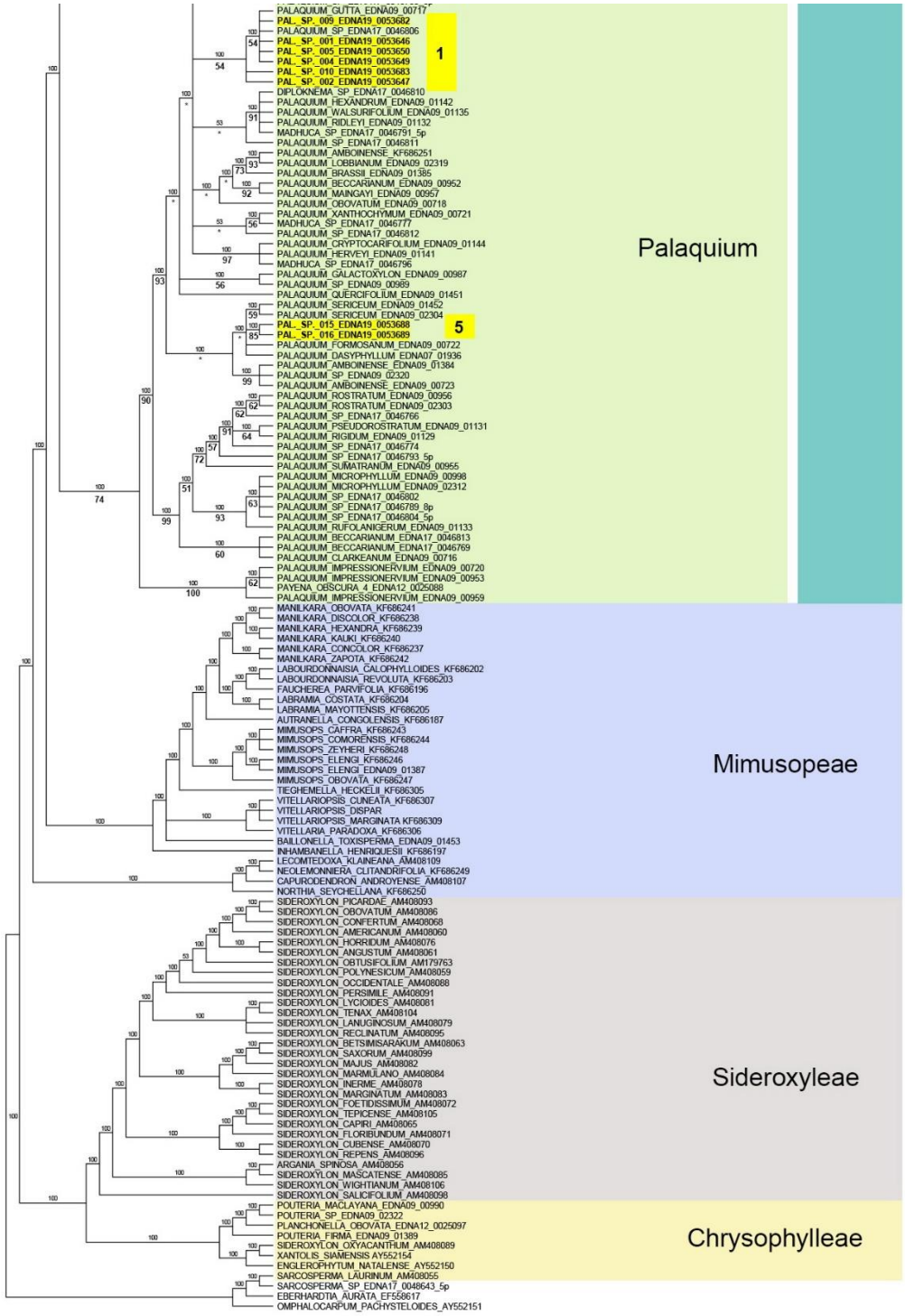


Figure 3.2.3 (part 2) 50% majority rule consensus tree produced from 17 equally most parsimonious trees (3036 steps, CI= 0.385, and RI =0.8175) for the Isonandreae matrix of 277 taxa. Branch consensus frequencies are shown above the branches and bootstrap support (BS) values are shown below for branches relevant for the newly added samples. New accessions and the taxon group are marked in yellow.

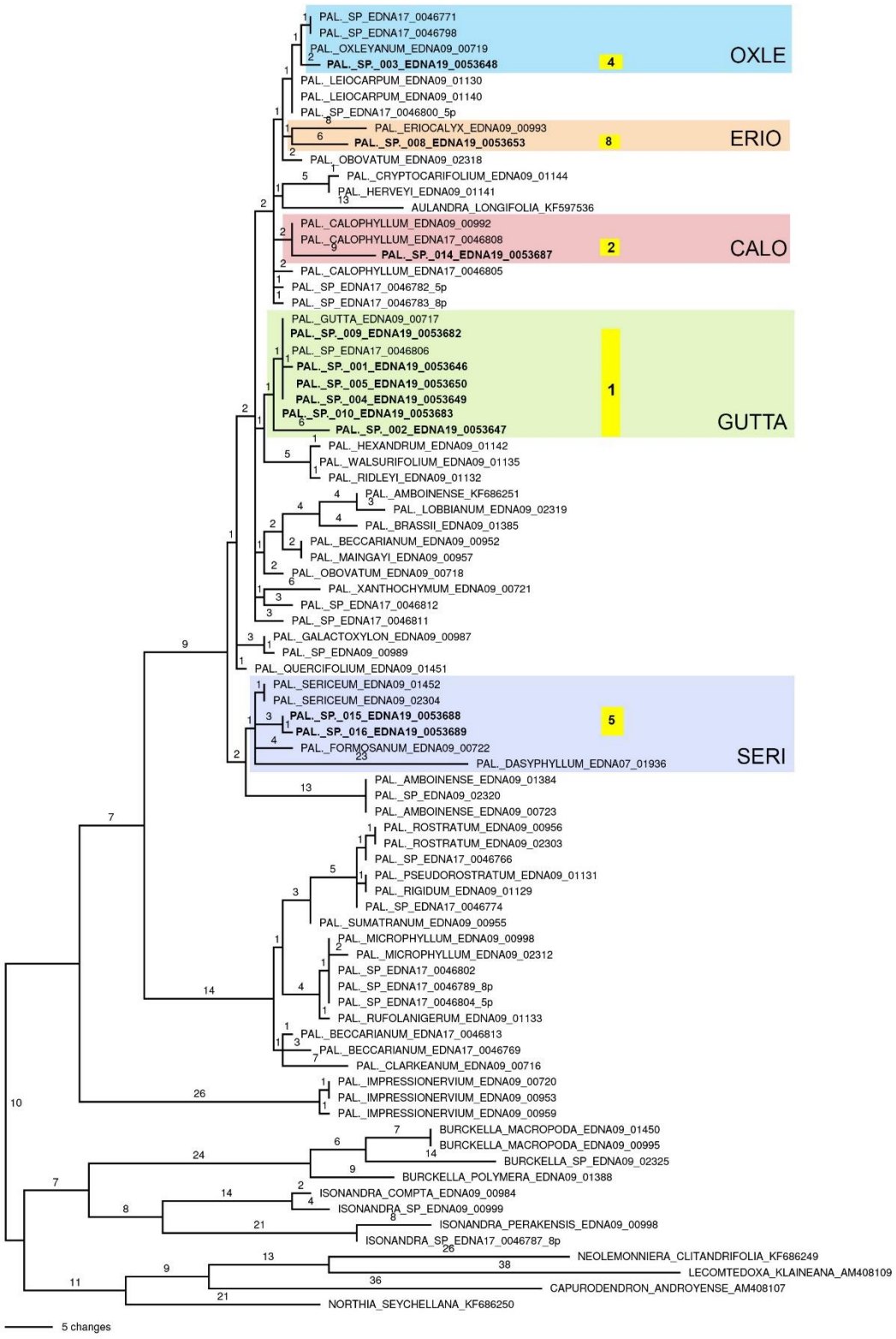


Figure 3.2.4 Phylogram of one of 139 equally most parsimonious trees (546 steps, CI= 0.685, and RI =0.8680) for the *Palaquium* matrix of 82 taxa. Branch length shows the number of character change (divergence) along each branch. New accessions are marked in bold with yellow highlight on the Taxon number.

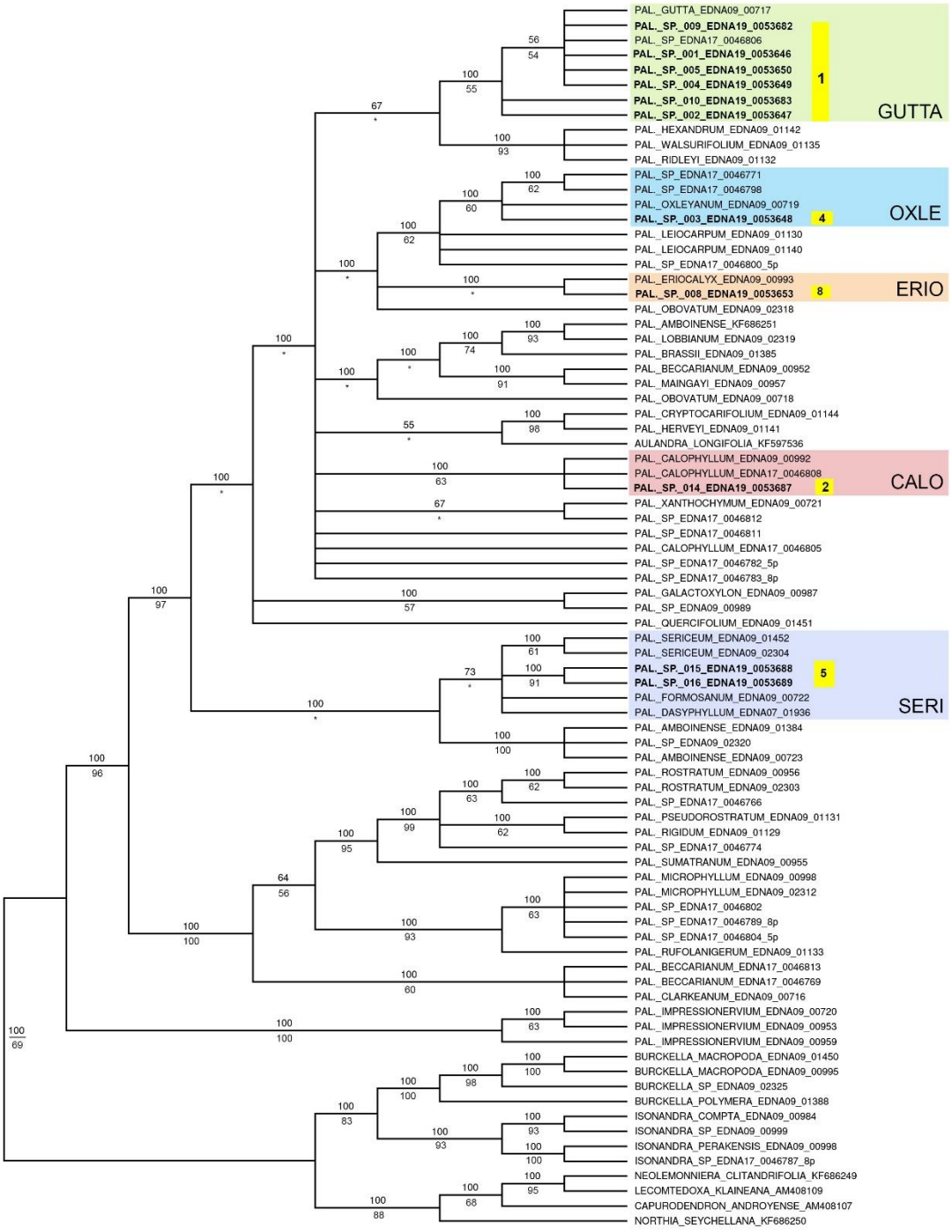


Figure 3.2.5 50% majority rule consensus tree based on 139 equally most parsimonious trees (546 steps, CI= 0.685, and RI =0.8680) produced from the *Palaquium* matrix of 82 taxa. Branch consensus frequencies are shown above and bootstrap support (BS) values below the branches. * indicates branches with bootstrap support values <50%. New accessions are marked in bold with yellow highlight on the Taxon number.

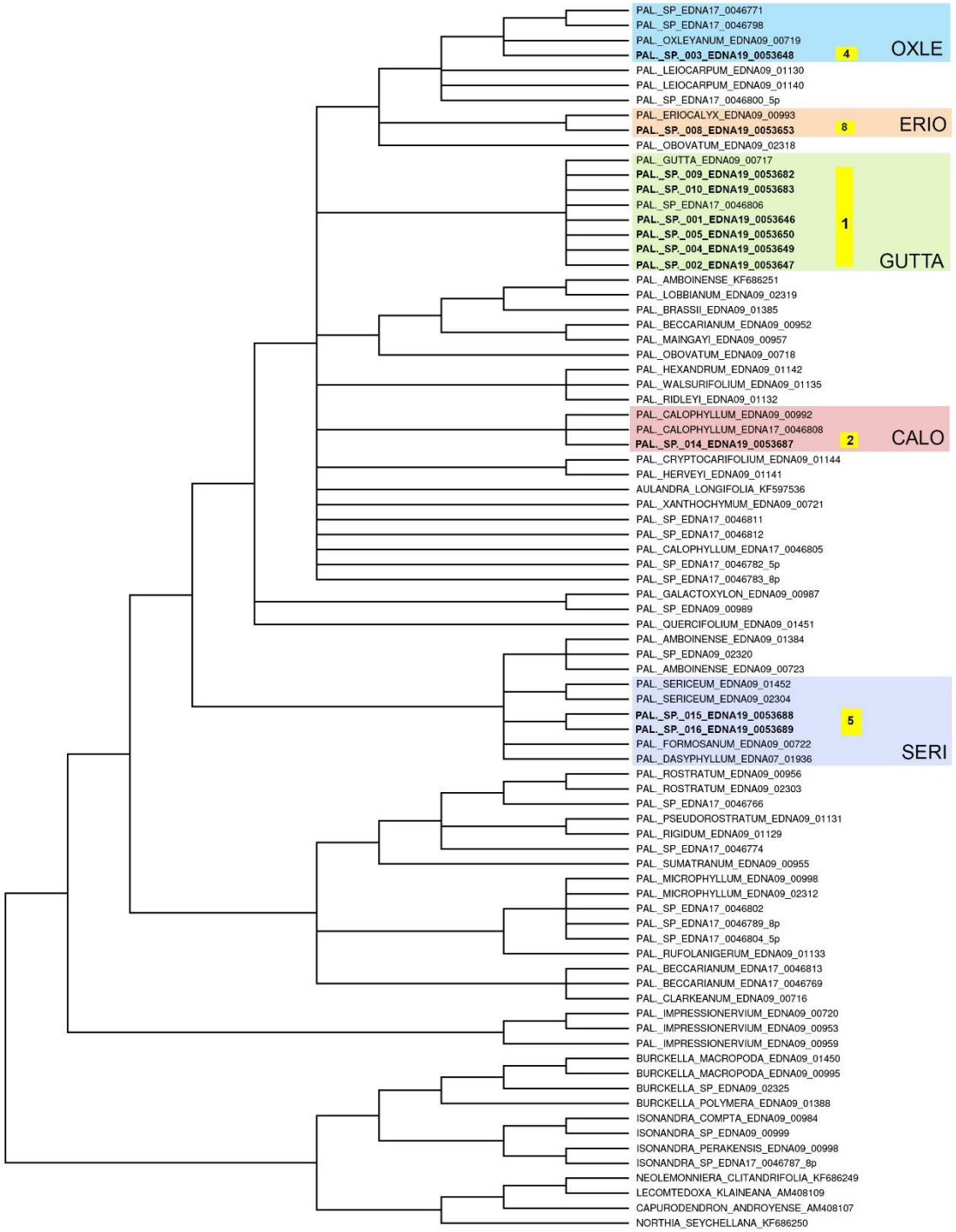


Figure. 3.2.6 Strict consensus tree based on 139 equally most parsimonious trees (546 steps, CI= 0.685, and RI =0.8680) produced from the *Palaquium* matrix of 82 taxa. New accessions are marked in bold with yellow highlight on the Taxon number.

3. 2. 3 Bayesian Inference analysis of the *Palaquium* matrix

MrModeltest 2.3 suggested different models for each partition of the ITS region. These were GTR+G for the spacers and K(80) for the 5.8S gene. The Bayesian analysis using the MrBayes programme ended with average standard deviation of split frequencies of 0.007759, which indicated good convergence of the two independent MrBayes runs. The acceptance rates and swapping frequencies also suggested a good mixing between the 4 chains in a run (Appendix C). Plotting the likelihood values against the generations showed that the burn-in of 25% was sufficient (Appendix D1 & D2).

The majority rule consensus tree topology from the Bayesian inference analysis of the *Palaquium* matrix using both models was congruent with that of the maximum parsimony majority rule tree but better supported estimates of phylogenetic relationships were found compared to the maximum parasimony analysis (Fig. 3.2.7). The phylogenetic tree of Bayesian inference analysis showed a strong support (0.95 PP) for the monophyly of *Palaquium*. All new accessions were placed in the same clades as in the parsimony analysis, with posterior probability values for the OXLE clade of 0.97, ERIO clade of 0.62, CALO clade 1, GUTTA clade of 0.98, and SERI clade of 0.92.

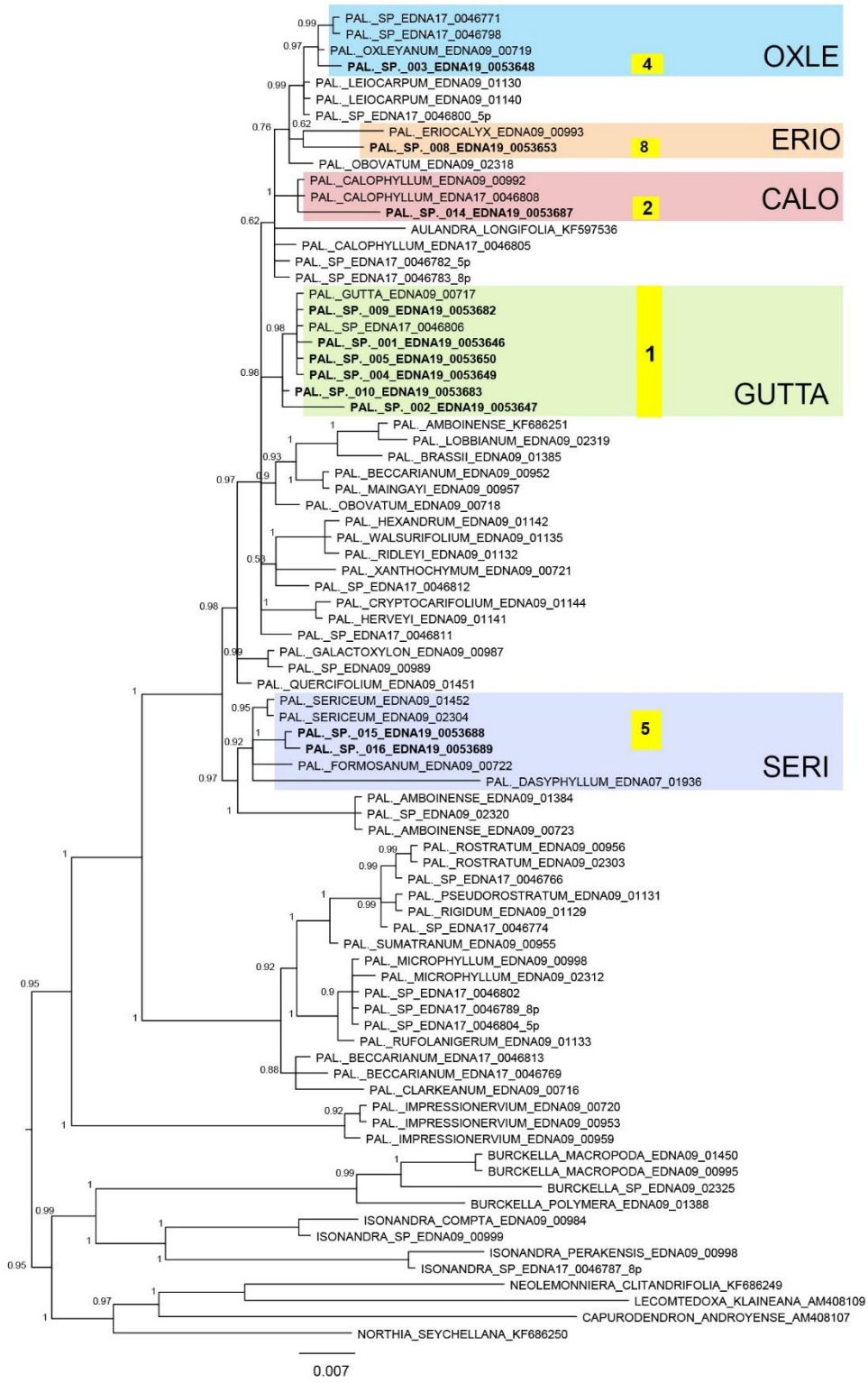


Figure. 3.3.7. A 50% Majority rule Bayesian consensus tree of *Palaquium* matrix showing the new accession in five sub-clades within the *Palaquium* clade and their posterior probability (PP). Branch lengths are the average length across all trees. New accessions are marked in bold with yellow highlight on the Taxon number.

3. 3 Morphological characters & phylogenetic support

A summary table of the result of observation on forma grouping, vegetative characters assessment, and phylogenetic analysis is given in Table 3.3.1. There are 35 herbarium specimens, 3 forma, 9 taxa, 17 DNA sample (EDNA) and 6 clades (including *Payena* clades). It should be noted that two herbarium specimens of DNA samples (PW-E1102 and PW-E1098) were missing and have been found atthe end of the project. Their data were excluded from the morphology table, but a brief observation was conducted on both samples. One of the DNA samples had a herbarium specimen (PWH 413) located in KRB (Kebun Raya Bogor Herbarium, Indonesia). This was observed by my colleague and from a photo only.

Table 3.3.1. Summary table of forma, taxa and clades based on the specimens and the DNA sample. ***Forma***: Gen. = Genuinum; Sel. = Selendit; Bor = Borneensis; Unpl. = unplaced; ? = doubtfully.

No.	Bar Code	Collector	Coll. No.	EDNA No.	Forma	Taxon	Clade	Notes
1	E00013587	J. Sinclair	5474		Gen.	1		
2	E00230060 (isotype)	Lobb	290		Gen.	1		
3	E00230061 (isotype)	Lobb	290		Gen.	1		
4	E00277821	G. Wray	1100		Gen.	1		
5	E00290117	Wilkie, P. & Gwee, A. T.	PW504	EDNA19_0053646	Gen.	1	GUTTA	
6	E00290128	Wilkie, P. Khoo, MS, Leong, PKF & Ibrahim A.	PW536	EDNA19_0053650	Gen.	1	GUTTA	
7	E00304060	Wilkie, P. & Imin, K, Kueh, HL.	FRI 52855		Gen.	1		
8	E00304198	Wilkie, P.	FRI 52919		Gen.	1		
9	E00331748	s.n.			Gen.	1		
10	E00416389	Wilkie, P.	FRI 72142	EDNA19_0053686	Gen.	1	CALO	not in *Palaquium* analysis as poor quality sequence but in CALO in large analysis
11	E00646660	Wilkie, P. & Imin	PW842	EDNA19_0053649	Gen.	1	GUTTA	
12	E00664277	Wilkie P. , Anak Kapi, A.	PW862	EDNA19_0053647	Gen.	1	GUTTA	
13	E00898190	Wilkie, P, , Hutabarat P.	PW1001	EDNA19_0053683	Gen.	1	GUTTA	

No.	Bar Code	Collector	Coll. No.	EDNA No.	Forma	Taxon	Clade	Notes
14	H2012/0110112	Curtis	780		Gen.	1		
15	H2012/0110411	Curtis	B 3582		Gen.	1		
16	H2012/0110414	s.n.	Rec-B:1887		Gen.	1		
17	H2012/0110415	Lobb	-		Gen.	1		
18	H2012/0110416	Lobb	-		Gen.	1		
19	H2012/0110417	A. C. Maingay, M.D.	983		Gen.	1		
20	H2012/0110418	s.n.	-		Gen.	1		
21	H2012/0110419	P. F. Cockburn	FRI 7682		Sel.?	1		
22	H2012/0110420	T. C. Whitmore	FRI 12527		Gen.	1		
23	H2012/0110421	T & P	T&P362		Gen.	1		
24	K00639125 (type)	Lobb	290		Gen.	1		
25	K0077680 (type)	Oxley	s.n.		Gen.	1		
26	E00903606	Wilkie, P. *et al.*	SFC 07419	EDNA19_0053687	Unpl.	2	CALO	
27	E00913534	Rantai Jawa *et al*.	S 71048		Born.	3		
28	E00639765	Wilkie, P.	PW 822	EDNA19_0053648	Born.	4	OXLE	
29	E00903604	Wilkie, P. *et al.*	SFC 07443	EDNA19_0053689	Unpl.	5	SERI	
30	E00903605	Wilkie, P. *et al.*	SFC 07432	EDNA19_0053688	Unpl.	5	SERI	
31	E00898191	Wilkie, P., Hutabarat P.	PW1010	EDNA19_0053651	Gen.	6a	*PAYENA*	not in *Palaquium* analysis
32	E00898554	Wilkie, P. & Hutabarat	PW1024	EDNA19_0053652	Gen.	6a	*PAYENA*	not in *Palaquium* analysis
33	E00416341	Wilkie, P.	FRI75020	EDNA19_0053690	Gen.	6b	*PAYENA*	not in *Palaquium* analysis
34	E00834389	Markus, G.	SAN 151019		Unpl.	7		
35	E00898547	Wilkie, P., Hutabarat, P., Lancar & Idel	PW1031	EDNA19_0053653	Unpl.	8	ERIO	
36	-	Wilkie, P *et al.*	PW-E1102	EDNA19_0053684	Gen.	6b	*PAYENA*	specimen was missing and recently found.
37	-	Wilkie, P *et al.*	PW-E1098	EDNA19_0053685	Gen.	6b	*PAYENA*	specimen was missing and recently found.
38	-	Hutabarat *et al.*	PWH413	EDNA19_0053682	Gen.	1	GUTTA	herbarium specimen in KRB

4. DISCUSSION

4. 1 The circumscription of *Palaquium gutta* (Hook.) Baill.

The most recent taxonomic accounts of Sapotaceae, Ng (1972) and Pennington (1991), used flower characters (calyx, stamens, and inflorescence) to determine generic limits. They both highlighted that vegetative characters might not be very informative at the genus level and can lead to incorrect genus determination within the tribe or family. In this study this has been found to be the case with several sterile specimens being incorrectly identified to genus. The fact that many Sapotaceae specimens in herbaria are sterile, in part due to many specimens being collected by foresters who generally use vegetative characters but also the fact that many species of Sapotaceae produce flowers and fruits very infrequently (often only every 5-10 years) is problematic. In this study a detailed examination of the morphology of collections provisionally identified as *Palaquium gutta* and the molecular sequencing of the material has helped clarify the identification of specimens and illuminate potentially taxonomically useful characters.

A major problem in determining sterile specimen of *Palaquium gutta* (Hook.) Baill. was the highly diverse nature of the vegetative characters within family. The 35 specimens that were provisionally determined as *Palaquium gutta* showed very high morphological diversity both in gross and microscopic characters.

Based on morphological and molecular data 8 distinct taxa have been recognised in this study.

Taxon 1 corresponds with the species descriptions of Lam (1925; 1927), van Royen (1960) and Ng (1972) and is clearly true *Palaquium gutta* since it includes a type specimen and two isotype (*Oxley* s.n. and *Lobb* 290). Most of the specimens examined (25 out of 35 specimens-shown from No. 1 to 25 in table Tab. 3.3.1.) were in this taxon. The taxon was morphologically highly variable showing the most diverse leaf shape (oblong, elliptic and obovate), form (narrow oblong, oblong, elliptic, oblanceolate, and narrow obovate), leaf apex (convex, convex rounded, acuminate, and retuse), petiole length (0.7 - 6 cm), and the leaf length (7 - 18 cm). It is very common and yet confusing that the leaf forms and leaf apices are different in the same twig. The leaf size also could be significantly longer and larger on younger leaves compared to the older leaves. Dubard (1909) identified the remarkable habit and variation as the cause of

ambiguity with many similar species. This was later addressed by Lam (1925) who united some of the similar species together and recognising forma.

The hairs of this taxon were of the Group 1 type (Figure 3.1.10), short, curved, and uniform in colour (rarely had more than two colours on the same surface). However, this character was not useful for Taxon 1 circumscription since similar hairs were found in Taxon 3, 4, 7 and 8. Hair colour on the abaxial leaves varied from golden yellow, light brown, to coppery dark brown. The hair colour usually turned paler or whitish or greyish as they got older, and as such it was found to be inconsistent and uninformative in this study.

Taxon 1 had a large range in number of secondary veins (16 – 26 or more), acute-moderate to acute-wide in angle of divergence, and a simple or festooned or mixed brachidodromous venation pattern. Van Royen (1960) highlighted that the secondary veins joined very close to the margin or faded until inconspicuous, while Ng (1972) confirmed the presence an inconspicuous intramarginal vein. This study also supports the presence of brachidodromous venation but found the joined veins near the leaf margin were not clearly seen on young leaves or when the leaf surface was covered by dense hairs. The lighting direction during observation very much influenced the clarity of the venation around the margin.

The two most significant characters in the Taxon 1 are the presence of intersecondary veins and admedial ramifying tertiary veins (Fig.4.1). One or more than one perpendicular intersecondary vein per intercostal area was usually present and it was very rarely absent in an intercostal area. The intersecondary veins could be absent in some intercostal areas because of various reasons: the intercostal area was too narrow and did not provide enough space for intersecondary veins to develop, the intersecondary veins had not fully developed in young leaves, or they were just less visible due to hair thickness. It was found to be very important to check other leaves on the same twig to confirm the presence of this key character.

Any perpendicular interscondary veins found in Taxon 1 ended joining the secondary veins somewhere in the middle with angle < 90^{o} or basiflexed (Fig. 3.1.9 a). This was different to the intersecondary veins of Taxon 6 which ran parallel and joined the secondary veins in the looping area near the margin (Fig. 3.1.9 b). The presence of intersecondary veins in *Palaquium gutta* is supported by Ng (1972) who recognised it as "*one or more prominent tertiary nerves developing between and running almost parallel to each pair of secondaries"*.

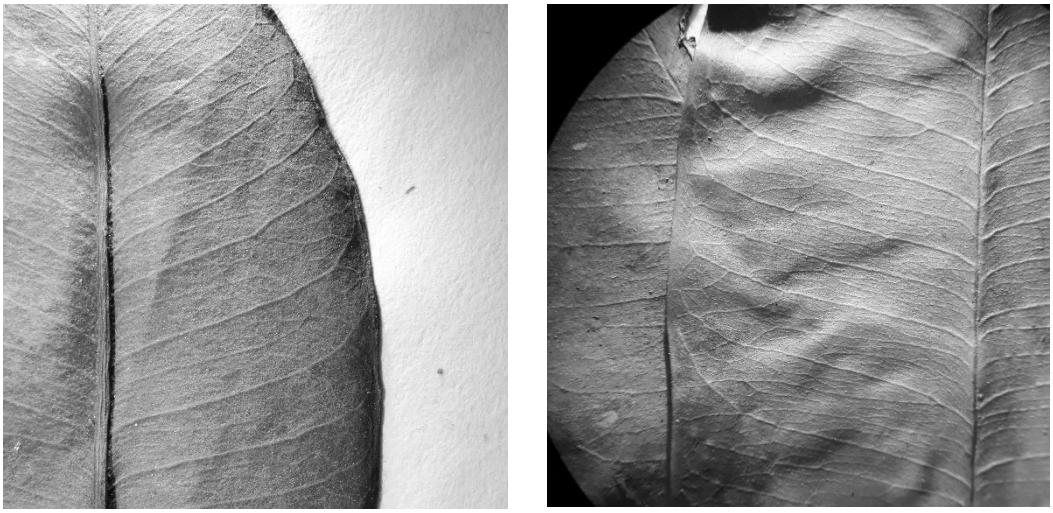


Figure 4.1 *Palaquium gutta* typical leaf venation

The second character, admedially ramified tertiary veins (Hickey, 1973; Ellis *et al.*, 2009) or descending from margin (or near margin) toward the midrib (Lam, 1925; Ng, 1972) is also indicative of this taxon. Tertiary venation patterns were unclear in van Royen (1960) who mentioned various patterns: transverse (percurrent) or subparallel, parallel to secondary venation (ramified), and reticulate, while Ng (1972) did not mentioned it at all. The confusion in patterns may be caused by the branch of ramified veins running from multiple points, including along secondary veins, and sometimes crossed each other. The ramified veins or the branches sometimes run across secondary veins and reach the adjacent secondary veins which make them looked like percurrent veins.

Morphologically there are two other taxa similar to Taxon 1: Taxon 4 and Taxon 6. Both taxa possessed intersecondary veins. However, despite also having admedially ramified tertiary veins, the intersecondary veins in Taxon 6 were more parallel than perpendicular, the distal veins were also parallel, never basifixed, and the marginal loops of the brachidodromous pattern were exclusively simple, never festooned or mixed. On the other hand, Taxon 4 had perpendicular intersecondary veins but these were usually absent on some or most of intercostal area (or totally absent) and the tertiary pattern was percurrent. Taxon 4 also never has more than 20 secondary veins, while Taxon 1 commonly has more than 20 secondary veins. Taxon 4, however, had percurrent tertiary venation which helps to easily separate it from Taxon 1.

All of the specimens placed in this group and that had sequence data available fell within the **GUTTA** clade of the phylogenetic analysis and supports recognition of this group as separate from others. The proposed close relationship using intersecondary venation and tertiary veination patterns with Taxon 4 and Taxon 6 is not supported. Taxon 4 being placed in a separate clade, OXLE clade, with *Palaquium oxleyanaum* and other, as yet unidentified, specimens. This suggests that venation pattern may have possibly evolved more than once across the genus. Taxon 6 was nested within the *Payena* clade, outside of the *Palaquium* clade (see Fig. 3.2.2; 3.2.3). Morphologically, other than characters of intersecondary veins and brachidodromous venation, Taxon 6 hairs were considerably longer (Group 2, 3 and 4 type) compared to Taxon 1 (Group 1 type). This suggests that intersecondary veins might be shared in the ancestor of two genera, *Palaquium* and *Payena*. It would be interesting to make an assessment of intersecondary vein across all genera in the tribe.

The results of this study clearly circumscribe *Palaquium gutta* morphologically by a combination of those two characters. The absence of which in any specimens would keep them out of Taxon 1. This is in disagreement with Lam's forma concept which is discussed below.

4. 2 Elucidating Lam's Forma Concept

It is clear from this study that there is overlapping morphology between the forma defined by Lam (1925; 1927). Placing specimens into forma (Fig 3.1.1.) was not easy and some specimens fitted more than one forma description. For instance, placing *Cockburn* FRI7682 by leaf size placed it in forma Selendit, but using both transverse and parallel tertiary veins placed it in forma Genuinum. Since leaf shape, leaf apex shape and leaf size were variable, the venation pattern was prioritised in the placement of specimens into two major groups - both forma Borneensis and Selendit had mostly transverse tertiary veins, while Genuinum and Vrieseanum could have both parallel and transverse, or exclusively parallel. Within the two groups, Borneensis-Selendit or Genuinum-Vrieseanum, two forma were separated by characters of leaf size and leaf apex shape. In other words this study found forma Selendit to be a small leaved version of Borneensis, and Vrieseanum a small leaved version of Genuinum without any transverse veins. This corresponds to van Royen (1960).

Excluding the unplaced group of specimens, this research placed the specimens into two forma only, forma Borneensis, and forma Genuinum. The uncertain specimen in Selendit afterward was placed in forma Genuinum by prioritising tertiary venation pattern over leaf size.

Forma Genuinum comprised all specimens of Taxon 1 and Taxon 6. Taxon 1 was confirmed both morphologically and in the molecular phylogenetic analysis as *Palaquium gutta*. It confirms that the parallel tertiary veins in the forma concept of Lam (1925) refers to the ramified tertiary veins of Hickey (1973) and Ellis *et al.*, (2009), and that transverse veins on Genuinum according to Lam could be just be branches of ramifying veins running across the intercostal area. Molecular data placed Taxon 6 in a *Payena* clade (Fig 3.2.2; 3.2.3) and subsequent morphological re-examination of the specimens confirmed the specimens in this taxon to indeed be *Payena* sp. This highlights a major issue with using morphology only to identify sterile specimens in the family. The parallel intersecondary veins, simple brachidodromous and longer hairs are the diagnostic of the Taxon 6 and on careful inspection can be separated from *Palaqium gutta* which has perpendicular intersecondary veins, simple or festooned brachidodromous, and shorter hairs. However, without a range of specimens from each taxon to compare the distinction is very difficult and placement in other genera is possible.

Forma Borneensis comprised two specimens from two taxa, Taxon 3 (provisionally identified as *P. leiocarpum*) and Taxon 4 (provisionally identified as *P. oxleyanum*). Both taxa were morphologically close to each other. The new sequence of Taxon 4 fell in the OXLE clade (60% BS; 0.97 PP), with another *P. oxelyanum* previously sequenced. No new sequence data was available for *P. leiocarpum* (Taxon 3) however looking at specimens of previously sequenced samples the identification was confirmed. The molecular phylogeny supports the morphological study and suggests that *P. oxleyanum* is sister to *P. leiocarpum*. However it places both taxa in a clade separate from the GUTTA clade containing true *Palaquium gutta* (Taxon 1). Clade support however is not strong in the parsimony analysis (although it is stronger in Bayesian analysis) and better sampling and sequence data from other regions including chloroplast regions may better illuminate relationships between these two taxa and taxon 1. Both Taxa 3 and 4 had venation patterns that place them in forma Borneensis but they are distinguished from each other by the number of secondary veins and presence of intersecondary veins. Taxon 3 tended to have secondary veins of 20 or more, while Taxon 4 mostly had less than 15, but never more than 20. From this it is concluded that Taxon 4 (provisionally *P. oxleyanum*) represented more closely Lamb's forma Borneensis.

Lam (1925) separated *Palaquium leiocarpum* Boerl. (Taxon 3) from *P. gutta* by having longer pedicel and glabrous fruit. Lam also recognised *P. leiocarpum* var. *longe-acuminatum* Boerl. (Boerlage, 1900) as belonging to *Palaquium gutta* forma Genuinum by the presence of "*the tertiary nerves are more erect than the secondary ones and mutually connected by longitudinaly stretched reticulation*". In this study these are identical to the perpendicular intersecondary veins and ramified tertiary veins which are the defining characters of *Palaquium gutta* (Taxon 1). It confirms that *P. leiocarpum* which does not have any intersecondary veins, is distinct from *P. gutta* but without noticing the absence of this character it is easily confused with *P. gutta*.

Surprisingly, Lam (1925; 1927) listed *Palaquium oxleyanum* in page 106 and 413 respectively, as species not examined or checked by himself and it is possible that he never realised that *P. oxleyanum* could be conspecific with *P. gutta* forma Borneensis. The herbarium material of the newly sequenced specimen of Taxon 4 (*Wilkie* PW822) was found to have been collected from the same tree in Bogor Botanic Garden from which seven specimens from BO (BO 1246196 – BO 1246202) had been made of (garden collection number of IV.D.39). These specimens were claimed by BO as type specimens of *P. gutta* forma Borneensis. The attached note on the specimens indeed show that it had been determined by Lam as *P. gutta* forma Borneensis. This strongly suggests that *P. gutta* forma Borneensis is conspecific with *P. oxleyanum*. This is also supported by Ng (1972) who put all *P. gutta* forma Borneensis into *P. oxleyanum* due to the significant difference in transverse (percurrent) tertiary venation.

Van Royen (1960) highlighted one of the main reason for his decision to unite the forma Borneensis and Forma Genuinum was finding the parallel veins among the specimen of *P. gutta* forma Borneensis which was exclusively defined by transverse (percurrent) veins only. The parallel veins that he mentions is most likely identical to the intersecondary veins that sometimes occur in *P. oxleyanum*. The unique combination of percurrent veins and intersecondary veins was found in this study as the defining characters of *P. oxleyanum*. However, it is interesting that van Royen (1960) described *P. oxleyanum* in the same publication without noticing that these characters were identical to *P. gutta* forma Borneensis.

Further observation of the type specimen of *P. oxleyanum* Pierre, and two species previously placed in *P. gutta* forma Borneensis (*P. borneense* Burck and *P. treubii* Burck) showed that all

of them possessed percurrent tertiary veins, intersecondary veins are absent on some or most of intercostal area, and less than 20 of secondary veins which made them identical and separated from *P. gutta*. Further molecular data might support the relationship of these three species. However, there is a chance that Lam examined and included other species beside *P. oxleyanum* in *P. gutta* forma Borneensis, therefore further investigation in BO herbarium where most of the specimens are held is needed.

Forma Selendit, as most closely related to forma Borneensis, is also considered to be *P. oxleyanum* differing in leaf size only. Based on observation from specimens of *P. oxleyanum* from E and K, the leaf length varied from 7 to 21.5 cm. Van Royen (1960) described the leaf length as 10.5 – 23 cm, while Ng (1972) extend the range to 8 – 28 cm. It is possible that the specimens of forma Selendit that Lam examined were actually just small leaved specimens of *P. oxleyanum*.

To conclude, the forma concept of Lam (1925; 1927) has not been found to be useful with the morphological characters used to distinguish them highly variable. Here we recognise true *Palaquium gutta* to include forma Genuinum and forma Vrieseanum and forma Borneensis and forma Selendit to be *Palaquium oxleyanum*.

4. 3 Easily confused species with *Palaquium gutta* (Hook.) Baill. circumscription

In this study several sterile specimens were provisionally identified as *Palaquium gutta* but could not be placed into any of Lam's forms, and did not have the two key morphological characters of *P. gutta* identified in this study. Molecular sequence analysis also indicated them to be separate species. The morphology and molecular analysis of these specimens is discussed under the taxon in which this study has placed them.

Palaquium calophyllum (Teijsm. & Binn.) Pierre ex Burck

Taxon 2 (*Wilkie* SFC 07419) exhibited either elliptical or narrow obovate leaf shape with consistent acuminate apex. The secondary veins were less than 15 with an acute narrow angle of divergence. It is one of two taxa (Taxon 2 and Taxon 5) with eucamptodromous venation with percurrent tertiary pattern. The hairs were belong to the Group 3 type which are much longer than the hairs of Taxon 1. The hairs also comprised two colours, scattered darker hairs

occurring above the regular hairs. This is a micro character that never occurs in *Palaquium gutta*. Likewise, the diagnostic characters of Taxon 2 that was easy to recognise with bare eyes is the darker hairy thick midrib and secondary veins on the abaxial surface (Fig. 4.2).

Figure 4.2 *Palaquium calophyllum* typical leaf venation

In the phylogenetic tree Taxon 2 fell in the clade **CALO** containing two previously sequenced *P. calophyllum*. The clade is well supported according to Bayesian analysis (1 PP), but weak supported in parsimony analysis (63% BS) and could collapse into a polytomy together with clade GUTTA, OXLE, and ERIO (Fig. 3.2.5). The Bayesian analysis supports the morphological study and suggests that *P. calophyllum* (CALO clade) is closely related to the *P. oxleyanum* (OXLE clade) and *Palaquium* sp. (ERIO clade) which share percurrent tertiary veins.

***Palaquium leiocarpum* Boerl.**

Taxon 3 (*Rantai Jawa et al.* S 71048) was the only specimens that had a wide obovate leaf form. It was one of three taxa (including Taxon 1 and 6) which had more than 20 secondary veins. The angle of divergence of the secondary veins was similar to Taxon 1, acute wide, but the venation was mixed simple and festooned brachidodromous with percurrent tertiary pattern, rarely intersecondary veins. Hairs were short, and belonged to the Group 1 type. The diagnostic characters of this taxon are the wide obovate leaf and percurrent venation without intersecondary veins (Fig.4.3).

Figure 4.3 *Palaquium leiocarpum* typical leaf venation

The relationship of Taxon 3 (*P. leiocarpum*) with *P. gutta* has already been discussed above. No new sequence data was produced for Taxon 3, however it was morphologically matched with *P. leiocarpum* specimens previously sequenced and composed a clade together with OXLE clade (Fig. 3.2.5; 3.2.7). Clade support was strong (0.99 PP) in Bayesian analysis, but weak in Parsimony analysis (62% BS). *P. leiocarpum* and *P. oxleyanum* are morphologically closely related by having percurrent tertiary venation and similar hair type. Morphologically and from ITS sequence data they are both separate from *P. gutta*.

***Palaquium oxleyanum* Pierre**

Taxon 4 (*Wilkie* PW822) has narrow obovate leaves, a convex rounded leaf apex with mucro present, brachidodromous venation with presence of intersecondary veins and short hairs that shared with Taxon 1 (*P. gutta*). However it is separated by the consistent characters of having secondary veins 15 or less, percurrent tertiary venation, and intersecondary veins are absent on some or most of intercostal area (unlike P. gutta that almost always has at least one in each intercostal area) (Fig. 4.4).

The relationship of *P. oxelyanum* and *P. gutta* forma Borneensis has been discussed under Taxon 1. ITS sequence data of both sample of *P. oxleyanum* (**OXLE** clade) and *P. leiocarpum* composed a clade that separate them from *P. gutta*. Morphologically both species were

distinguished from *P. gutta* by the percurrent tertiary veins. *P. oxleyanum* is separated from *P. leiocarpum* by the presence of intersecondary veins and the number of secondary veins.

Figure 4.4 *Palaquium oxleyanum* typical leaf venation

Palaquium oxleyanum was recognised by Pierre (1885) from a specimen sent by Oxley that he claimed as *P. gutta*. Pierre regarded it as different species and named it *Palaquium oxleyanum* after the collector name. This suggests that Oxley the collector of true *P. gutta* did not recognise the difference between them.

***Palaquium sericeum* Lam**

Taxon 5 (*Wilkie et al.* SFC 07443, SFC 07432) always has a narrow oblong leaf shape with acuminate apex. Taxon 5, like Taxon 2, exclusively had a narrow acute angle of divergence of the secondary venation, eucamptodromous venation, and a percurrent tertiary venation pattern (Fig. 4.5). The number of secondary veins could reach almost 20 but can be less than 15. The hairs belong to the Group 3 or 4 type, which is long or very long and having scattered darker hairs above the whitish regular ones. The other diagnostic characters of Taxon 5 are the darker hairs covering the midvein and secondary veins on the abaxial surface (similar to Taxon 2) and the cordate or rounded leaf base which is not found in any other taxa studied for this project.

Figure 4.5 *Palaquium sericeum* typical leaf venation

Sequence data of Taxon 5 places it in the **SERI** clade containing *P. sericeum*, *P. formosanum*, and *P. dasyphyllum*. Examining herbarium specimens and images it was morphologically confirmed as *P. sericeium* by having a cordate or rounded base, while *P. formosanum* had wide obovate leaves with cuneate or convex base, and *P. dasyphyllum* had similar leaf shape with a convex leaf base, never a cordate one. Clade support was strong (0.92 PP) in Bayesian analysis, but there was no support in the Parsimony analysis. However the SERI clade was sister to the larger clade containing the OXLE, ERIO, CALO and GUTTA clades (Fig. 3.2.5; 3.2.7) and it is clearly separate from *Palaquium gutta*.

***Payena acuminata* (Blumea) Pierre and *Payena maingayi* Clarke**

Taxon 6 (*Wilkie et al.* PW1010, PW1024, FRI 75020) has a large range of leaf shapes: narrow oblong, oblong, narrow elliptic, and oblanceolate with acuminate apex. Secondary veins were usually more than 26 with an acute wide angle. They were the only specimens that had a simple brachidodromous venation, admedially ramifying tertiary veins, and intersecondary parallel to major secondary veins, which are the characters that distinguished them from *P. gutta* (Fig.4.6).

It was noted that two different hair colours were found: long golden brown hairs (Group 3 type) and very long whitish grey (Group 4 type) with scattered darker hairs above on both (the same as Taxon 5). The hair size and colour was quite consistent between different leaves. Based on these character, Taxon 6 was likely composed of two different species.

Taxon 6 was nested in the *Payena* clade in Isonandreae matrix tree (Fig. 3.2.2; 3.2.3) which was well supported in the Parsimony analysis (93% BS) (a Bayesian analysis was not undertaken for the larger matrix). The new sequence data, included the two missing herbarium specimens that were only found towards the end of the research period (PW-E1102; PW-E1098) were nested in a clade containing *Payena acuminata* and *Payen maingayi* with a good support (97% BS). The diagnostic characters mentioned above is likely to be the diagnostic character of genus *Payena* (following Ng (1972).

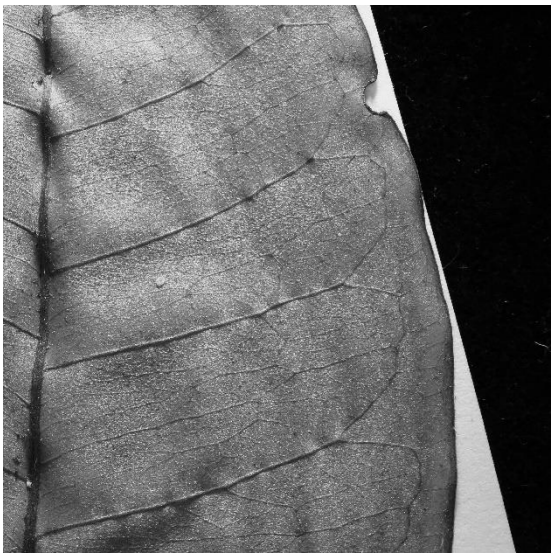


Figure 4.6 *Payena acuminata* and *Payena maingayi* typical leaf venation

Taxon 6a (*Wilkie et al.* PW1010, PW1024) has hair character that match *Payena acuminata*, while the hairs of **Taxon 6b** (*Wilkie et al.* FRI 75020, PW-E1102; PW-E1098) match *Payena maingayi*. Within the clade, both species were placed randomly which indicated confusion in identifying the sterile specimens. Ng (1972) confirmed that both species were difficult to separate without flowers.

***Palaqium* sp. 1**

Taxon 7 (*Markus* SAN 151019) has distinctive narrow elliptic and oblanceolate leaves with acuminate apex. The secondary venation was a mix of simple and festooned brachidodormous veins without intersecondary veins (Fig. 4.7). The tertiary vein pattern was percurrent. The hairs

were golden intermediate or slightly longer than *P. gutta*. From the venation characters, it was similar to Taxon 4, except that the leaf shape was significantly different. It has not yet been able to identify this specimen successfully and no sequence data is available, but based on morphology it is not considered to be *Palaquium gutta.*

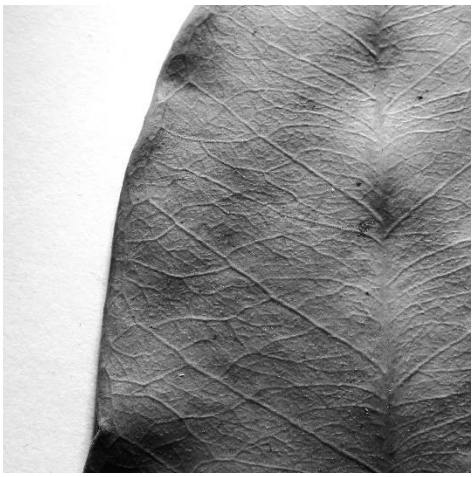


Figure 4.7 *Palaquium* sp.1 (Taxon 7) typical leaf venation

***Palaqium* sp. 2**

Taxon 8 (*Wilkie* PW1031) has an oblong or narrow obovate leaf shape, convex or convex-rounded leaf apex, a wide acute angle of divergence in the secondary venation and intermediate hairs just like Taxon 7. It is very similar to Taxon 4 morphologically by having percurrent tertiary pattern and number of secondary veins less than 15. However, the brachidodromous venation were always festooned and the secondary veins were always wide acute in angle of divergence that distinguish it from Taxon 4 (Fig. 4.8).

ITS sequence data analysis places Taxon 8 in the **ERIO** clade with a specimen identified as *P. eriocalyx* (Fig. 3.2.5; 3.2.7). However, it is significantly different to this specimen in morphology. Further investigation is needed to better understand its placement given the very different morphology of both specimens. The clade support for the ERIO clade is weak in both the Bayesian and Parsimony analysis but it is sister to a clade containing the OXLE clade and *P. leiocarpum* accessions. It is clearly not *P. gutta* based on morphology and molecular data

but what species of *Palaqium* it is needs further investigation of a wide range of other *Palaquium* species.

Figure 4.8 *Palaquium* sp. 2 (Taxon 8) typical leaf venation

5. CONCLUSION AND FUTURE RESEARCH

This study has provided a better understanding of the circumscription of *Palaquium gutta* (Hook.) Baill. ITS sequence data analysis has revealed the monophyly of *P. gutta* which is supported morphologically by the perpendicular intersecondary veins and admedially remified tertiary veins. However, this species has indeed a wide range and highly variable vegetative characters such as leaf size, leaf shape and form, leaf apex, and number of secondary veins which were among the less informative characters but added to confusion with other species.

This investigation does not support Lam's forma concept (1925; 1927) but helped re-circumscribe *Palaquium gutta* and recognise *P. gutta* forma Borneensis as *Palaquium oxleyanum* Pierre. It is suggested that *Palaquium gutta* circumscription follows van Royen circumscription (1960) and that we exclude *P. gutta* forma Borneensis and all its synonyms from *P. gutta.*

The ITS region proved to be quite informative in resolving phylogenetic relationships within the genus and family of Sapotaceae. The phylogeny supports the monophyly of tribe Isonandreae (Pennington, 1991), and monophyly of most genera in Isonandreae. Bayesian analysis strongly supports the phylogeny within *Palaquium* clade. However, the *Palaquium* clade support was weak according to MP analysis and some clades and subclades could easily collapse into polytomy. More investigation is needed to assess species identifications in the larger phylogeny and to assess sequence data quality as well as improving sampling and obtaining sequence data from both nuclear and chloroplast DNA.

An important point highlighted during this study is the importance of observing and carefully diagnosing micro-characters in sterile specimens, such as venation, on *Palaquium* or Sapotaceae species to understand the venation pattern and variation. Observing the venation on herbarium specimen can be challenging since specimen cannot be flipped over or taken off the sheet, thus the angle and intensity of lighting play an important role in observation. It is also important to work on as many samples as possible in order to have a better comparison within and between species and genera. For example, Lam who looked at many specimen for his 1925 & 1927 publications, still failed to recognise *P. oxleyanum* because he simply never examined the species directly. The difficulty with using micro-morphological characters underlines the

potentially important role molecular sequence data can have in helping identifying sterile material with confidence.

Last but not least, in order to understand the nature of a family is very important to help distinguish whether the variation in characters are consistent and truly informative or just general variation of the species. In Sapotaceae, particularly in *Palaquium*, it is very common to find different leaf size, shape and apex on the same twig, or different density, texture, and colour of hairs on the same tree.

Future research

In order to fill the gap and gain better evidence and understanding of the circumscription of *Palaquium gutta* and other morphologically similar *Palaquium* species based on vegetative characters, further work needs to be conducted in the following areas:

1. **Expand herbarium sampling** by examining all specimens of *Palaquium gutta* including the forma and related species in BO and KRB herbaria and to include existing living specimens in botanic gardens. Examining the specimens directly will give more confidence in the result and conclusion.
2. **Expand DNA evidence** by conducting further molecular work on both nuclear and chloroplast regions to get a more confident phylogenetic estimate and better support. Also add more samples of species to see if it provides better resolution in the phylogeny.
3. **Add morphological evidence** by observing hair type and structure within species *P. gutta* and genus *Palaquium* with SEM (scanning electron microscope) to see whether the hair characters are taxonomically informative.
4. **Produce species revision and vegetative characters based identification keys** to establish better species circumscription of *Palaquium gutta* and reduce misidentification in herbarium specimens.

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APPENDICES

Appendix A

Figure A.1 The 35 physical specimens from the E and K herbaria provisionally determined as *Palaquium gutta* (Hook.) Baill.

E00013587 / J. Sinclair 5474

E00230060 / Lobb 290

E00230061 / Lobb 290

E00277821 / G. wrav 1100

E00290117 / Wilkie & Gwee PW504

E00290128 / Wilkie *et al.* PW536

E00304060 / Wilkie *et al.* FRI 52855

E00304198 / Wilkie *et al.* FRI52919

E00331748 / s.n.

E00416341 / Wilkie FRI75020

E00416389 / Wilkie FRI72142

E00639765/ Wilkie PW822

E00646660 / Wilkie PW842

E00664277 / Wilkie PW862

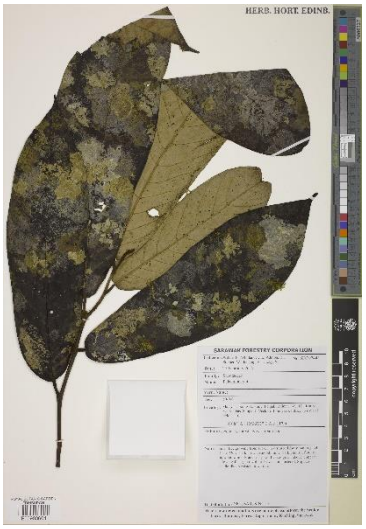
E00834389 / Markus SAN151019

E00898190 / Wilkie & Hutabarat PW1001

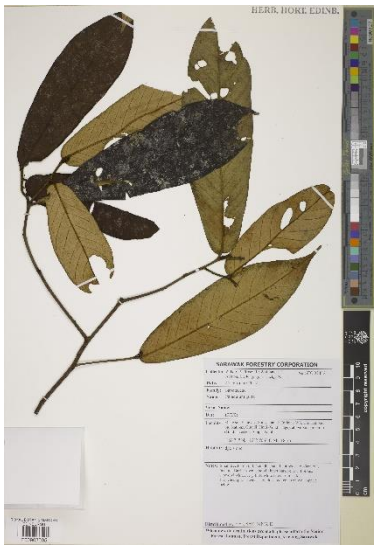
E00898191 / Wilkie *et al.* PW1010

E00898547 / Wilkie *et al*. PW1031

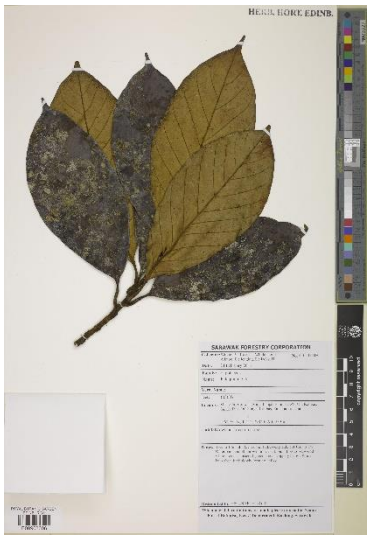
E00898554 / Wilkie & Hutabarat. PW1024



E00903604 / Wilkie *et al.* SFC 07443



E00903605 / Wilkie *et al.* SFC 07432



E00903606 / Wilkie *et al*. SFC 07419

E00913534 / Rantai Jawa *et al.* S71048

H2012/01014-11 / Curtis B3582

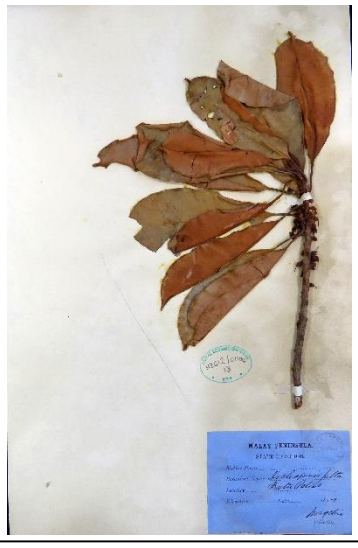
H2012/01101-12 / Curtis 780

H2012/01014-14/ rec-B:1887

H2012/01014-15 / Lobb s.n.

H2012/01014-16 / Lobb s.n.

H2012/01014-17 / Maingay 983



H2012/01014-18 / s.n.

H2012/01014-19 / Cockburn FRI7682

H2012/01014-20 / Whitmore FRI12527

H2012/01014-21 / T&P 362

K00639125 / Lobb 290

K0077680 / Oxley s.n. (TYPE)

Appendix B

Table B.1 Data for the newly acquired sequenced samples of *Palaquium gutta*.

All samples are from silica gel dried samples. (E) = Edinburgh Herbarium

No	Species name	Sequencing ID	Accession Number	Collector	Collector Number	Country
1	*Palaquium gutta*	Pal._ sp._001	EDNA19_0053646	Wilkie, P & A.T. Gwee	PW504 (E)	Singapore
2	*Palaquium gutta*	Pal._ sp._002	EDNA19_0053647	Wilkie, P & A. Anak Kapi	PW862 (E)	Malaysia
3	*Palaquium gutta*	Pal._ sp._003	EDNA19_0053648	Wilkie, P	PW822 (E)	Indonesia
4	*Palaquium gutta*	Pal._ sp._004	EDNA19_0053649	Wilkie, P & K. Imin	PW842 (E)	Malaysia
5	*Palaquium gutta*	Pal._ sp._005	EDNA19_0053650	Wilkie, P, M.S. Khoo, P.K.F. Leong & A. Ibrahim	PW536 (E)	Malaysia
6	*Palaquium gutta*	Pal._ sp._006	EDNA19_0053651	Wilkie, P, P. Hutabarat, Lancar & idel	PW1010 (E)	Indonesia
7	*Palaquium gutta*	Pal._ sp._007	EDNA19_0053652	Wilkie, P, P. Hutabarat, Lancar & Basuki	PW1024 (E)	Indonesia
8	*Palaquium gutta*	Pal._ sp._008	EDNA19_0053653	Wilkie, P, P. Hutabarat, Lancar & idel	PW1031 (E)	Indonesia
9	*Palaquium gutta*	Pal._ sp._009	EDNA19_0053682	Hutabarat, P & Wilkie, P.	PWH413 (E)	Indonesia
10	*Palaquium gutta*	Pal._ sp._010	EDNA19_0053683	Wilkie, P & P. Hutabarat	PW1001 (E)	Indonesia
11	*Palaquium gutta*	Pal._ sp._011	EDNA19_0053684	Wilkie, P, P.W.K. Hutabarat, Firmansyah, Hendra, Mansyah & Asgar	PW-E1102 (E)	Indonesia
12	*Palaquium gutta*	Pal._ sp._012	EDNA19_0053685	Wilkie, P, P.W.K. Hutabarat, Firmansyah, Hendra, Mansyah & Asgar	PW-E1098 (E)	Indonesia
13	*Palaquium gutta*	Pal._ sp._013	EDNA19_0053686	Wilkie, P, Y.C. Chan, Mohd. Hairul, M.A. & A. Norazmi	FRI72142 (E)	Malaysia
14	*Palaquium gutta*	Pal._ sp._014	EDNA19_0053687	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07419 (E)	Malaysia
15	*Palaquium gutta*	Pal._ sp._015	EDNA19_0053688	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07432 (E)	Malaysia
16	*Palaquium gutta*	Pal._ sp._016	EDNA19_0053689	Wilkie, P, R. Ragai, W. Lawrence, A.C.J. Bernier, E.A. Jenging & J.A. Ngabong	SFC07443 (E)	Malaysia
17	*Palaquium gutta*	Pal._ sp._017	EDNA19_0053690	Wilkie, P, Siti Munirah, Mohd. Hairul, M.A. & Nazre	FRI75020 (E)	Malaysia

Appendix C

Characteristics of the BI analysis of the *Palaquium* matrix on 82 samples:

Average standard deviation of split frequencies: 0.007759

Analysis completed in 2 hours 10 mins 50 seconds
Analysis used 7849.70 seconds of CPU time
Likelihood of best state for "cold" chain of run 1 was -4173.15
Likelihood of best state for "cold" chain of run 2 was -4177.05

Acceptance rates for the moves in the "cold" chain of run 1:

With prob.	(last 100)	chain accepted proposals by move
45.0 %	(26 %)	Dirichlet(Tratio{2})
27.5 %	(20 %)	Dirichlet(Revmat{1})
37.1 %	(30 %)	Slider(Revmat{1})
25.7 %	(32 %)	Dirichlet(Pi{1})
26.7 %	(27 %)	Slider(Pi{1})
30.6 %	(17 %)	Multiplier(Alpha{1})
31.1 %	(31 %)	ExtSPR(Tau{all},V{all})
23.4 %	(24 %)	ExtTBR(Tau{all},V{all})
37.3 %	(36 %)	NNI(Tau{all},V{all})
16.1 %	(18 %)	ParsSPR(Tau{all},V{all})
26.3 %	(23 %)	Multiplier(V{all})
56.0 %	(54 %)	Nodeslider(V{all})
25.0 %	(19 %)	TLMultiplier(V{all})

Acceptance rates for the moves in the "cold" chain of run 2:

With prob.	(last 100)	chain accepted proposals by move
44.7 %	(25 %)	Dirichlet(Tratio{2})
27.9 %	(21 %)	Dirichlet(Revmat{1})
37.4 %	(22 %)	Slider(Revmat{1})
25.6 %	(27 %)	Dirichlet(Pi{1})
27.1 %	(24 %)	Slider(Pi{1})
30.3 %	(25 %)	Multiplier(Alpha{1})
31.2 %	(28 %)	ExtSPR(Tau{all},V{all})
23.5 %	(24 %)	ExtTBR(Tau{all},V{all})
37.4 %	(38 %)	NNI(Tau{all},V{all})
16.2 %	(13 %)	ParsSPR(Tau{all},V{all})
26.3 %	(31 %)	Multiplier(V{all})
56.1 %	(59 %)	Nodeslider(V{all})
24.8 %	(34 %)	TLMultiplier(V{all})

Chain swap information for run 1:

	1	2	3	4
1		0.35	0.07	0.01
2	332637		0.39	0.10
3	333378	332853		0.42
4	333523	333810	333799	

Chain swap information for run 2:

	1	2	3	4
1		0.36	0.08	0.01
2	333600		0.39	0.09
3	332934	332959		0.42
4	333596	333242	333669	

Upper diagonal: Proportion of successful state exchanges between chains
Lower diagonal: Number of attempted state exchanges between chains

Chain information:

ID	Heat
1	1.00 (cold chain)
2	0.91
3	0.83
4	0.77

Heat = 1 / (1 + T * (ID - 1))
(where T = 0.10 is the temperature and ID is the chain number)

Appendix D

Figure D.1 Scatterplot of likelihood values against generation time for run1 of the MrBayes analysis of the *Palaquium* matrix of 82 samples.

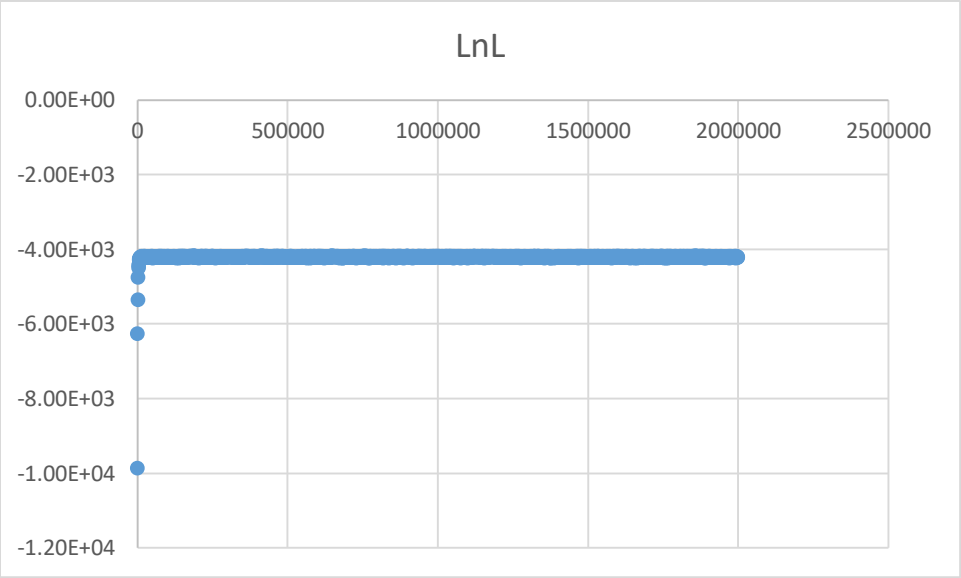
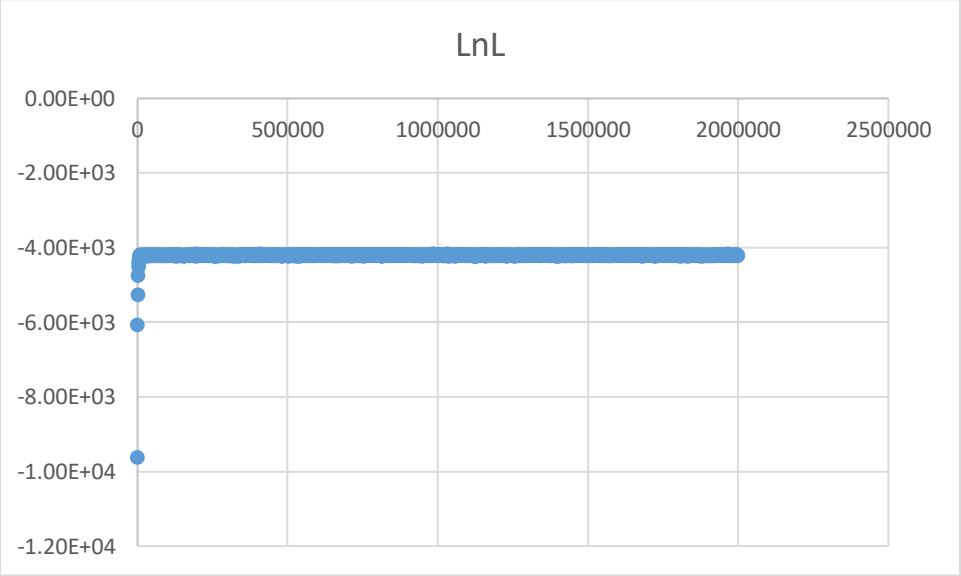


Figure D.2 Scatterplot of likelihood values against generation time for run2 of the MrBayes analysis of the *Palaquium* matrix of 82 samples.



Appendix E

Figure E.1 (part 1) Bootstrap values from the parsimony bootstrap analyses using 10,000 replicates on Isonandreae matrix tree. Bootstrap values less than 50% are not shown on the branches

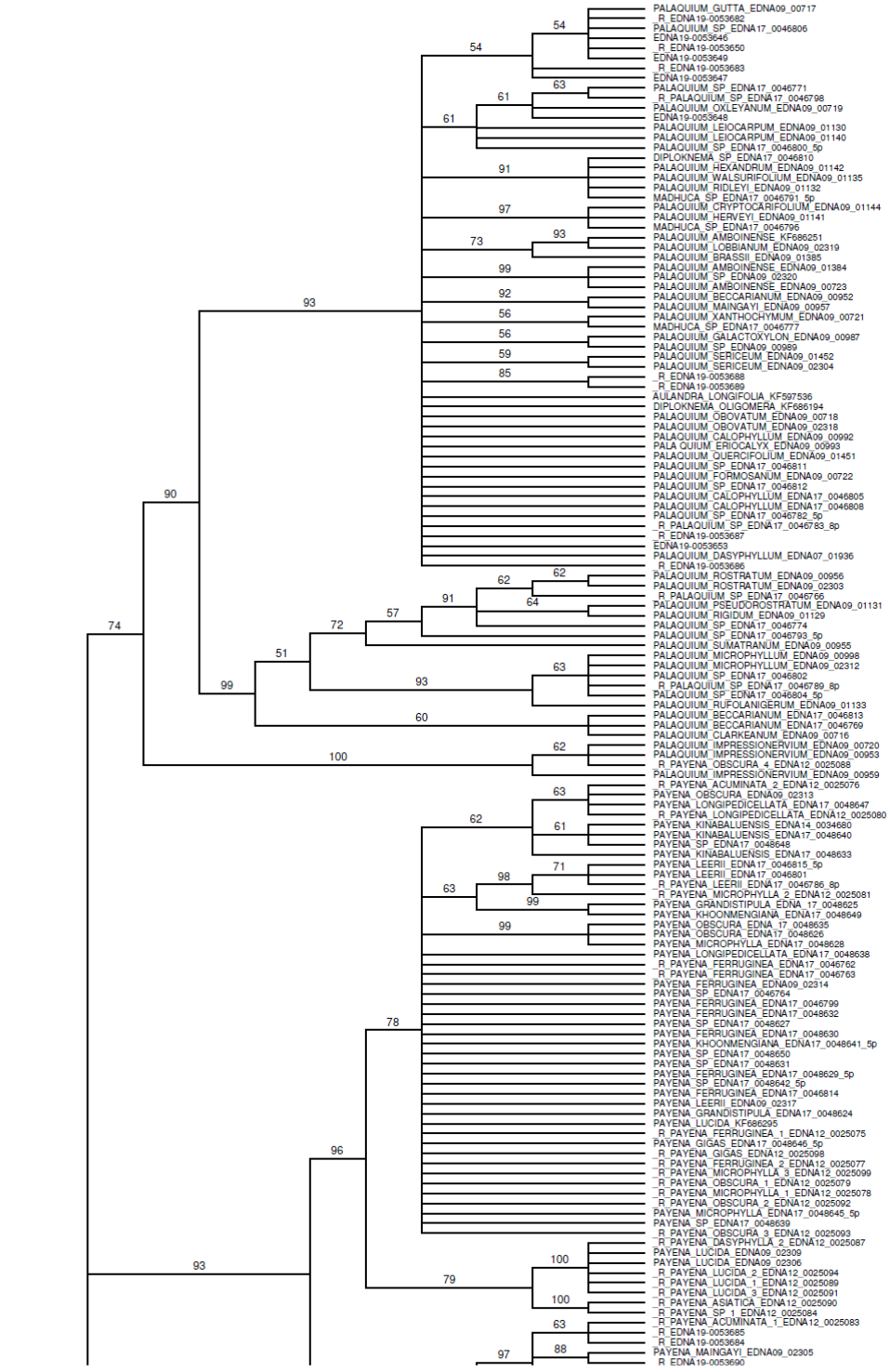


Figure E.1 (part 2) Bootstrap values from the parsimony bootstrap analyses using 10,000 replicates on Isonandara matrix tree. Bootstrap values less than 50% are not shown on the branches

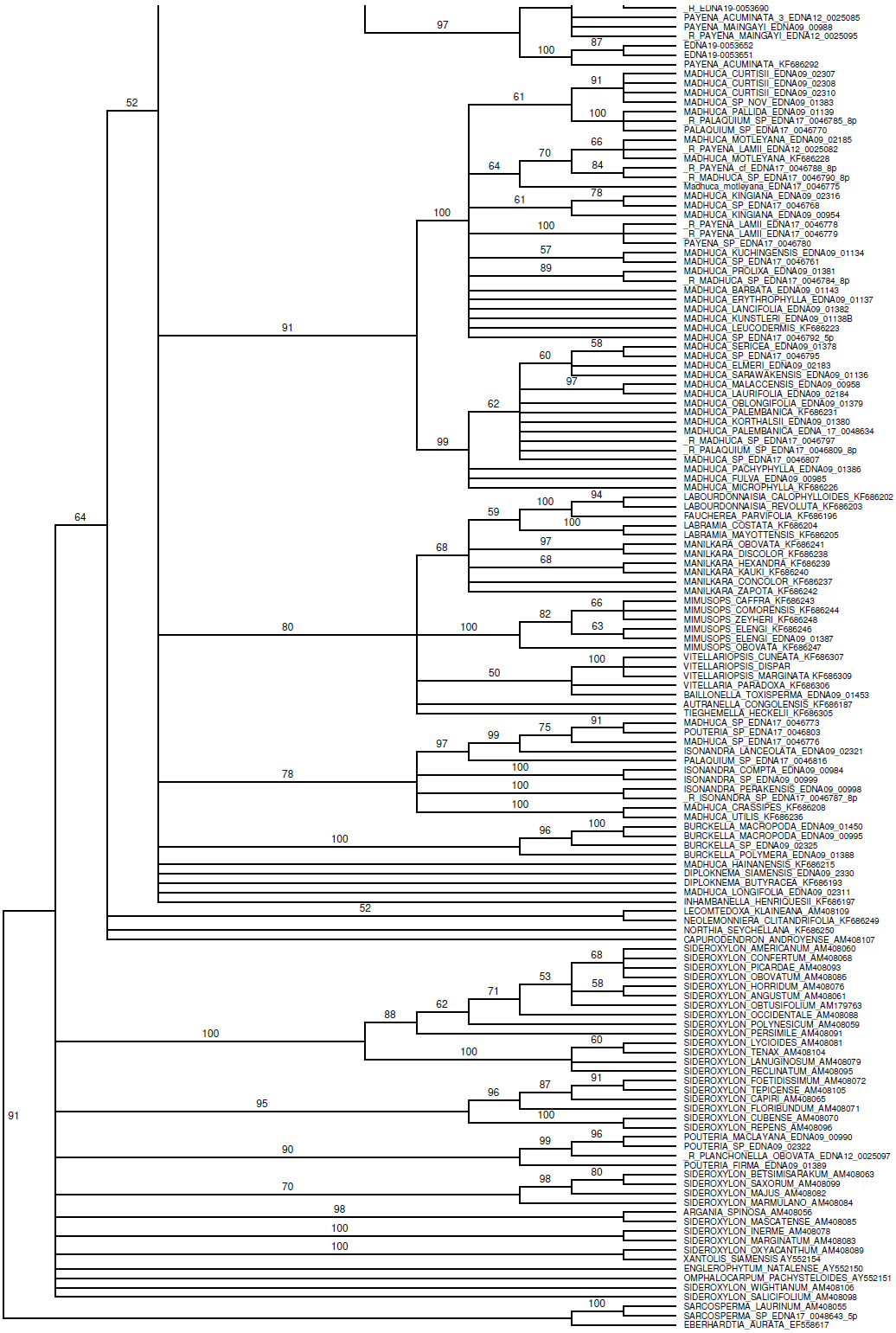


Table F.1 List of Synonyms of *Palaquium gutta* (Hook.) Baill. and the protologues

✓= had accessed; × = had not accessed/ not found; Type = type specimen; Non-type = any specimen identified as

No	Species name	Protologue	Protologue access	Herbarium access
1	*Isonandra gutta* Hook.	London J. Bot. 6: 463 (1847).	✓	Type
2	*Dichopsis gutta* (Hook.) Benth. ex Murton	J. Soc. Arts 26: 938 (1878).	×	
3	*Croixia gutta* (Hook.) Baehni	Boissiera 11: 110 (1965).	×	
4	*Isonandra percha* Hook.	London J. Bot. 6: 610 (1847).	✓	×
5	*Isonandra gutta* var. oblongifolia de Vriese	Tuinb.-Fl. 3: 225 (1856).	×	×
6	*Isonandra acuminata* Miq.	Fl. Ned. Ind. Eerste Bijv.: 581 (1861).	✓	×
7	*Dichopsis oblongifolia* Burck	Rapp. Expl. Recherch Gutta-Percha: 17 (1885).	×	Non-type
8	*Palaquium acuminatum* Burck	Ann. Jard. Bot. Buitenzorg 5: 40 (1885).	✓	Type
9	*Palaquium borneense* Pierre	Bull. Mens. Soc. Linn. Paris 1: 499 (1885).	✓	Type
10	*Palaquium borneense* Burck	Ann. Jard. Bot. Buitenzorg 5: 26 (1885).	✓	Type
11	*Palaquium formosum* Pierre	Bull. Mens. Soc. Linn. Paris 1: 498 (1885).	✓	Type
12	*Palaquium gloegoerense* Burck	Ann. Jard. Bot. Buitenzorg 5: 40 (1885).	✓	Type
13	*Palaquium malaccense* Pierre	Bull. Mens. Soc. Linn. Paris 1: 498 (1885).	✓	Type
14	*Palaquium oblongifolium* (Burck) Burck	Ann. Jard. Bot. Buitenzorg 5: 25 (1885).	✓	Non-type
15	*Palaquium obscurum* Burck	Ann. Jard. Bot. Buitenzorg 5: 40 (1885).	✓	Type
16	*Palaquium princeps* Pierre	Bull. Mens. Soc. Linn. Paris 1: 499 (1885).	✓	×
17	*Palaquium selendit* Burck	Ann. Jard. Bot. Buitenzorg 5: 41 (1885).	✓	Type
18	*Palaquium treubii* Burck	Ann. Jard. Bot. Buitenzorg 5: 27 (1885).	✓	Type
19	*Palaquium vrieseanum* Burck	Ann. Jard. Bot. Buitenzorg 5: 28 (1885).	✓	Type
20	*Palaquium fulvosericeum* Engl.	Bot. Jahrb. Syst. 12: 511 (1890).	✓	Type
21	*Palaquium gutta* var. *sessiliflora* Boerl.	Bull. Inst. Bot. Buitenzorg 5: 21 (1900).	✓	×
22	*Dichopsis borneensis* (Burck) Fox	Rep. Bot. Gard. Singapore 1901: 6 (1902).---	✓	×
23	*Dichopsis treubii* (Burck) Fox	Rep. Bot. Gard. Singapore 1901: 6 (1902).	✓	×
24	*Palaquium ellipsoideum* Becc.	For. Borneo: 560 (1902).	✓	Type
25	*Palaquium optimum* Becc.	For. Borneo: 154, 558 (1902).	✓	Type
26	*Palaquium tammadek* Becc.	For. Borneo: 559 (1902).	✓	×
27	*Palaquium croixianum* Pierre ex Dubard	Mém. Soc. Bot. France 16: 13 (1909).	✓	×
28	*Palaquium gutta* var. *oblongifolium* (Burck) H.J.Lam	Bull. Jard. Bot. Buitenzorg, sér. 3, 7: 29 (1925).	✓	Non-type
29	*Palaquium gutta* f. selendit (Burck) H.J.Lam	Bull. Jard. Bot. Buitenzorg, sér. 3, 7: 29 (1925).	✓	Non-type
30	*Palaquium gutta* f. borneense (Burck) H.J.Lam	Bull. Jard. Bot. Buitenzorg, sér. 3, 8: 389 (1927).	✓	Type
31	*Palaquium gutta* f. vrieseanum (Burck) H.J.Lam	Bull. Jard. Bot. Buitenzorg, sér. 3, 8: 391 (1927).	✓	Non-Type