Cardamoms of South East Asia: phylogeny and taxonomy of the genus *Elettaria* Maton

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Illustration on the front page: From White (1811)

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Helena Båserud Mathisen

01.12.2014

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Abstract

Elettaria Maton is a small genus in the pantropical Zingiberaceae, comprising 11 species. Molecular data analyses have been widely used to reconstruct evolutionary history and to infer phylogenetic relationships, but to date no published molecular studies have included any representatives of *Elettaria*. In this study, I test the monophyly of *Elettaria* by using the nuclear ribosomal internal transcribed spacer (ITS) region and the chloroplast *trnL-F* region. DNA samples were collected during my own fieldwork in Borneo and from capsules of cardamom sold commercially. In addition, I also benefited from samples collected during other expeditions and I also downloaded sequences from GenBank®. All samples for each DNA region were sequenced and aligned in one big matrix, before two different phylogenetic analyses were implemented: the maximum parsimony analysis and the maximum likelihood analysis. The produced cladograms revealed that *Elettaria* is not a monophyletic clade, but falls into minimum three separate clades. The *Elettaria* clade 3 contains the type species and is therefore the true *Elettaria* clade likely only to contain species from India and Sri Lanka. *Elettaria* clade 1 includes species from Sundaland. Several morphological differences separate clade 1 from the true cardamom. Anther dehiscence and fruit type seems good characters to separate the species within clade 1. Several samples from Sulawesi, including *Elettariopsis kandariensis*, are also included in clade 1 and the analayses indicate that the origin of these is likely to be from Sundaland after crossing Wallace's Line. *Elettaria* clade 2 consists of samples of a sterile but commercially important plant that is presently impossible to identify and, despite its distinct smell of cardamom, is unrelated to *E. cardamomum*.

1 Introduction

1.1 Ginger family

Zingiberaceae, commonly known as the ginger family, is one of eight families in Zingiberales and accounts for over 1200 species (Mabberley, 2008). It has a pantropical distribution with centre of diversity in South East Asia, but one also find genera in Africa (*Aframomum* K.Schum., *Aulotandra* Gagnep., *Renealmia* L. and *Siphonochilus* J.M.Woods & Franks) and South America (*Renealmia*) (Figure 1.1). Autapomorphies of the family are 1) the labellum, which is formed by a fusion of the lateral staminodes of the inner staminal whorl, 2) two epigynous nectariferous glands placed at the base of the style, and 3) the presence of essential or ethereal oils (Kress, 1990). Several species are well known due to their frequent use in cooking, medicine and for decoration (Lamb *et al.*, 2013).

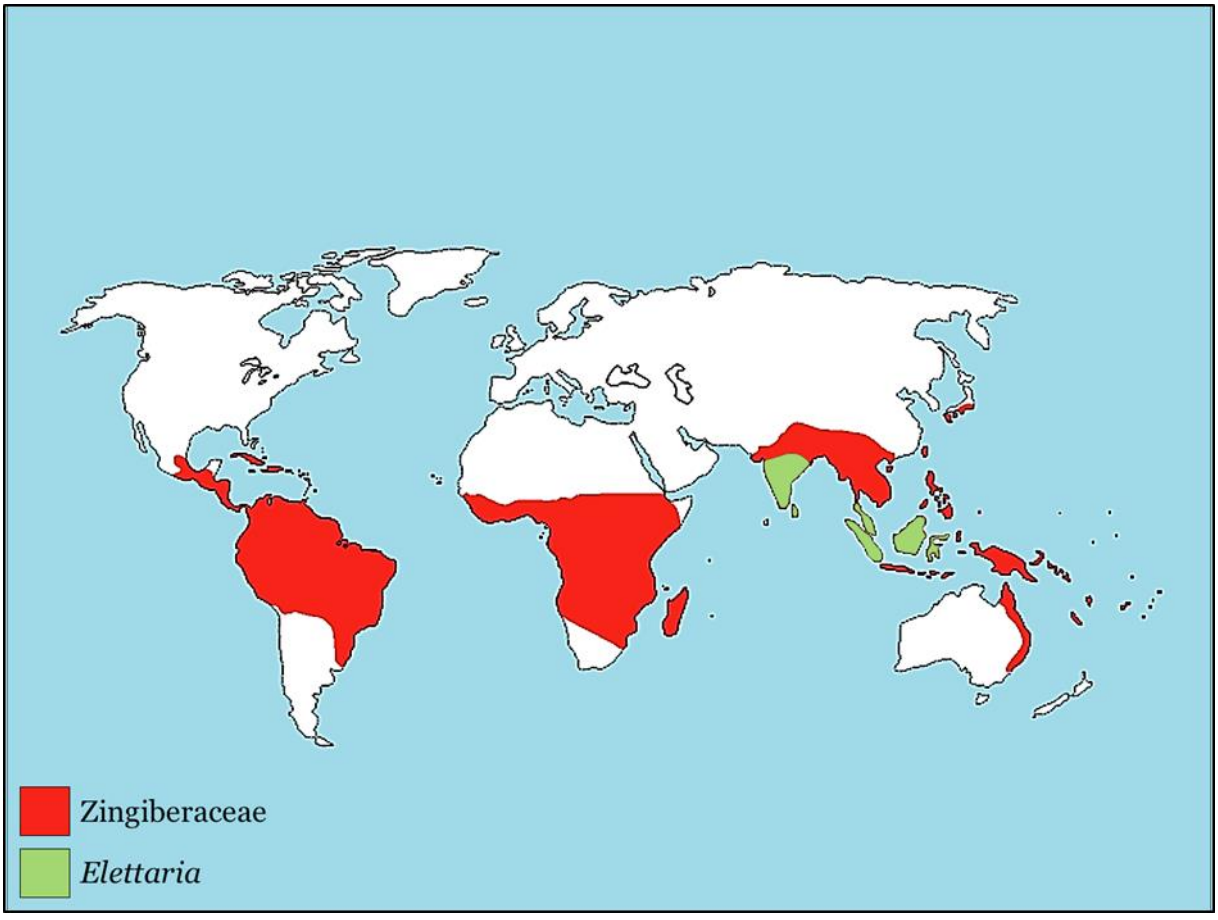

Figure 1.1 Map displaying the distribution of the Zingiberaceae (the ginger family) and the genus *Elettaria* (Based on the map from Stevens 2001).

1.2 Study genus

Elettaria Maton is one of 53 genera in Zingiberaceae (Kress, 1990). The genus is characterized by a long prostrate inflorescence, which usually are more or less embedded in the soil, bearing several-flowered cincinni with tubular bracteoles and globose or ellipsoid fruits (Holttum, 1950, Smith, 1986) (Figure 1.2). It is distributed

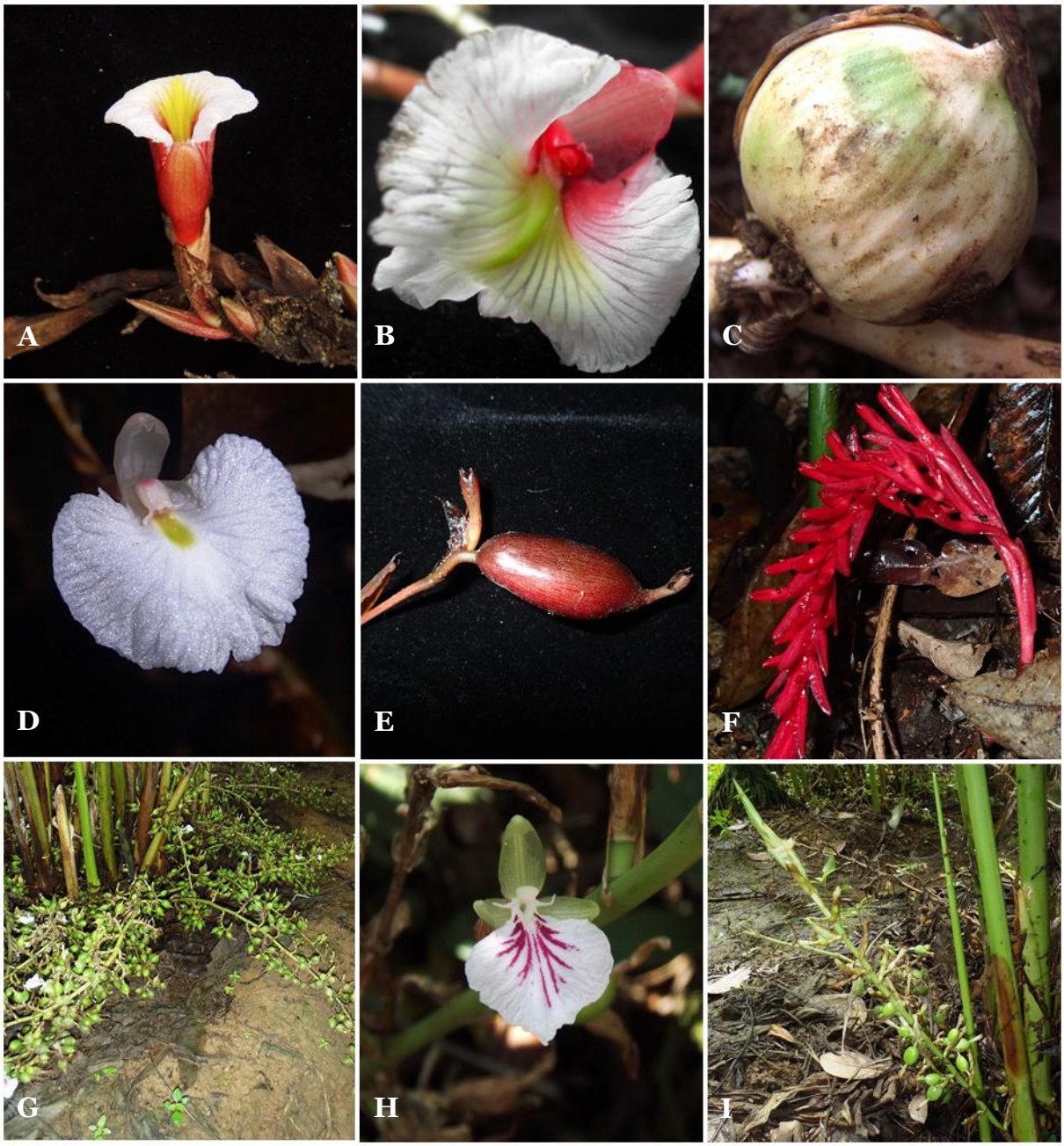

Figure 1.2 Representative floral, fruit and inflorescence structures of species of *Elettaria*. **A.** *Elettaria* sp. **B. and C.** *E.* sp. **D.** and **E.** *E. surculosa*. **F.** *E. rubida*. **G.- I.** *E. cardamomum*. (Photo Axel Dalberg Poulsen).

(Figure 1.1) from south India, Sri Lanka and to parts of Malesia: Peninsular Malaysia, Sumatra, Borneo and Sulawesi (Smith, 1986). The first written record of true cardamom (*Elettaria cardamomum* (L.) Maton) is in "*Hortus Indicus Malabaricus"* by Rheede *et al.* (1692). The plates from the book constitute the holotype of the genus (Figure 1.3), even though it did not get its official scientific name until 1811 by William George Maton, after he read Mr David White's description of the plant from the Malabar region in India (White, 1811).

Elettaria cardamomum is today a renown and beloved spice in kitchens all over the world and therefore of high economic importance. One can also find important spices in the related genus *Amomum* Roxb., such as black cardamom (*A. subulatum* Roxb.). It is also, likewise true cardamom, widely used in Southeast Asia (Lamxay and Newman, 2012). Currently, 11 species of *Elettaria* are accepted (The Plant List 2013) , but *Elettaria* has been revised several times, since it was recognized in 1811. In 1904, Schumann only recognized *E. cardamomum* and *E. major* (Gaertn.) Abeyw. (today known as the recognized *E. ensal* (Gaertn.) Abeyw.) in the genus.

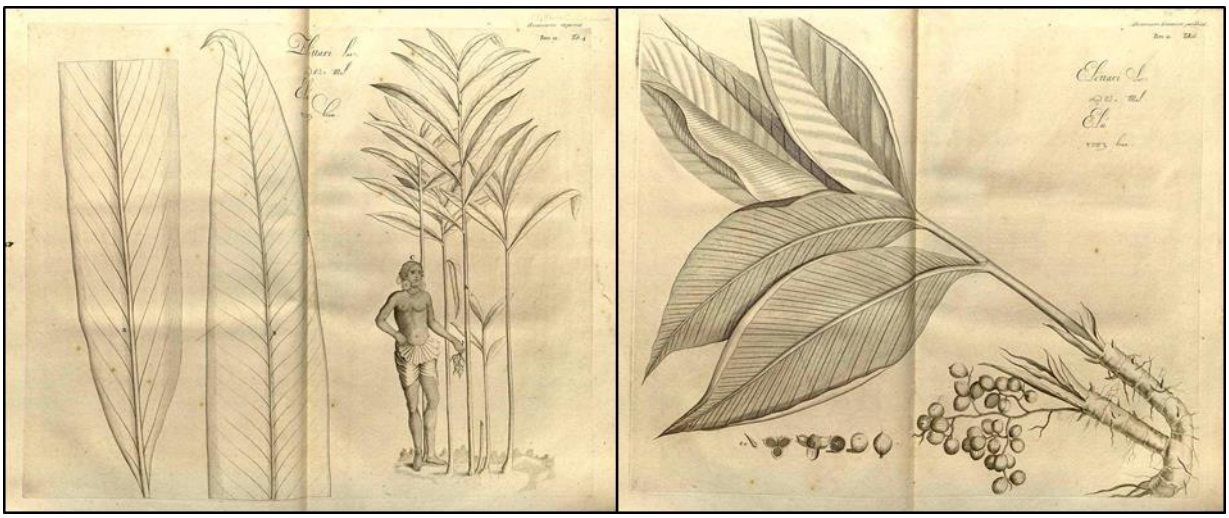

Figure 1.3 Plates of the holotype for the genus *Elettaria* (from the book by Rheede *et al.* 1692)

Based on morphology, Holttum (1950) transferred *Elettariopsis longituba* Ridl. to *Elettaria*. He indicated that several other species should be included (*Elettariopsis multiflora* Ridl., *Elettariopsis aquatilis* Ridl., *Cyphostigma surculosum* K.Schum. and sp.nov. from Borneo), but he did not take formal action. He suggested that the most important characteristic for *Elettaria* is the prostrate inflorescence axis with 2-ranked sheats and cincinni in the axils. He pointed out several similarities between *Elettaria* and the genus *Cyphostigma* Benth.: same type of inflorescence, fusion of

calyx and corolla, the position of the flower and the tubular bracteoles. He therefore indicated that *Cyphostigma* might be derived from *Elettaria*. He also proposed that *Elettaria cardamomum* and the Malaysian *Elettaria* might represent parallel evolution (Holttum, 1950). The next revision were done by Burtt and Smith, when they transferred *Elettariopsis surculosa* (K. Schum.) Ridl. in 1972 and Smith (1982) described *Elettaria rubida* R.M.Sm.. In 1986, Smith further transferred *Elettariopsis multiflora* Ridl. and finally, Sakai and Nagamasu (2000) described four new species (*E. brachycalyx, E. kapitensis, E. linearicrista, E. longipilosa*) and attempted to make a new combination of *Elettariopsis stoloniflora* (K. Schum.) Ridl., but used the wrong epithet *stolonifera*. In this thesis the correct combination, *Elettaria stoloniflora* S.Sakai & Nagam. is used.

In the article from 2000, Sakai and Nagamasu proposed that anther dehiscence might be an important character when distinguishing species. In their studied species from Borneo, they found four main types (Figure 1.4); *E. longituba* (Ridl.) Holttum have a simple pore without a flap (Figure 1.4 A), while *E. stoloniflora* and *E. rubida* R.M.Sm. have short slits (Figure 1.4 B), *E. kapitensis* S.Sakai & Nagam. show dehiscence throughout the entire length of the thecae (Figure 1.4 C), and finally *E. surculosa* B.L.Burtt & R.M.Sm., *E. brachycalyx* S.Sakai & Nagam., *E. longipilosa* S.Sakai & Nagam. and *E. linearicrista* S.Sakai & Nagam. have a pore covered with a hairy flap (Figure 1.4 D)

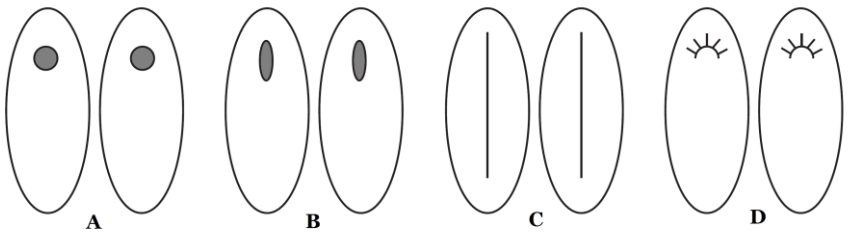

Figure 1.4 A drawing showing the different anther dehiscence patterns found in *Elettaria*. A: pore without a flap (*E. longituba*); B: short slits *(E. stoloniflora* and *E. rubida*); C: dehiscence throughout the entire length of the thecae (*E. kapitensis*) and D: pore with a hairy flap (*E. surculosa*, *E. brachycalyx*, *E. longipilosa* and *E. linearicrista*).

1.3 Molecular studies

Molecular data analyses have been widely used to reconstruct evolutionary history and to infer phylogenetic relationships within several angiosperm families (e.g., Liden *et al.* 1997; Oxelman *et al.* 1997; Andersson and Chase 2001; Hahn 2002; Borchsenius et al 2012). This is also the case for Zingiberaceae (e.g.,Ngamriabsakul *et al.* 2000; Rangsiruji *et al.* 2000a; Rangsiruji *et al.* 2000b; Wood *et al.* 2000; Kress *et al.* 2002; Pedersen 2004; Williams *et al.* 2004). The molecular study made by Kress *et al.* in 2002 focused on resolving the phylogenetic relationships among the genera in Zingiberaceae, using the nuclear ribosomal internal transcribed spacer (ITS) and the chloroplast *mat*K regions. The study resulted in the proposal of a revised classification of the family, with four subfamilies; Alpinioideae, Zingiberoideae, Tamijioideae and Siphonochiloideae and the four tribes Alpinieae, Riedelieae, Zingibereae and Globbeae. It did not include any samples of the genus *Elettaria*, however they suggested that the genus be placed in the tribe Alpinieae based on morphological features. After this publication, several studies have further tried to unravel the phylogenetic difficulties among the genera and also internally in genera (e.g., Kress *et al.* 2005; Xia *et al.* 2004; Kress *et al.* 2007). The latest and most detailed phylogeny were presented by Kress et al. (2007).The proposed phylogeny of Alpinioideae is pictured in figure 1.5.

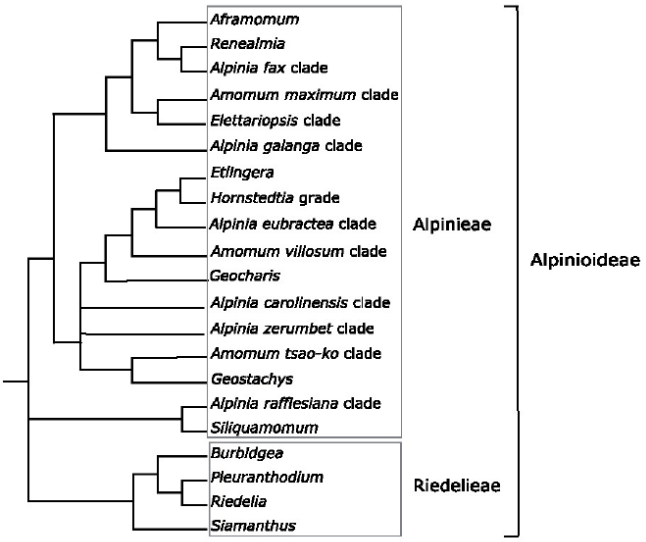

Figure 1.5 Proposed phylogeny of the Alpinioideae. The major clades have been reduced into single branches for clarity (from the article by Kress *et al.* 2007).

1.1 Aims of the study

To date, no published molecular studies have included any representatives from *Elettaria*. The aim of this study will be to:

1. Test the monophyly of the genus *Elettaria* using molecular analyses.
2. Place *Elettaria* within the tribe Alpinieae and indicate sister relations.
3. Elucidate structures within the genus.
4. Evaluate if the anther dehiscence types reflect the evolutionary history.

2 Materials and Methods

2.1 Collection

Species of the genus *Elettaria* and other relevant genera from Alpinieae, were collected during fieldwork with my supervisor, Axel Dalberg Poulsen, in the Malaysian state of Sarawak, Borneo (Figure 2.1). The sampling was conducted within a period of 30 days in February and March 2014. Each plant was documented on site by photographs and measurements including growth form and number of leafy shoots, the length and number of leaves per leafy shoot, the diameter and colour of the base, sheath characters, the length and other characteristics of the inflorescence and the colour, shape and diameter of the flower and/or the fruit. A fresh leaf sample was taken according to "The Tea Bag Method" described by Wilkie *et al.* (2013). Finally, the entire plant was collected and pressed as voucher specimen (Figure 2.2).

Figure 2.1 Collection localities: The red dots represent collections from February/March 2014, while the yellow dots indicate other collections of *Elettaria* made by Axel Dalberg Poulsen *et al.* on both sides of the important biogeographical boundary, Wallace's Line (Based on the map by Chris Van Nice).

Figure 2.2 Documenting the collection of *Elettaria longituba*, Kubah NP, Sarawak, Malaysia (Poulsen *et al.* 2938). This species has ovoid subterranean fruits that involve a lot of digging to excavate (Photo Axel Dalberg Poulsen).

Collected plants were kept in plastic bags inside hessian sacks for protection against the sun while in the field. Later, the plants were pressed and dried at 55-65°C in an oven at the Botanical Research Centre (BRC) at Semenggoh Wildlife Centre in Sarawak (Figure 2.3). Flowers and/or fruits were separately preserved in alcohol. The first set was deposited at the Sarawak Herbarium (SAR). Excess sets were divided between The Royal Botanic Garden, Edinburgh (E), The Natural History Museum in Oslo (O), and The Singapore Herbarium (SING) (Table 1).

Figure 2.3 The method used for pressing and drying the voucher specimens at the BRC at Semenggoh Wildlife Centre, Sarawak.

In addition, DNA samples were taken from cultivated plants and herbarium specimens from The Royal Botanic Garden in Edinburgh and The Natural History Museum in Oslo. Previously collected DNA samples of *Elettaria*, from localities in Sulawesi and Sumatra (Figure 2.1), have also been included in this study. Finally, I extracted DNA by scraping capsules from commercially sold cardamom (*Elettaria cardamomum*) with a scalpel blade.

2.2 DNA extraction

Genomic DNA was isolated from silica-dried leaf samples or herbarium specimens using the DNeasy® Plant Mini Kit (Qiagen®) according to the manufacturer's protocol, with following two modifications: At step one: Approximately one cm^2 of dried leaf material was put in a crushing tube together with two tungsten carbide beads and grounded to fine powder in a Crushing mill (MM301, Retsch® GmbH & Co) at 23Hz for 2 x 30 seconds. In step 11, the amount of AE buffer was reduced from 100 µL to 50 µL.

2.3 PCR amplification and cycle sequencing

The molecular analyses were based on chloroplast *trn*L-F sequences (*trn*L intron and the *trn*L-*trn*F intergenic spacer) and nuclear ribosomal internal transcribed spacer (ITS) sequences (ITS1, 5.8S and ITS2). The DNA regions were amplified using the AmpliTaq® DNA polymerase buffer II kit (Applied Biosystems®). The ITS region was amplified using the universal primers "ITS4" and "ITS5"(White *et al.*, 1990), while the *trn*LF region was amplified in two sections using the universal primers "c" and "d" and "e" and "f" (Taberlet *et al.*, 1991). All extracted DNA-samples were set up in 12.5 µL PCR reactions containing 1.25 µL 0.4 g/L BSA, 1.25 µL 25 mM Magnesium chloride ($MgCl_2$), 1.25 µL Taq polymerase buffer, 0.25 µL 10 mM dNTP's, 0.5 µL 10 µM of each primer, 1 µL extracted DNA, 0.08 µL of Taq polymerase and 6.42 µL sterile deionized water (MilliQ water).

PCR was performed on a thermal cycler with the following protocol for the ITS region: pre-denaturation at 95°C for 2 minutes followed by 25 cycles with denaturation at 95°C for 45 seconds, annealing at 55°C for 30 seconds, synthesis at 72°C for 30 seconds and a final elongation step at 72°C for 6 minutes, before stored at 12°C. The protocol for *trn*LF region was the same with one exception; the annealing temperature was set to 57°C.

The quality of the PCR products was assessed by running a gel electrophoresis on a 1% Agarose gel at 90V for 30 minutes. Successful PCR products were purified by adding 2µL 10 times diluted ExoSAP-IT® (Affymetrix® (USB Products®)), incubated at 37°C for 45 minutes and then the temperature was raised to 80°C for 15 minutes for inactivation of the hydrolytic enzymes in ExoSAP-IT. This purification step was

done to remove redundant primers and unincorporated dNTPs which may interfere when sequencing the PCR product.

Cycle Sequencing was performed with BigDye® Terminator (BDT) v1.1 and v3.1 cycle sequencing kit (Applied Biosystems®). 1 µL of purified PCR-product were added to 6.3 µL MilliQ water, 1.8µL buffer, 0.5 µL of either reverse or forward primer and 0.4 µL BigDye v1.1 or v3.1. The strips were placed in a thermal cycler with pre-denaturation at 96°C for 1 minute, followed by 30 cycles with denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds and a final elongation step at 60°C for 6 minutes before stored at 15°C.

The products were further purified by ethanol precipitation. It was added 1 µL 125 mM EDTA, 1 µL 3M sodium acetate and 25 µL 96% ethanol to each sample, followed by 15 minutes of incubation at room temperature before the centrifugation step at 5500 rpm for 25 minutes in a plate centrifuge (Rotanta 46 RS (Andreas Hettich GmbH & Co. KG)), which held 4°C. The centrifugation step separated the liquid from the matter, hence forming a DNA containing pellet at the bottom of each well. The excess liquid was removed by inverting the plates on lint-free paper and placed in the plate centrifuge at 400 rpm for 20 seconds. Further, 35 µL of 70% ethanol was added to the wells and the centrifugation steps were repeated, followed by 2-3 minutes in a vacuum centrifuge, for removal of any remaining liquid.

The dried samples were re-suspended in 11 µL Hi-Di™ Formamide (Applied Biosystems®) and 10 µL of the solution were transferred to an ABI plate. The capillary electrophoresis was run on ABI prism 3130*xl* Genetic Analyzer (Hitachi, Applied Biosystems®) at the DNA laboratory of The Natural History Museum, Oslo, Norway

2.4 Alignment

First I used CodonCode Aligner version 4.2.7 (CodonCode Corporation) to assemble the forward and reverse sequence pairs and manually edited the sequence pairs for any discrepancies by checking the chromatogram pane. Uncertain bases in the beginning and the end of the sequences were also deleted. This was done to create a single consensus sequence for each sample. Further, the consensus sequences were

aligned using Clustal W (Larkin *et al.*, 2007) with default settings. The multiple sequence alignments were done together with downloaded sequences from GenBank® (Table 2). Manually adjustments were done and the finished aligned matrices were exported as a FASTA-file without gaps.

2.5 Molecular analyses

The sequences were realigned in *MEGA* version 6.06 (Tamura *et al.*, 2013) using Clustal W, with default settings, and again manually adjusted before phylogeny reconstruction were conducted. I performed separate maximum likelihood analyses for the ITS-region and the *trn*L-F-region, using the Tamura-Nei method (Tamura and Nei, 1993). The phylogeny was tested with the bootstrap method set to 1000 replications. Gaps/Missing data were treated as complete deletions. The optimal tree inference was found using the Nearest-Neighbour-Interchange (NNI) heuristic.Later, I also performed maximum parsimony analyses for both sequence regions. The same settings as for the maximum likelihood analyses were used with one exception; the optimal tree inference was found using heuristic searches performed with Subtree-Pruning-Regrafting (SPR). The resulting maximum likelihood and maximum parsimony phylogenetic trees for each sequence region were merged into one tree topology, and *Burbidgea schizocheila* were set as outgroup, after examination of the proposed phylogeny of Alpinieae by Kress *et al.* (2002).

2.6 Anther dehiscence type and fruit form

Pickled flowers were examined with respect to anther dehiscence type. The different types were photographed and a symbol for the different forms were inserted in the ITS phylogenetic tree. Finally, I also included symbols for what kind of fruit form each available sample had.

Table 1. List of all extracted and sequenced DNA samples in this study.

Name	Collector	Coll.no.	Country	State	Locality	dd.mm.yy	Latitude	Longitude	Herbarium*
Aframomum melegueta K.Schum.	Mathisen	HM03	Cote d'Ivoire	-	Cultivated at O*	Des. 14	-	-	O
Alpinia galanga (L.) Willd. (1)	Mathisen	HM05	Norway	-	Cultivated at O*	Des. 14	-	-	O
Amomum cerasinum Ridl.	Poulsen & Mathisen	2945	Malaysia	Sarawak	Kubah National Park	20.02.14	1° 36' 42.6" N	110° 11' 32.6" E	SAR, E
Amomum coriaceum R.M. Sm.	Poulsen & Mathisen	2946	Malaysia	Sarawak	Kubah National Park	20.02.14	1° 36' 42.6" N	110° 11' 32.6" E	SAR, E
Amomum dictyocoleum K.Schum	Poulsen & Mathisen	2936	Malaysia	Sarawak	Semenggoh Forest Reserve	17.02.14	1° 24' 5.0" N	110° 18' 53.7" E	SAR, E
Amomum sp.	Poulsen & Mathisen	2965	Malaysia	Sarawak	Gunung Mulu National Park	28.02.14	4° 2' 36" N	114° 52' 31.5" E	SAR, E
Burbidgea schizocheila Hackett (1)	Newman *et al.*	2540	Malaysia (Cult.)	Sabah	Cultivated at E*	06.12.12	-	-	E
Elettaria A	Poulsen *et al.*	2672	Indonesia	Central Sulawesi	Kebun Kopi	07.03.08	0° 43' 20" S	119° 58' 53" E	AAU, BO, E, SING
Elettaria B	Poulsen *et al.*	2750	Sulawesi	South Sulawesi	Montane forest	16.01.09	2° 15' 26" S	120° 47' 47.9" E	AAU, BO, E
Elettaria C	Poulsen *et al.*	2738	Sulawesi	Central Sulawesi	Open area near garden	14.01.09	1° 56' 14.7" S	120° 47' 30.7" E	BO, E
Elettariopsis kandariensis Loes.	Poulsen *et al.*	2790	Sulawesi	Southeast Sulawesi	Boroboro Range	01.07.09	4° 9' 9.2" S	122° 29' 42.5" E	AAU, BO, CEB, E
Elettaria D	Poulsen *et al.*	2811	Sulawesi	Central Sulawesi	Forest patch near trail in garden area	11.02.09	0° 59' 7.9" N	121° 36' 0" E	BO, E
Elettaria E	Poulsen *et al.*	2677	Indonesia	Central Sulawesi	Palayan	08.03.08	1° 4' 29" N	120° 53' 25" E	AAU, BO, E, SING
Elettaria F	Poulsen *et al.*	2681	Indonesia	Central Sulawesi	Along road to G. Tinombala.	09.03.08	0° 37' 48" N	120° 40' 2" E	BO, E
Elettaria G	Poulsen *et al.*	2431	Indonesia	Riau	Bukit Tigapulu National Park	30.07.06	0° 51' 0" S	102° 31' 0" E	AAU, ANDA, BO, E,
Elettaria H	Poulsen *et al.*	2973	Brunei	Belait District	Kg. Teraja	05.03.14	4° 17' 9.01" N	114° 25' 44.6" E	BRUN, E
Elettaria I	Poulsen & Mathisen	2964	Malaysia	Sarawak	Gunung Mulu National Park	06.07.05	4° 2' 30.9" N	114° 52' 42.8" E	SAR, E, O
Elettaria longipilosa S.Sakai & Nagam.	Poulsen & Mathisen	2953	Malaysia	Sarawak	Lambir Hills National Park	06.07.05	4° 14' 14" N	114° 3' 50.2" E	SAR, E, O
Elettaria longituba (Ridl.) Holttum	Poulsen & Mathisen	2938	Malaysia	Sarawak	Kubah National Park	17.02.14	1° 36' 38" N	110° 11' 49.3" E	SAR, E, O, SING
Elettaria rubida R.M.Sm.	Poulsen & Mathisen	2966	Malaysia	Sarawak	Gunung Mulu National Park	06.07.05	4° 2' 35.5" N	114° 52' 30.1" E	SAR, E
Elettaria stolonifera (K. Schum.)	Poulsen &	2942	Malaysia	Sarawak	Trail between Kubah Natinal	19.02.14	1° 36' 39.8" N	110° 10' 20.8" E	SAR, E, O

S. Sakai & Nagam.	Mathisen				Park & Matang Wildlife Centre				
Elettaria surculosa (K. Schum.) B.L.Burtt & R.M.Sm. (1)	Poulsen & Mathisen	2939	Malaysia	Sarawak	Kubah National Park	17.02.14	1° 36' 37.6" N	110° 11' 47.2" E	SAR, E, O, SING
Elettaria surculosa (K.Schum.) B.L.Burtt & R.M.Sm. (2)	Poulsen & Mathisen	2947	Malaysia	Sarawak	Kubah National Park	20.02.14	1° 36' 44.6" N	110° 11' 32.5" E	SAR, E
Elettaria cardamomum (L.) Maton 1	Poulsen & Mathisen	2979	Scotland	-	Cultivated at E*	29.04.14	-	-	E
Elettaria cardamomum (L.) Maton 2	Borgen & Poulsen	1979-96	Norway	-	Cultivated at O*	08.06.09	-	-	O
Elettaria cardamomum (L.) Maton 3	Poulsen & Mathisen	2977	Scotland	-	Cultivated at E*	28.04.14	-	-	E
Elettaria cardamomum (L.) Maton 4	Poulsen & Mathisen	2978	Scotland	-	Cultivated at E*	29.04.14	-	-	E
Elettaria cardamomum (L.) Maton 5	Mathisen	HM02	India	Western India	DNA extracted from capsules	Des. 14	-	-	O
Elettaria cardamomum (L.) Maton 6**	Mathisen	HM01	Guatemala	-	DNA extracted from capsules	Des. 14	-	-	O
Elettariopsis sp.	Poulsen & Mathisen	2948	Malaysia	Sarawak	Kubah National Park	20.02.14	1° 36' 44.6" N	110° 11' 32.5" E	SAR, E
Elettariopsis kerbyi R.M.Sm. (1)	Poulsen & Mathisen	2956	Malaysia	Sarawak	Lambir Hills National Park	26.02.14	4° 12' 8.2" N	114° 2' 40.5" E	SAR, E
Etlingera inundata S.Sakai & Nagam. (1)	Poulsen & Mathisen	2954	Malaysia	Sarawak	Lambir Hills National Park	25.02.14	4° 14' 12.3" N	114° 4' 3.1" E	SAR, E
Hornstedtia leonurus (J.König) Retz. (2)	Poulsen & Mathisen	2958	Malaysia	Sarawak	Lambir Hills National Park	26.02.14	4° 12' 9.5" N	114° 2' 38" E	SAR, E, SING
Hornstedtia scyphifera (J.König) Steud.	Poulsen & Mathisen	2967	Malaysia	Sarawak	Gunung Mulu National Park	01.03.14	4° 2' 40" N	114° 49' 28" E	SAR, E, O
Renealmia alpinia (Rottb.) Maas (1)	Mathisen	HM04	Guatemala	-	Cultivated at O*	Des. 14	-	-	O

* Herbaria abbreviation according to Index Herbariorum

** Only sequenced for the *trn*L-F region

Table 2. List of all sequences downloaded from GenBank.

Name	Collector	Coll.no.	Country	ITS	trnL-F	Herbarium*
Aframomum limbatum (Oliv. & T.Hanb.) K.Schum	D. Harris	5764	Cameroon	FJ848584	FJ848629	E
Aframomum longiligulatum Koechlin	D. Harris	9024	Congo	FJ848580	-	E
Aframomum longiligulatum Koechlin	D. Harris	5668	Central African Republic	-	FJ848639	E
Aframomum verrucosum Lock	D. Harris	5665	Central African Republic	FJ883014	-	E
Aframomum verrucosum Lock	A.D. Poulsen	1327	Uganda	-	FJ848660	C, K, MHU
Aframomum angustifolium K.Schum.	A.D. Poulsen	1356	Uganda	FJ848620	-	C
Aframomum angustifolium K.Schum.	W.J. Kress	3403	Madagascar	-	FJ848632	US
Aframomum sceptrum Oliv. & D.Hanb.	D. Harris	8359	Gabon	FJ848576	FJ848621	E
Alpinia abundiflora B.L.Burtt & R.M.Sm.	A. Weerasooriya	-	Sri Lanka (Cult.)	AY742334	KF748154	K
Alpinia fax B.L.Burtt & R.M.Sm.	A. Weerasooriya	-	Sri Lanka (Cult.)	AY742348	KF748153	K
Alpinia galanga (L.) Willd (2)	A.Rangsiruji	3	Cultivated in E	AY424739	AY424775	E
Amomum coriaceum R.M. Sm. (2)	S. Sakai *et al.* - unpublished	-	-	AB097240	-	-
Amomum dimorphum M.F.Newman	S. Sakai *et al.*- unpublished	-	-	AB097244	-	-
Burbidgea schizocheila Hackett (2)	A. Rangsiruji & M. Newman	19851903	Borneo (Cult.)	AY769821	AY769784	E
Elettariopsis kerbyi R.M. Sm. (2)	W. J. Kress	96-5746	Borneo	AF478746	-	US
Elettariopsis smithiae Y.K.Kam	W. J. Kress	99-6313	Thailand	AY352013	-	US
Elettariopsis unifolia (Gagnep.) M.F.Newman	M. Newman	747	Vietnam	AY769832	AY769795	E
Etlingera inundata S.Sakai & Nagam. (2)	S. Sakai *et al.* - unpublished	-	Borneo	AB097233	-	-
Geocharis fusiformis (Ridl.) R.M.Sm.	L.B. Pedersen	1141	Tenom Orchid Centre (Cult.)	AF414487	-	C
Hornstedtia leonurus (J.König) Retz (1)	S. Sakai *et al.* - unpublished	-	-	AB097237	-	-
Hornstedtia sanhan M.F.Newman	M. Newman	202	Vietnam	AY769844	AY769807	E
Paramomum petaloideum (S.Q.Tong) T.L.Wu	W. J. Kress	#95-5508	China	AF478771	-	US
Plagiostachys strobilifera (Baker) Ridl.	S. Sakai	361	Sarawak	AB097252	-	KYO
Plagiostachys oblanceolata Gobilik & A.L.Lamb	Julius *et al.*	ATW34	Borneo	DQ507848	-	BORH, HYO
Renealmia alpinia (Rottb.) Maas (2)	Lombardi	3644	Brazil	DQ122863	DQ444491	U
Renealmia battenbergiana Cummins	AN	44	Ghana	DQ427031	DQ444515	HLA
Renealmia cernua (Sw. ex Roem. & Schult.) J.F.Macbr.	Maas	9410	Costa Rica	DQ427023	DQ444525	U
Renealmia pluriplicata Maas	Maas	9444	Costa Rica	DQ427019	DQ444518	U

*Herbaria abbreviation according to Index Herbariorum

3 Results

3.1 Sequence alignments

ITS-region sequences downloaded from GenBank varied from 412 bp (*Alpinia abundiflora* B.L.Burtt & R.M.Sm.) to 637 bp (*Etlingera inundata* S.Sakai & Nagam. (2)). The sequenced unaligned ITS-region sequences varied from 598 bp (*Elettaria surculosa* (K.Schum.) B.L.Burtt & R.M.Sm. (1)) to 641 bp (*Alpinia galanga* (L.) Willd. (1)). The complete matrix had a total aligned length of 677 bp.

*trn*L-F-region sequences downloaded from GenBank varied from 746 bp (*Renealmia cernua* (Sw.ex Roem. & Schult.) J.F.Macbr.) to 907 bp (*Hornstedtia sanhan* M.F.Newman), and the aligned length of the matrix was 981 bp. The unaligned *trn*L-F-region sequences varied from 837 bp (*Elettaria cardamomum* (3)) to 922 bp (*Amomum dictyocoleum* K.Schum.),

3.2 Phylogeny reconstruction

3.2.1 ITS-region

The maximum parsimony analysis based on the ITS data set resulted in three most parsimonious trees (MPTs) of 477 steps each. Of 677 characters, 210 were parsimony informative. After setting the cut-off value = 50 %, they all got the same tree topology. The maximum likelihood analysis gave almost the exact same tree topology (Figure 3.1).

The ingroup taxa comprise two major monophyletic lineages that have rather low support: clade A (bootstrap value (BT 63/64) and clade B (BT 52/66). In clade A, three collections of *Elettaria cardamomum* (BT 100/100) and two species of *Plagiostachys* Ridl. (BT 100/100) constitute a highly supported clade (BT 98/99) that resolves as sister to the rest of the clade, also well supported (BT 99/92). *Geocharis fusiformis* (Ridl.)R.M.Sm. resolves as a poorly supported sister (BT 54/--) to the rest of the clade, which further subdivides into four clades that form a polytomy. 1) A highly supported clade consisting of *Hornstedtia* Retz., *Amomum* and

Etlingera Giseke (BT 95/93), 2) the poorly supported clade consisting of *Amomum* and *Elettariopsis* Baker in HooK.f. (BT 52/-), 3) *Amomum coriaceum* R.M.Sm. (BT 100/100) and 4) finally the highly supported clade consisting of several species of *Elettaria*, one *Amomum* and one sample identified as *Elettariopsis* (BT 92/92*)*. The *Amomum dimorphum* M.F.Newman resolves as the sister to the rest of this group, with high support (BT 98/95). The clade is subdivided into two clades with high support (BT 90/85 and BT 100/99),

Clade B is subdivided into four clades that form a polytomy. 1) A poorly supported clade consisting of *Elettaria*, *Alpinia* and *Aframomum* (BT 55/--), 2) a highly supported clade of *Renealmia* (BT 100/99), 3) a clade consisting of *Elettariopsis* and *Paramomum* S.O.Tong (BT 100/99), where *Paramomum* resolves as sister to the *Elettariopsis* species (BT 69/58), and 4) finally, a highly supported clade of *Alpinia galanga* (100/100).

3.2.2 *trn*L-F region

The maximum parsimony analysis based on the *trn*L-F data set resulted in four most parsimonious trees (MPTs) of 85 steps each. Of 981 characters, 78 were parsimony informative. The maximum likelihood analysis gave almost the exact same tree topology (Figure 3.2).

3.2.3 Comparison of the nuclear and chloroplast cladograms

The cladogram for the *trn*L-F-region had lower resolution than the tree based on the ITS-region. The ingroup formed one large polytomy and it was therefore few discrepancies. However, some incongruent results were found: the most species rich clade of *Elettaria* was split in two clades in the trnL-F cladogram. *Elettaria* 1a has low support (BT --/51) and *Amomum cerasinum* Ridl. is resolved as sister. *Elettaria* 1b has low support (BT 52/--) and *Amomum dictyocoleum* is included in this unresolved group.

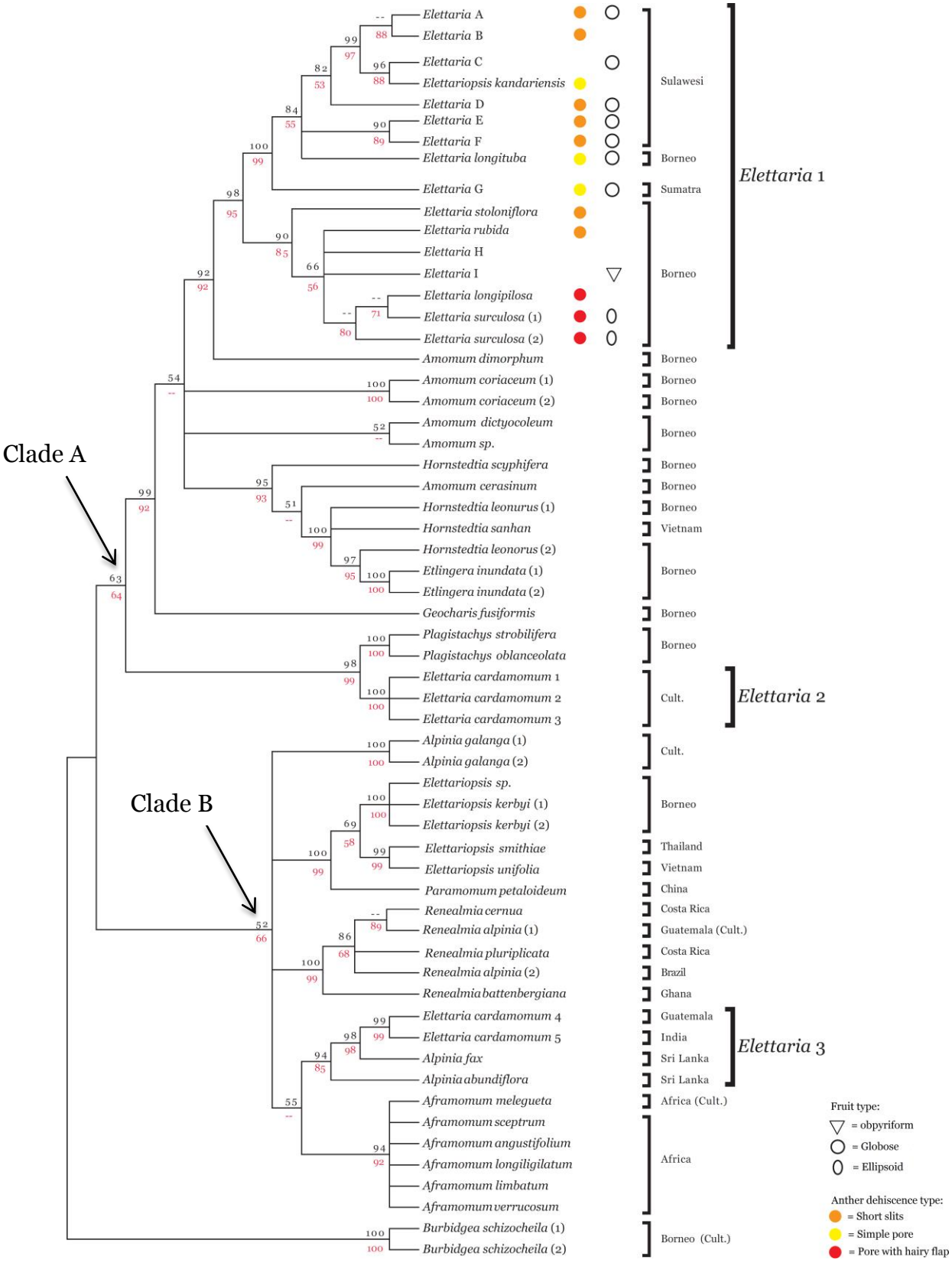

Figure 3.1 Phylogeny of *Elettaria* and related genera based on ITS-region sequence data. The black bootstrap values are from the maximum parsimony analysis and the red values are from the maximum likelihood analysis. Different fruit types and anther dehiscence types are indicated with assigned symbols.

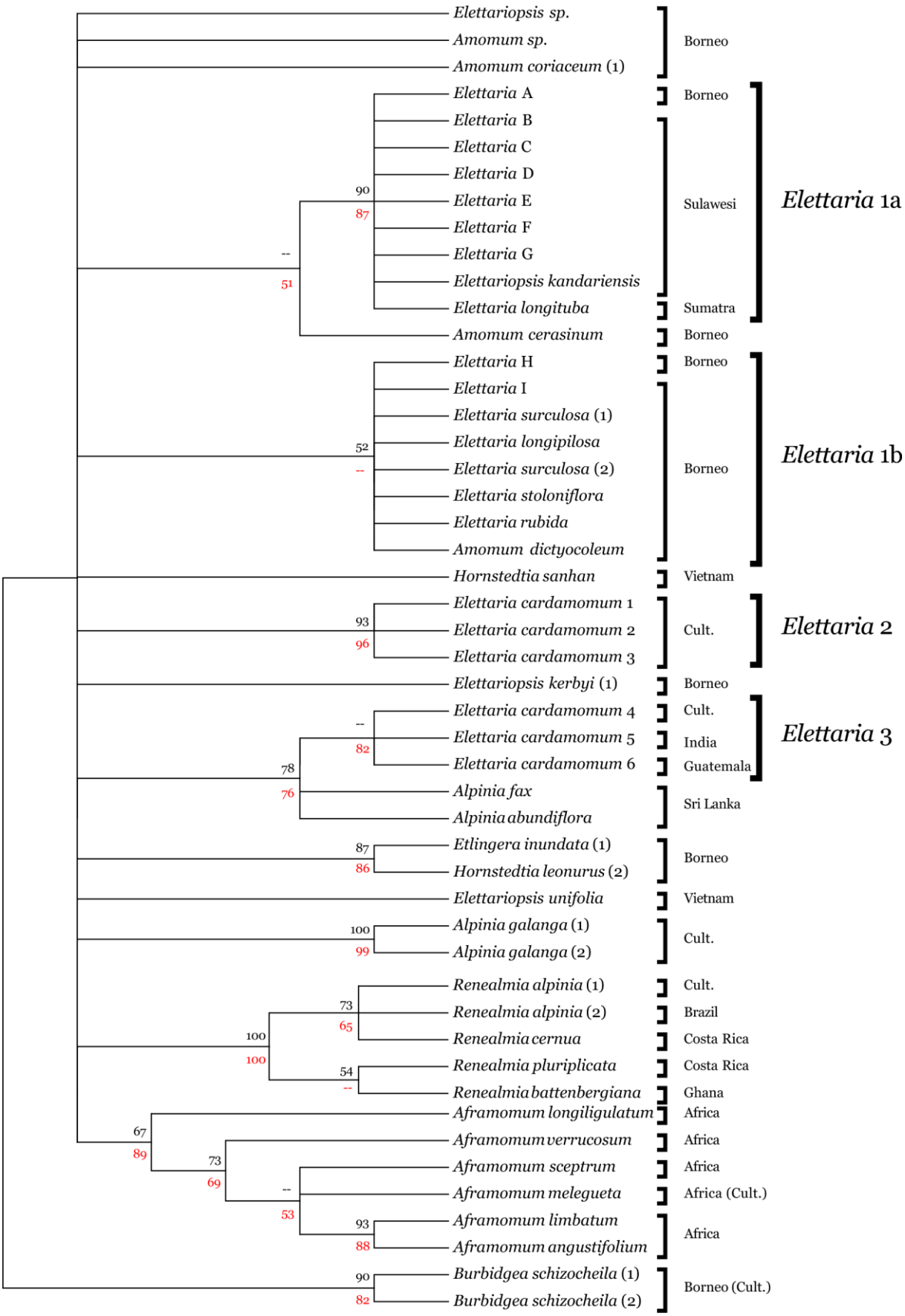

Figure 3.2 Phylogeny of *Elettaria* and related genera based on *trn*L-F-region sequence data. The black digits are from the maximum parsimony analysis and the red numbers are from the maximum likelihood analysis.

4 Discussion

4.1 Monophyly of the genus *Elettaria*

The included samples determined to belong to the genus *Elettaria,* do not come out as one monophyletic clade in my analyses, but fall into at least three independent clades (figure 3.1 and 3.2). The phylogenetic tree from the ITS-region is far more resolved, and with better support than the one produced from *trn*L-F. This is not surprising because chloroplast DNA is known to evolve slower (e.g., Drouin *et al.* 2008)), and may explain why the *Elettaria* clade 1 in the ITS-tree splits into two (*Elettaria* 1a and *Elettaria* 1b) though with low support (figure 3.2).

The type species of *Elettaria*, *E. cardamomum*, is found in the *Elettaria* clade 3, which together with *Alpinia fax* B.L.Burtt & R.M.Sm. and *A. abundiflora* B.L.Burtt & R.M.Sm. form a highly supported clade. As the type belongs in this clade, this is the clade for true cardamom. It is convenient that this highly traded species maintains nomenclatural stability. *Elettaria cardamomum* originates from India, which corresponds well with the distribution of *A. fax* and *A. abundiflora,* making this an Indian – Sri Lankan clade. Kress *et al.* (2007) named this clade the "*Fax* clade". Had Kress *et al.* included a sample of *E. cardamomum* in their study, they would have realized that the correct name for this is the "*Elettaria clade".* Both *A. fax* and *A. abundiflora* were originally described in *Elettaria*, as *E. involucrata* Thwaites and *E. floribunda* Thwaites, before ending up in the polyphyletic genus *Alpinia* (Kress et al., 2007) . Based on my analyses, the two names should be resurrected. The low support values makes it difficult to conclude anything certain about sister relationships of the *Elettaria* clade 3, but it does not contradict the results by Kress et al. (2007) where *Renealmia* is resolved as sister. As previously mentioned, Holttum (1950) highlighted several morphological similarities between *Elettaria* and the monotypic genus *Cyphostigma* endemic to Sri Lanka. It is unfortunate that DNA material of *Cyphostigma pulchellum* was not included in this study, but due to its geographical distribution, it is likely to be a synonym of *Elettaria* though this needs to be confirmed. This needs to be confirmed in further studies.

Elettaria clade 1, forms a well-supported clade. It consists of samples collected by myself and others in Sulawesi, Borneo and Sumatra. *Amomum dimorphum*, also

from Borneo, is resolved as sister to the *Elettaria* clade 1. It is interesting that this relative species rich clade comes out separately from *Elettaria* clade 3, where the type belongs. For the first time indicating that prior revisions (Holttum 1950; Burtt and Smith 1972; Smith 1982; Smith 1986; Sakai and Nagamasu 2000), based on morphology, resulted in a non-monophyletic genus delimitation. My results show that the generic name of *Elettaria* should not be applied for the species in this clade. Holttum (1950) already noted that *E. cardamomum* has inflorescences above ground with short corolla tubes, while the included species from Borneo, Sumatra and Sulawesi (Appendix I) have subterranean inflorescences. There are further morphological differences such as longer corolla tubes, lacking tooth-like staminodes, having a semi-tubular base to the labellum (not flat), labellum white with yellow-green band sometimes with red bands (white with branched purple lines), anther often crested (ecristate). Furthermore, the fruits of taxa from *Elettaria* clade 1 are ellipsoid, obpyriform, or globose, whereas *E. cardamomum* have ellipsoid fruits (Table 3). Species from Sulawesi, including species currently placed as *Elettariopsis kandariensis* Loes. belong to the same genus as taxa from Sundaland and await a new name. The question is what the name of the genus should be for these taxa. No obvious old names seem applicable.

Table 3. Morphological characteristics: summarizing the differences between *Elettaria* clade 1 and *Elettaria cardamomum*.

Character	*Elettaria* clade 1	*E. cardamomum*
1 Inflorescence	Long, prostrate, more or less subterranean	Long, erect, above the ground, leaning against the soil when ripe
2 **Flower:**		
Corolla tube	Long corolla tubes	Short corolla tubes,
Colouring	yellow-green bands, sometimes also with red bands	Purple branched in centre
Staminodal theet	No	Two
Labellum	More or less semitubular at base, often with anther crest	Flat, ecristate
Anther	Simple pore, pore with hairy flap, short slits or dehiscence throughout the entire length of the thecae	Dehiscence troughout the entire length of the thecae
3 Fruit	Ellipsoid, globose or obpyriform	Ellipsoid

Elettaria clade 2 constitutes three cultivated plants from the greenhouses at the botanical gardens in Edinburgh and Oslo, which was named *E. cardamomum*. They all have a distinct smell of cardamom, however given the present result these determinations are obviously not correct (Figure 4.1). None of the three plants have flowered yet, making determination difficult. These plants have been cultivated for at least 40 years, with no known origin, making determination even more problematic. Given this surprising result, ITS-sequences were run through the BLAST nucleotide search engine at GenBank® (Benson et al., 2009) and they matched a 100% with several species from the genus *Alpinia* and *Plagiostachys* (eg., *Alpinia calcarata* Roscoe, *A. chinensis* Roscoe and *Plagiostachys parva* Cowley). One should be reluctant in trusting these results as they match with more than one species. Some of the uploaded sequences on GenBank might be incorrect determined or there might simply be very little variation between some of the species. The present analysis includes the type of *Alpinia*, *A. galanga*, and it is unlikely that the mystery cardamom belongs to this genus.

Fig.4.1 Since 1979, a sterile plant has been presented to visitors of the Victoria House, Natural History Museum in Oslo as *Elettaria cardamomum*. My molecular work shows the identification is not correct and the plant has been removed (Photo Axel Dalberg Poulsen).

4.2 Morphological variation and biogeography within clade 1

Sakai and Nagamasu (2000) emphasized anther dehiscence as an important diagnostic character for the genus *Elettaria,* after morphological studies of species from Borneo. The sister to all taxa from Sulawesi, *Eelettaria longituba* (from Borneo), has anthers which open by simple pores and globose fruits. All verifiable collections from Sulawesi, had the same fruit type as *E. longituba*, but just one of the examined collections from Sulawesi, *Elettariopsis kandariensis,* had the same anther dehiscence type (simple pore). The remaining collections from Sulawesi had another common anther dehiscence type; short slits. This may indicate that the origin of the Sulawesi species is likely to be from Sundaland after crossing Wallace's Line, though the relative low support on internal structure does not allow firm conclusions. There seems also to be great variations of the anther crests, which indicate that more taxa are present among the included material from Sulawesi, but further morphological examination is required to determine how many new species may be involved.

5 Concluding remarks

This study has shown that samples determined to belong to the genus *Elettaria* is not a monophyletic clade, but fall into at least three independent clades. The type species of *Elettaria* (*Elettaria cardamomum*) is found in the well-supported *Elettaria* clade 3, together with *Alpinia abundiflora* and *A. fax,* and forms the true *Elettaria* clade. As a consequence, the initial names of *A. abundiflora* and *A. fax*, *E. involucrata* and *E. floribunda,* should be resurrected. The low support values makes it difficult to conclude anything certain about sister relationships, but they do not contradict prior results, where *Renealmia* is resolved as sister to the previously Fax clade. The *Elettaria* clade 1 should be recognized at generic rank, and a new combination should be published for the taxa within it, including species from Sulawesi currently named *Elettariopsis kandariensis*. *E. longituba* is resolved as sister to the Sulawesi species and the anther dehiscence types and the fruit types indicate that the origin of the Sulawesi species is likely to be from Sundaland after crossing Wallace's Line. On the other hand, the cultivated *Elettaria* clade 2 is difficult to determine due to lack of floral material. When using BLAST, the ITS-sequence matches with species in several genera. Determination must await a plant to flower.

Although the study shows clear evidence of non-monophyletic genus delimitation, further studies are required to support the result. The use of additional DNA markers will increase the molecular support. It will be beneficial to add more chloroplast and nuclear markers. One should also include additional closely related species, like *Cyphostigma pulchellum,* to be sure their place in the Tribe. Additional examinations of morphology, with emphasize on floral structure, will also be necessary to be able to distinguish between potentially new species.

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APPENDIX I: COLLECTIONS

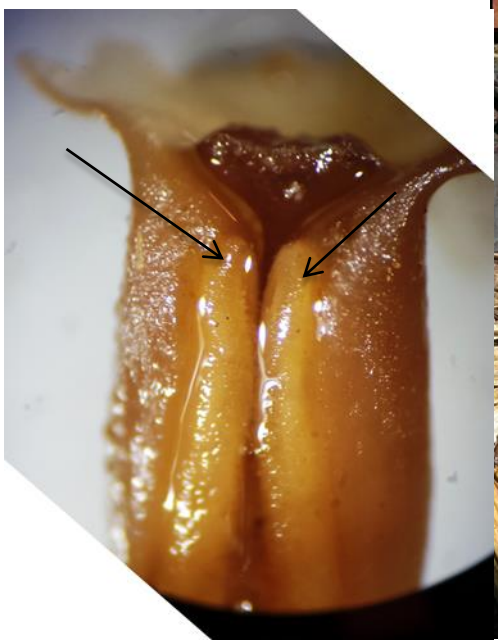
Species of *Elettaria* collected in the wild from which DNA-samples were extracted and included in the phylogenies. For each, notes on locality, coordinates, elevation, description and deposition of herbarium sets are presented. Photographs from the wild by A.D. Poulsen. Microscopic photographs of pickled anthers by H.B. Mathisen.

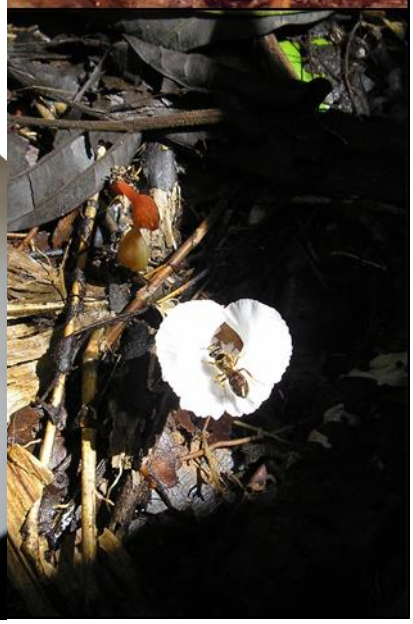
Poulsen 2431– *Elettaria* G

Indonesia. Sumatra, Riau, Bukit Tigapulu National Park. (0° 51' 0" S; 102° 31' 0" E);
alt. 130 m; 30 July 2006.

Notes: Terrestrial herb to 3.5 m, in clump. Base of leafy shoot and sheath pale brown. Ligule green, bilobed. Petiole to 3 cm. Lamina to 75 x 18 cm, plicate, pubescent beneath. Inflorescence long-creeping. Calyx red. Corolla lobes pale reddish brown. Labellum white with yellow-green basal centre. Anther crest white. Fruit subterranean, rounded, cream to pale green.

Deposited at: AAU, BO, E, Andalas University. Material in spirit. Photographed. DNA sampled.





Poulsen 2672 – *Elettaria* A

Indonesia. Central Sulawesi, Kebun Kopi.
Roadside in secundary forest and plantation area.
(0° 43' 20" S; 119° 58' 53" E);
alt. 750 m; 07 March 2008.

Notes: Terrestrial herb in loose clump. Leafy shoot to 6.2 m long. Base of leafy shoot to 8 cm diam., greenish brown. Sheath greenish brown. Ligule twisted with callus at base. Petiole 0–9 cm long, longest at uppermost leaf. Lamina to 90 x 20 cm, mid-green; beneath pale green, pubescent. Inflorescence to 2.5 m long, subterranean, scales cream to pale red. Flowers single, to 12 cm long. Bracteole 5–6 cm long, pale red in upper 2 cm. Calyx 9.5 cm, red in upper 5 cm. Corolla lobes pale green. Labellum white, red in centre, terminal lobe 3-lobed, with yellow-green patch. Stamen red. Fruit 3.5 x 3 cm, subterranean, ridged. Cream to pale reddish.

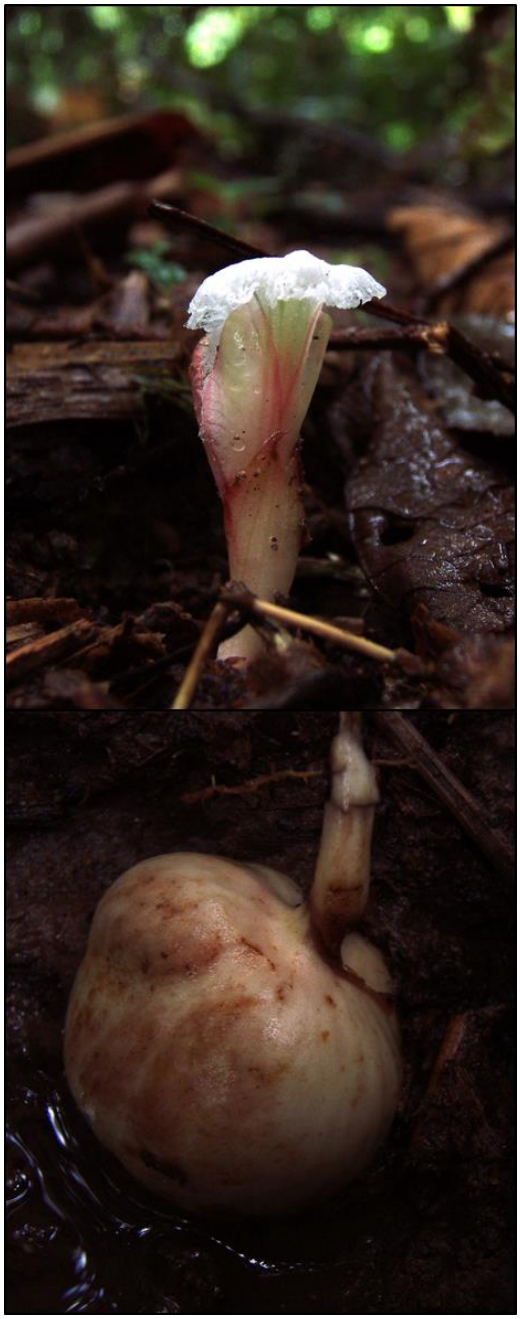
Deposited at: AAU, BO, E, SING. Material in spirit. Photographed. DNA sampled.

Poulsen 2677 – *Elettaria* E

Indonesia. Central Sulawesi, Palayan.

Cocoa plantation.

(1° 4' 29" N; 120° 53' 25" E);
alt. 550 m; 08 March 2008.



Notes: Terrestrial herb in dense clump of 18 shoots. Leafy shoot to 6.7 m long. Base of leafy shoot to 7 cm diam., brown. Sheath brownish green. Ligule bilobed, dentate to 7 mm long. Petiole to 2 cm at uppermost leaf. Lamina to 92 x 16 cm, mid-green. Inflorescence subterranean, to 2.5 m long; scales cream to pale pink. Calyx reddish with pale yellow-green apex; lobes cream, pale greenish to rose. Labellum white, centre red, terminal lobe with yellow-green patch. Stamen dark red at base, white in upper 6 mm, crest white. Stigma red. Fruit 3 x 3.5 cm, cream (green where exposed). Vernacular name: baku tondo (Dondo language). Used medicinally mixed with coconut juice to treat sick children.

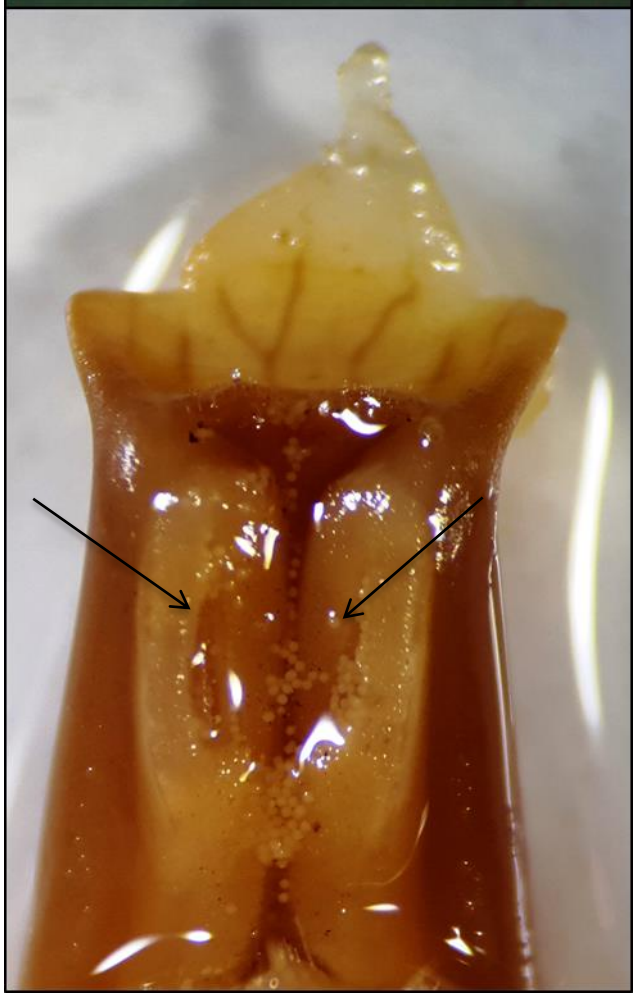
Deposited at: AAU, BO, E, SING. Material in spirit. Photographed. DNA sampled.

Poulsen 2681 – *Elettaria* F

Indonesia. Central Sulawesi, Along road to G. Tinombala. Open area.
(0° 37' 48" N; 120° 40' 2" E);
alt. 1040 m; 09 March 2008.

Notes: Terrestrial herb in loose clump. Leafy shoot to 4 m long. = Poulsen 2677.

Deposited at: BO, E. Material in spirit. Photographed. DNA sampled.



Poulsen 2738 – *Elettaria* C

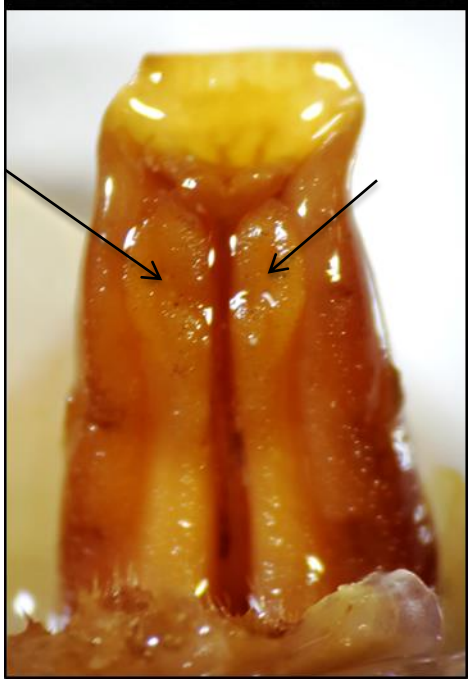
Indonesia. Central Sulawesi Open area near garden.
(1° 56' 14.7" S; 120° 47' 30.7" E);
alt. 500 m; 14 January 2009.

Notes: Terrestrial herb. Leafy shoot to c. 5 m long, in dense clump. Sheath greyish brown. Ligule bilobed, 7 mm long. Lamina sessile, to c. 80 x 19 cm. Inflorescence radical, creeping in the upper soil, to 1.5 m long. Calyx pale red in lower half, pale yellowish green in upper half or all plain pale red. Corolla tube cream, red in upper part dorsally, lobes reddish, transparent. Labellum white with yellow-green centre bordered by 2 red stripes. Stamen and stigma cream. Fruit subterranean, 2.2 x 2.9 cm, white.

Deposited at: BO, E. Photographed. Material in spirit. DNA sampled.

Poulsen 2750 – *Elettaria* B

Indonesia. South Sulawesi, Montane forest.
(2° 15' 26" S; 120° 47' 47.9" E);
alt. 1250 m; 16 January 2009.



Notes: Terrestrial herb in dense clump (5–7 cm between neighbouring shoots). Leafy shoot to 3 m long. Base to 2.5 cm diam., sheath brownish near base, higher up greenish, tesselated. Ligule 5 mm long. Petiole 1.5 cm long with swollen base. Lamina to 46 x 8 cm. Inflorescence from base, 1.4 m long, creeping along the ground, pale red when young. Calyx pale pink with pale green apex. Corolla lobes transparent red. Labellum white with yellow-green centre bordered by wine-red bands and patch. Stamen pale wine-red. Stigma wine-red. The taste of the centre of leaf base: bitter.

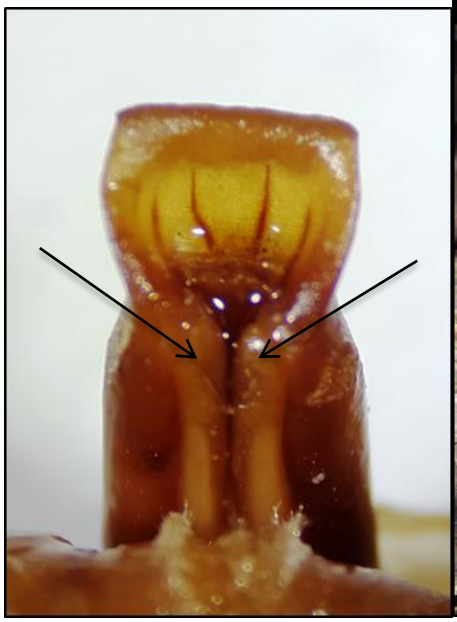
Deposited at: AAU, BO, E. Photographed. Material in spirit. DNA sampled.

Poulsen 2790 – *Elettariopsis kandariensis*

Indonesia. Southeast Sulawesi, Boroboro Range. Roadside, disturbed vegetation in forest, dominated by Dicranopteris.
(4° 9' 9.2" S; 122° 29' 42.5" E);
alt. 200 m; 11 February 2009.

Notes: Terrestrial herb in dense clump of 14 shoots. Leafy shoot 4.75 m long. Base of leafy shoot to 5 cm diam. Sheath pale greenish brown with distinct swollen mucro. Ligule to 3 mm long. Lamina sessile, to 70 x 16 cm, narrowly obovate, mid-green, not plicate, pubescent beneath. Inflorescence spreading in upper soil to 1.5 m from base of leafy shoot. Calyx pale pink. Corolla lobes transparent red. Labellum white with yellow centre bordered by 2 red lines in lower half. Stamen white with short crest. Stigma white.

Deposited at: AAU, BO, CEB, E. Photographed. Material in spirit. DNA sampled.



Poulsen 2811 – *Elettaria* sp.

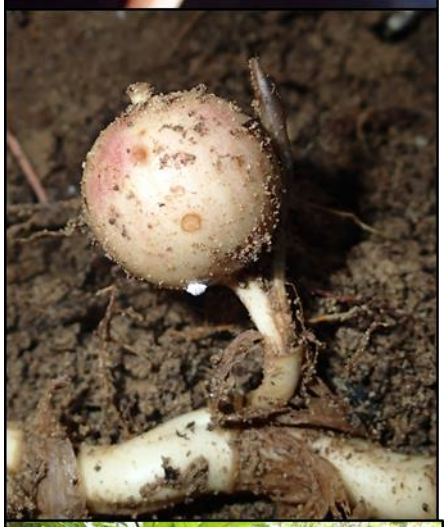
Indonesia. Central Sulawesi, Forest patch near trail in garden area.
(0° 59' 7.9" N; 121° 36' 0" E);
alt. 40 m; 22 February 2009.

Notes: Terrestrial herb of few shoots to 10 cm apart. Leafy shoot 3 m long. Base of leafy shoot to 4.5 cm diam., red. Sheath green, mucro swollen. Ligule to 3 mm long, dark purple when young. Petiole c. 1 cm long, base swollen. Lamina to 68 x 13 cm; pubescent beneath. Inflorescence radical, long-creeping in the soil. Corolla pale red. Labellum white with red centre basally and green patch in centre of terminal lobe. Stamen white (incl. crest). Fruit subterranean, globose, to 2.5 x 2.5 m, cream to pale green.

Deposited at: BO, E. Photographed. Material in spirit. DNA sampled.

Poulsen 2938 – *Elettaria longituba* (Ridl.) Holttum

Malaysia. Sarawak, Kubah National Park.
Old secondary forest, just above the stream, Sungai Cina. (1o36'38"N; 110o11'49.3"E);
alt. 200 m; 18 February 2014.

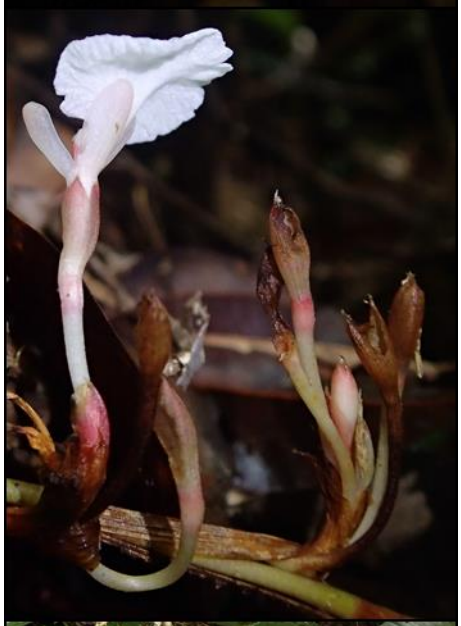


Notes: Terrestrial herb, in loose clump. Leafy shoots 5.5-9 cm apart, to 2.7 m long, with up to 20 leaves; base to 3.5 cm in diam., yellowish brown; sheath glabrous, smooth, greenish brown in lower parts, green at apex; ligule to 7 mm long, central split, greenish brown; petiole 1.5-3.5 cm long, with a swelling adaxially; lamina to 62 x 12.5 cm, narrowly obovate, mid-green above, pale beneath, slightly plicate, pubescence beneath, base oblique, apex acuminate. Inflorescence radical, long creeping. Peduncle at soil surface and subterranean, 2 m long, peduncular axis brown-yellowish tinged pink. Bracts brown-yellowish, pink when fresh, scales short lived, brown. Flower singular, lower bracteoles brown, tubular, calyx pale pink, apex pale greenish brown in the upper 1 cm, corollar tube white, corollar lobes transparent greenish, labellum white, to 17 mm broad, obovate angled 90 degrees in the middle, yellow central line
10 x 2.5 mm, anther to 4 mm long and pale red, crest 2 mm long and white undulating, stigma pale pink (white). Fruit 2-2.5 cm in diam., pale yellowish, sometimes with a slight pinkish tinge.

Deposited at: SAR, E, O, SING. Material in spirit. Photographed. DNA sampled.

Poulsen 2939 – *Elettaria surculosa* (K. Schum.) B.L. Burtt & R.M. Sm.

Malaysia. Sarawak, Kubah National Park.
Old secondary forest, by old skid trail.
(1o36'37.6"N; 110o11'47.2"E);
alt. 150 m; 18 February 2014.



Notes: Terrestrial herb, in loose clump. Leafy shoots to 7 cm apart, to 1.8 m long, with up to 25 leaves; base to 2 cm in diam., pale brown, red when young; sheath smooth, shiny, glabrous, mid/green; ligule to 5 mm, entire or asymmetrically encised; petiole 5-10 mm long; lamina to 28 x 7 cm, elliptic, base slightly oblique, apex acuminate, midgreen above, pale beneath, shiny smooth glabrous. Inflorescence radical, long creeping, above ground or in the leaf litter, to 80 cm long. Bracteoles pale red. Calyx pale reddish brown. Peduncle 1.5-2 mm, pale yellowish. Bracts reddish-brown when fresh, orangebrown when old. Flower white, yellow-green line to 9 x 1 mm. Stamen white, red at base.

Deposited at: SAR, E, O, SING. Material in spirit. Photographed. DNA sampled.

Poulsen 2942 – *Elettaria stolonifera* (K. Schum.) S. Sakai & Nagam.

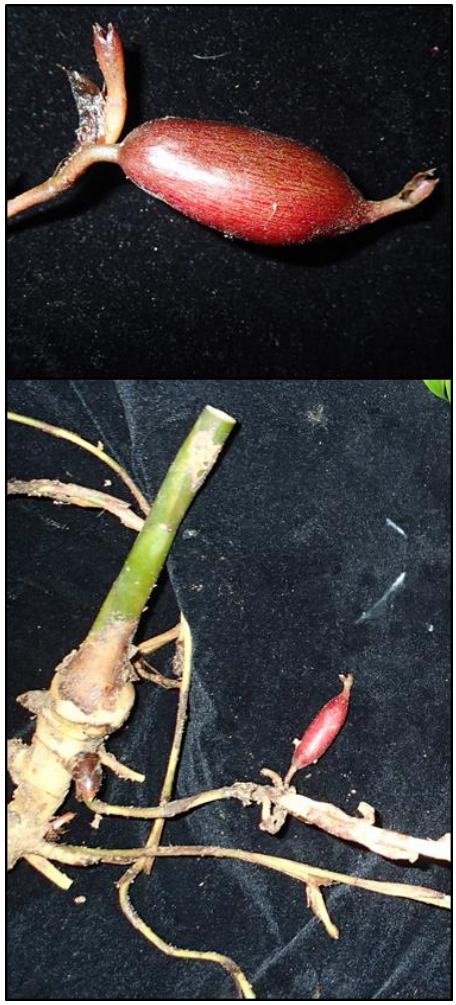
Malaysia. Sarawak, trail between Kubah National Park and Matang Wildlife Centre.
Lowland mixed dipterocarp forest, on a ridge.
(1°36'39.8"N; 110°10'20.8"E);
alt. 90 m; 19 February 2014.

Notes: Terrestrial herb. Rhizome creeping. Leafy shoots 15-17 cm apart, 85-105 cm long, with up to 15 leaves; base to 1 cm in diam.; sheath pale green, smooth glabrous; ligule to 3 mm long, entire, yellowgreen; lamina to 24 x 2.5-3 cm, subsessile, dark green above, pale green beneath, shiny, glabrous, base cuneate. Inflorescence radical, to 38 cm long, horizontal, in leaf litter, buds white.

Deposited at: SAR, E, O. Photographed. DNA sampled.

Poulsen 2947 – *Elettaria surculosa* (K. Schum.) B.L. Burtt & R.M. Sm.

Malaysia. Sarawak, Kubah National Park.
Mixed dipterocarp forest,near stream.
(1o36'44.6"N; 110o11'32.5"E);
alt. 280 m; 20 February 2014.



Notes: Terrestrial herb. Rhizome creeping and subterranean. Leafy shoot to 14 com apart, to 2.65 m long; base to 4 cm in diam., yellow-green; sheath glabrous, brownish-green; ligule to 9 mmm long; lamina to35 x 9 cm, narrowly obovate, mid-green, glabrous, smooth, pale green beneath. Inflorescence radical to 1.3 m long in leaf litter or terminal to 22 cm long. Fruit 40 x 17 mm, reddish brown, seeds not ripe.

Deposited at: SAR, E. Photographed. DNA sampled.

Poulsen 2953 – *Elettaria longipilosa* S. Sakai & Nagam.

Malaysia. Sarawak, Lambir National Park, Sungai Liku. Riverbank. (4o14'14"N; 114o3'50.2"E); alt. 50 m; 25 February 2014.

Notes: Terrestrial herb, in very loose clump. Leafy shoots to 10 cm apart, 70-150 cm long; base to 1.13 mm in diam., pale yellowish-brown; sheath pale green; ligule to 6 mm long, entire, pale green, densely hairy; lamina to 33 x 6 cm, narrowly obovate, dark green, pale beneath and pubescent, base cone-shaped, apex tapering. Inflorescence radical. Horizontal, more or less embedded in the soil, 17-28 cm long. NB. beta = sterile individual, gamma = second fertile individual

Deposited at: SAR, E, O. Photographed. DNA sampled

Poulsen 2964 – *Elettaria* sp.

Malaysia. Sarawak, Mulu National Park, path to Gunung Mulu. Near trail.
(4o2'30.9"N; 114o52'42.8"E);
alt. 900 m; 28 February 2014.

Notes: Terrestrial herb. Rhizome creeping, 0.4-1 cm thick, pale yellowish, subterranean, scales brown. Leafy shoot to 1.8 m long, leafless in lower half, with up to 23 leaves, 10-30 cm apart; base 2 cm in diam., green to reddish brown; sheath greyish-green; ligule to 4 mm, greenish-brown, slightly uneavenly bilobed, pubescent; petiole to 2 mm; lamina to 26.5 x 7 cm, mid-green, shiny, pale beneath, base cuneate, slig htly oblique, apex acuminate. Inflorescence radical, subterranean to 70 cm long. Fruit pedicell 5 cem, to 2.7 x 1.7 cm, irregularly egg-shaped, 6 ridges, beaked, to 7 cm long, from pale reddish-brown to burgundy.

Deposited at: SAR, E, O. Material in spirit. Photographed. DNA sampled.

Poulsen 2966 – *Elettaria rubida* R.M. Sm.

Malaysia. Sarawak, Mulu National Park, path to Gunung Mulu. On steep slope, in primary forest. (4o2'35.5"N; 114o52'30.1"E);
alt. 650 m; 28 February 2014.

Notes: Terrestrial herb. Rhizome creeping and subterranean, pale yellowish brown, scales red when exposed. Leafy shoot to 1.6 m long, leafless in lower half, 15-17 cm apart; base to 2.5 cm in diam., pale yellow, red when exposed; sheath dark brownish-green, smooth, glabrous; ligule to 3 mm long; lamina subsessile, callus (3 mm), obovate, to
33 x 13 sm, deeply plicate (corrugated), mid-green, pale beneath; base cuneate, apex apiculate 1.5 cm long. Inflorescence radical, to 40 cm long; peduncle mostly exposed, lax; bracts pale pink to red. Flower bud yellow.

Deposited at: SAR, E. Photographed. DNA sampled.

Poulsen 2973 – *Elettaria* sp.

Brunei, Belait District, Kg. Teraja.
In primary forest on sandy soil on bank of small stream. (4017'9.01"N;114025'44.6"E);
alt. 60 m; 05 March 2014.

Notes: Terrestrial herb. Rhizome creeping. Leafy shoots to 15 cm apart, to 2.6 m long, with up to 25 leave; base to 4 cm diam., cream to pale brow; sheath mid-green; ligule to 7 mm long, entire, green; petiole to 5 mm long; lamina to 44 x 12 cm, narrowly ovate to elliptic, mid-green, pale brownish green beneath. Inflorescence radical, creeping ± embedded in soil, to 3.4 m long, axis pale green, scales pale brown; pedicel cream, ovary pale red, calyx pale red with green apex, corolla lobes very pale yellow-green, labellum white except for yellow-green center, stamen white with pale red base. Fruits to 4 x 1.7 cm, ellipsoid, reddish brown.

Deposited at: BRUN, E. Material in spirit. Photographed. DNA sampled.