Grasping the Nettle

Untangling a complex of Urticaceae genera from the Tropics

Tom Wells

21-Aug-17

ROYAL
BOTANIC
GARDEN
EDINBURGH

THE UNIVERSITY OF EDINBURGH
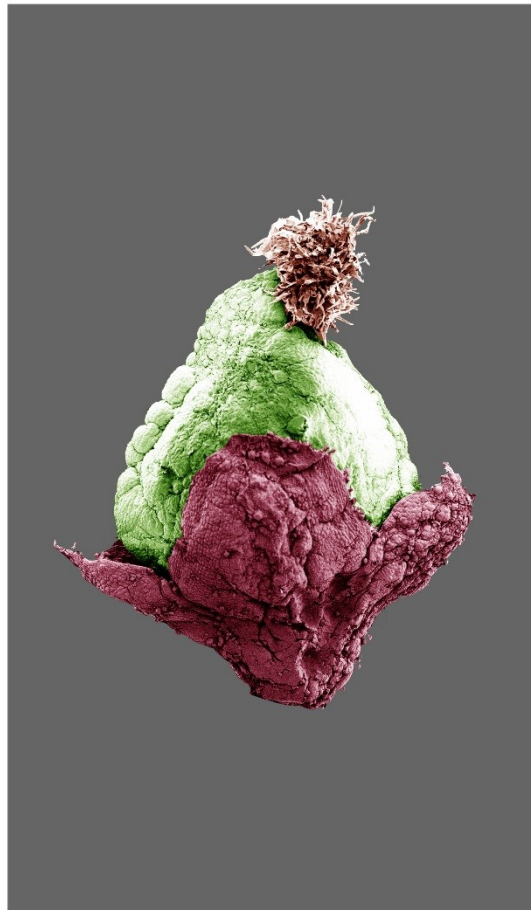
Thesis submitted in partial fulfilment of the requirements for the degree of MSc in the Biodiversity and Taxonomy of Plants

- 1 -

Abstract:

Urera Gaudich. is a genus of large nettles (Urticaceae) from Tropical Africa and Latin America, with one species endemic to Hawaii. Since its description it has been dogged by a long history of doubts about its taxonomic circumscription, and more recent phylogenetic work at the family (Wu et al. 2013) and tribe level (Kim et al. 2015) has shown it to be paraphyletic, with three other genera nested within it: the African arid specialist *Obetia* Gaudich., the Hawaiian endemic *Touchardia latifolia* Gaudich., and *Poikilospermum* Zippelius ex Miquel, a South East Asian vine previously considered a member of the former *Cecropiaceae* Berg. For this study, a more densely sampled phylogeny was combined with detailed morphological analysis, including use of the Scanning Electron Microscope, confirming the paraphyletic structure and providing circumscriptions and a resulting recommendation for updating the taxonomy. In the process, putative evolutionary trends were also analysed, such as biome shifts and range expansions, and the development of morphological traits including stinging hairs, perianth and stigma morphology, secondary woodiness, and a climbing habit.

Acknowledgements:

The number of people who have helped me with this project has become somewhat of a running joke amongst my fellow students, and it is true that I owe a huge debt of gratitude to all those whose advice and assistance I have relied upon over the last three months.

Firstly, I would like to thank my supervisor Alex Monro, who conceived the project in the first place, and has provided me with help, support and prompt and invaluable feedback throughout, even during the birth of his first child. I have learned a great deal from Alex, both about a group of plants I never anticipated knowing so well, and about the taxonomic process in general. I feel extremely lucky to have been able to do so.

Mark Hughes and Peter Moonlight both helped me with matters phylogenetic and biogeographic, as well as providing general advice on the direction of my work, and Louis Ronse de Craene kindly offered his insights on the floral morphology.

In the labs, Andrew Hudson was extremely generous in allowing me to use the University facilities without which the project may never have gone ahead, and in providing help, advice and suggestions without which I would have been lost. Frieda Christie was unimaginably patient in taking me through the processes involved with the CPD and SEM, and although it was mostly a terrifying combination of delicate and expensive equipment, I enjoyed it very much.

Suzanne Cubey and Robyn Drinkwater were very generous with their time, helping me with sampling and digitising of Herbarium material at RBGE, as was Jacek Wajer at the BM.

Andy Ensoll was also very kind in lending his formidable propagation skills, such that I might actually get to see a living plant, and not just dried bits of them.

I would also like to thank all the members of staff at RBGE and beyond who have taught us over the course of the last year and have made the entire experience so enjoyable and fulfilling. This project would not have been possible without the frightening volume of knowledge you have helped us to learn in such a short space of time.

I was also extremely fortunate that I was able to meet the financial requirements of the course through the generosity of my parents and the Humanitarian Trust.

Finally, I would like to thank my partner Ngaio for her patience, support and inspiration, and without whom I would never have started nor completed this course.

CONTENTS

1. INTRODUCTION

- 5 -

1.1 Introduction to Urticaceae

The Nettle family *Urticaceae* is widely distributed, but found mostly in the Tropics, with some members of the tribes Urticeae and Boehmerieae adapted to temperate latitudes, and a few to arid ones (Friis 1989). Variously herbs, shrubs, lianas and small trees, the tribes Urticeae, Boehmerieae and Cecropieae tend to be disturbance specialists predominantly found at the margins of forests, while Elatostemeae are shade specialists found in dense forest (Monro Pers. Comm.). The family is currently thought to contain some 2000 species, with a high level of morphological diversity, but united by a highly reduced unisexual, apetalous floral morphology, possessing a single stigma and a pseudomonomerous carpel with a single basal or sub-basal, orthotropous ovule (Wu et al., 2013). This reduction is a result of a wider process related to adaptation to a wind pollination syndrome across the predominantly wind-pollinated former order "Urticales" and the wider Rosids (Ronse de Craene 2012). As a result the Urticaceae present difficult taxonomic challenges and can often be hard to accurately identify or determine, with many characters requiring a microscope to be seen clearly (Wu et al. 2013).

The most comprehensive and definitive taxonomic studies of the family remain those carried out by Hugh Algernon Weddell in the mid-19th century, and while his tribal classifications have been largely upheld by more recent systematic reviews (Friis, 1989) and molecular phylogenetic studies (e.g. Hadiah et al. 2008, Wu et al. 2013, Kim et al. 2015) the placement and delimitation of some genera is in urgent need of revision (Wu et al. 2013, Kim et al. 2015) with many species hard to place (Monro, Pers. Comm).

Perhaps the most widely recognised members of the family are the stinging nettles, from the tribe Urticeae. Taxa distinguished by their stinging hairs, these 12 genera and the 220 odd species are also united by their 4-parted female perianth, frequently with one pair of tepals larger than the other, and are traditionally of economic importance for their fibres and use in herbal medicine (Kim et al. 2013).

1.2 Taxonomic Overview of *Urera* Gaudich.

A genus of Urticeae with particularly interesting patterns of morphology and a clearly polyphyletic classification (Kim et al. 2013) is a group of large stinging nettles from across the tropics, for which Charles Gaudichaud-Beaupré (1830) first proposed the name *Urera* Gaud. on the basis of material he collected of twelve species of the then *Urtica baccifera* L., *Urtica madagascariensis* Juss. ex Poir*., Urtica alceifolia* Poir. *Urtica javaensis* Poir., *Urtica gigantea* Poir., *Urtica palmata* Forssk., *Urtica lamiifolia* Juss., *Urtica parietariifolia* Deless. ex C. Presl, and *Urtica frutescens* Deless, plus three other species of which he was less certain. Only four of these taxa remain members of *Urera.* The characters he outlined for the genus were alternate leaves, 4- or 5-merous male flowers with a globose pistillode, 3- or 4-merous irregular female flowers with a capitate-globose stigma, and a compressed, obliquely ovate achene often enclosed in fleshy tepals.

Weddell's (1852, 1854) first treatments of the family validly published Gaudich's *Urera*, which he divided into two groups based on stigma morphology. The first group, with a capitate-penicillate morphology, contained the majority of the species, while the second group was based on a lanceolate or filiform stigma and contained *U. laciniata*, plus two species later recognised as members of the genera *Laportea* Gaudich. and *Dendrocnide* Miq.. In Weddell's next treatment, (1856) and in his contribution to de Candolle's Prodomus (1869) he divided the genus into unnamed sections, first between those with dichotomously branched cymose inflorescences and those with paniculate ones, and then between the Neotropical and Palaeotropical species. In the same publication he described the new monotypic genus *Scepocarpus* Wedd. on the basis of material collected by Mann in Fernando Po. He described it almost identically to *Urera*, but with a tubular perianth rather than a four-lobed one. In doing so he apparently overlooked the fact that he had described three African species, *U. obovate* Benth*., U. acuminata* (Poir.) Gaudich. ex Decnea nd *U. cameroonensis* Wedd., with the same feature, and Bentham (1880) later united the two genera.

Since Weddell's final treatment (DC, 1869) however, *Urera* has only been revised through regional treatments and piecemeal works, leaving the application of names and general understanding of the diversity poor (Steinmann 2005). As noted by Monro (2006) regional floral treatments such as these are unlikely to illuminate monophyletic groups or evolutionary relationships in complex genera. Friis (1993) recognised the need for revision of *Urera*, following on from Killip's (1960) assertion that "the American species of *Urera* are greatly in need of taxonomic revision", and Burger (1977) who agreed the Neotropical taxa wanted "careful monographic study", going on to give this rather ominous warning: "Whoever chooses to revise the Neotropical species of this genus will encounter some of the most baffling patterns of variation that the Neotropical flora has to offer." Meanwhile, Friis (1985) described the taxonomy of the African members of *Urera* as somewhat arbitrary and based on fluctuating characters. He hypothesised that the differences in habit, leaf morphology, and perianth fusion in particular mean that the African and Neotropical species should probably be separated, but that further studies ought to be carried out first.

More recently, Steinmann (2005) stated that this "neglected" genus required further research. Meanwhile, Monro & Rodriguez (2006) described the confusion and widespread misidentification of specimens caused by a lack of keys, similarity of morphology, and overlapping characters between the species.

Friis (1993) suggested there are around 35 species of *Urera* worldwide, while Steinmann (2005) inferred there might be 14 species across Mexico and Central America, and Monro & Rodrigues (2009) listed 12 species for Mesoamerica. From my analysis there appear to be at least an additional two good species found only in South America: *U. altissima* Lillo. and *U. aurantiaca* Wedd.. For the African members of the genus, Friis (1985) listed three species of *Urera* for Tropical East Africa, while Keay (1958) listed twelve for Tropical West Africa, but no pan-African treatment has been attempted since Rendle's (1917). One species, *U. glabra* Wedd., is endemic to Hawaii.

1.3 Recent Molecular work

These concerns about the taxonomy of *Urera* appear to have been well founded. Recent molecular phylogenies of the family (Wu et al. 2013) and the tribe Urticeae (Kim et al. 2015) show *Urera* to be clearly polyphyletic and paraphyletic. Some of the variation follows a marked geographic pattern, with the African and Latin American species sampled resolving as clearly separate clades, but indications are that the South American grouping is itself not a single unit either (Kim et al. 2015). What's more, three other genera appear to be nested within the traditional circumscription of *Urera* (Wu et al. 2013, Kim et al. 2015): the African arid specialist *Obetia* Gaudich., the Hawaiian endemic *Touchardia latifolia* Gaudich., and *Poikilospermum* Zippelius ex Miquel, a South East Asian vine previously considered a member of the former *Cecropiaceae* Berg (Monro 2006, Wu et al., 2013).
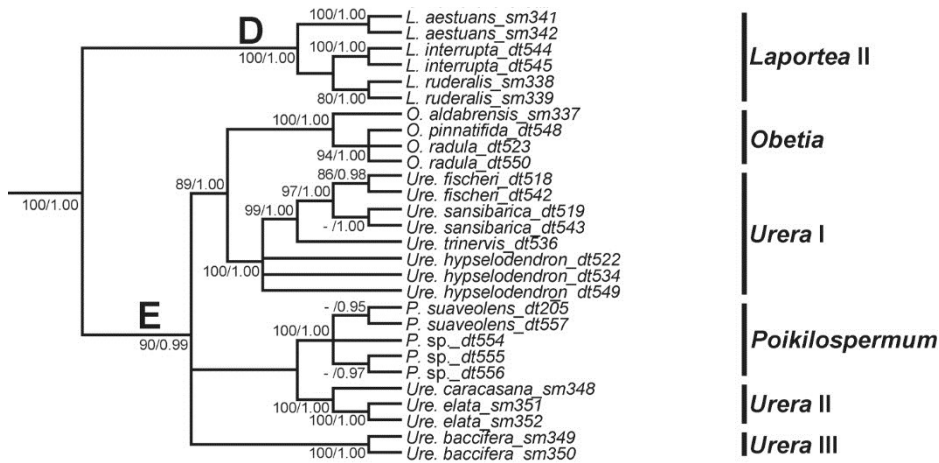

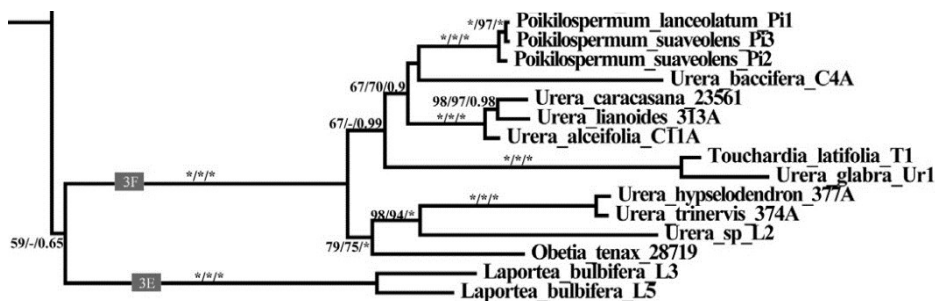*Fig. 1 Relevant clade from analysis in Kim et al. (2015)*

Fig. 2 Relevant clade from analysis in Wu et al. (2015)

1.4 Putative morphological patterns

The species currently assigned to *Urera* are relatively varied in their morphology, but have been defined by perianths becoming colourful and fleshy in fruit and to varying degrees surrounding a single, generally glabrous, elliptical achene, topped by a persistent subsessile stigma, which is usually penicillate-capitate, but can also be sub-lanceolate (Friis, 1993), while stinging hairs or spines are also common (Burger, 1977). Within this definition, hints about the structure presented in the phylogenies is visible in the past morphology-based systematic and taxonomic literature.

For example, from the limited sampling in the treatments of Wu et al. (2013) and Kim et al. (2015), *U. baccifera* (L.) Gaudich. ex Wedd. appears to be separate from a clade of the other Neotropical species including *U. caracasana (Jacq.)* Gaudich. ex Griseb*., U. simplex* Wedd.(syn. *U. elata* (Sw.) Griseb.), and *U. lianoides* A.K.Monro & Al.Rodr.. Kim et al. (2015) termed these Urera II and Urera III respectively, with Urera I formed by the African species. Morphologically, *U. baccifera* had already been observed to possess some distinguishing characters that stood it apart from the rest of the Latin American species. Most prominent of these is its development of markedly more potent stinging spines, which lend it its vernacular name "Chichicaste" and its utility as a cattle fence (Standley & Steyermarck, 1952). Burger (1977) described *U. baccifera* as easily distinguished by these stinging spines and the prominent teeth on its leaf margins. In the past authors have also noted the similarities between *U. baccifera* and *U. laciniata* Wedd*.,* and Monro & Rodrigues (2009) note that they most closely resemble each other, while Weddell (1856) wrote that *U. laciniata* had marked similarities with U. baccifera, but also possessed a unique stigma, far more similar to that of Laportea. However, *U. laciniata* was not included in any of the molecular studies of the genus so far conducted.

Wu et al. (2013) also noted that Weddell (1869) had originally placed *Poikilospermum* in Urticaceae, while Kim et al. (2015) point out that observations of their dimorphic wood fibres had been used to link the genera by Bonsen & Ter Welle (1984). The rest of the former Cecropicaceae *sensu* Berg

(1978) are now considered a part of Urticaceae once more, but forming a basal tribe Cecropieae, distantly related to *Poikilospermum* and *Urera* (Wu et al., 2013). Meanwhile although *Touchardia* was for a long time placed in the tribe Boemerriadadae, Friis (1989) noted the need for it to be moved and it has often been suggested that it is closely related to *U. glabra*, also endemic to Hawaii (Wagner, 1999). *Obetia madagascariensis* (Juss. ex Poir.) Wedd. was one of the original species placed in *Urera* by Gaudichaud, though its accrescent perianths' habit of remaining dry in fruit were later recognised as easily used to distinguish it (Friis, 1983).

Friis (1985) stated his suspicions that the African species of *Urera* differ significantly enough from the Neotropical ones to perhaps warrant separation. He based this assertion on his observation that they are lianas, in contrast to the shrubs and small trees of the Neotropics, and particularly on the fusion of the perianth found in all African taxa except *U. hypselodendron.*

1.5 Aims of the study

The aim of this study then is to provide a more densely sampled molecular phylogeny of *Urera* and the surrounding genera, and to use this to evaluate morphological characters for their phylogenetic informativeness through a process of "reciprocal illumination" (Scotland et al., 2003), thereby supporting the morphological circumscription and delimitation of this complex of taxa.

Meeting this aim would provide the foundations for a more stable taxonomy, as well as a framework within which to explore evolutionary trends within the family, such as biome shifts and the development of morphological traits including stinging hairs, fleshy fruits and a woody habit.

2. MOLECULAR ANALYSES

- 12 -

2.1 Molecular Methodology

Sampling

The phylogenetic analyses carried out in previous studies of Urticaceae by Hadiah et al. (2008), Wu et al (2013), and Kim et al. (2015), combined with regional taxonomic studies by Monro & Rodrigues (2009) Steinmann (2005) and Friis (1983; 1985) provided the basis for the taxa to be sampled in this study. My sampling was designed to complement the existing work, while providing as comprehensive as possible a taxonomic and geographic covering of the genus *Urera*, with a particular focus on the Latin American species, where the most up to date taxonomic work has been done, and previous work had highlighted the likelihood of polyphyly (Kim et al. 2015). An outgroup of *Laportea* was inferred from the work of Kim et al. (2015) and Wu et al. (2013), who established a well-resolved and well-supported clade of generic relationships in the tribe and wider family. It was also ensured that the Generotypes of the four genera were included. Sequences from previous studies were downloaded from Genbank (NCBI, 2016).

For my own sampling I aimed to include around 30 specimens sampled from herbarium material at BM, K & E. For *U. laciniata* I chose six specimens in total from across the range of the species; one from Central America and five from South America. For the more widespread *U. baccifera*, I chose twelve samples, again from across the range of the species. Wedell (1856) described three morphological varieties of the species and implied a related pattern of geographic/ecological distribution and this was also reflected in my sampling. For the remaining Latin American species, I chose a single sample for those with a restricted range such as *U. pacifica* or *U. altissima,* and two from across the range of more widespread species such as *U. caracasana* and *U. simplex*.

In addition to those African specimens of *Urera*, plus the *Obetia* and *Poikilospermum,* sampled by Wu et al. (2013) and Kim et al. (2015), I supplemented my own sampling with two specimens of *U. hypselodendron* Hochst. ex A. Rich*., and one each of *U. fischeri* Engl*., U. trinervis* (Hochst.) Friis & Immelman*, U. cameroonensis* Wedd*., P. cordifolium (Barg.-Petr.) Merr., P. scabinerium (Barg.-Petr.),*

P. lanceolatum (Trécul) Merr*., O. aldabrensis* Friis, and *O. tenax* Friis. This also allowed me to sample these taxa for the ndhF gene region, which had not been sampled in previous studies. My sampling therefore covered the entire geographic range of the genus and sister clade, representing just under half of the recognised species, and with multiple accessions for many of the more widespread species.

Table 1. Sampling (Including Genbank Samples)

Genus	Distribution	No. species sampled	Of likely	% of species	No. specimens in this study
Urera	Central & South America	13	16	81%	30
Poikilospermum	South East Asia	4	20	20%	4
Urera	Africa	5	20	25%	6
Touchardia	Hawaii	1	1	100%	1
Obetia	Africa	4	5	80%	4
Laportea (Outgroup)	Cosmopolitan	4	-	-	-

I selected three regions for the study: one nuclear (*nrITS*) and two plastid (*TrnL-F* & *ndhF*). *nrITS* and *TrnL-F* were selected to fit with the work previously undertaken, particularly that of Kim et al. (2015) whose sampling for the genus and surrounding clade was most comprehensive. Despite concerns about the potential for networks of paralogous relationships in *nrITS* to confound reconstruction of accurate phylogenies (Alvarez & Wendel 2003) I followed the advice of Feliner & Rossello (2007) that it remains the most practical region for analysis of phylogenies at the species level. The third region selected by Kim et al. (2015) for their study was the plastid region *rbcL*, but I chose instead *ndhF*, which has been shown to produce around three times the phylogenetic information of *rbcL* (Kim & Jansen, 1995) and almost as much as *rbcL, atpB* and *18s nrDNA* combined, with better support (Givnish et al., 2006).

DNA Isolation, PCR amplification & Sequencing

DNA was isolated from fragments of herbarium specimens using a protocol based on that of Doyle & Doyle (1987) and combined with an additional set of steps using the EZNA kit to further purify samples. Sarkinen et al. (2012) showed that this combination of CTAB + silica binding generated high yields and purity of DNA and the highest rates of PCR success when working with Herbarium Specimens. They state that this is a result of the removal of polyphenols and polysaccharides, which could otherwise have a negative impact on PCR results by inhibiting properties of primary and secondary chemicals.

Samples of roughly 1cm^2 of leaf material were placed in 1.5ml Eppendorf tubes with two steel ball bearings and shaken in a mixer mill at 30/s for two intervals of one minute, with the blocks reversed in between the cycles to ensure even mixing.

To each tube was then added 650µl of extraction buffer containing 2% cetyltrimethyl ammonium bromide (CTAB), and 2µl/ml of βmercapto-ethanol added just before use. The samples were then returned to the mixer mill for a further minute to ensure they were well mixed. They were then heated in a heating block at 65°C for 15-20 minutes, and allowed to cool for two minutes before I added 650µl of Chloroform (CHCl$_3$).

After being vortexed again, the samples were spun in the microfuge at 13000 xg for five minutes before I removed the upper aqueous layer to a clean tube. I then added 333µl of isopropanol, and mixed well by rocking the tube end-over-end to precipitate the nucleic acids.

After a further five minutes at maximum speed in the microfuge the nucleic acids formed a pellet at the base of the tube and the liquid was tipped away. The tubes were returned to the microfuge for a further few seconds to dry the sides and the last of the liquid was removed by pipette.

The pellet of nucleic acids was then dissolved in 200µl of TE with $1/1000^{th}$ volume of 10mg/ml of RNase. The dissolving was aided by constant mixing at room temperature, with additional occasional vortexing if the pellet did not easily dissolve.

Once dissolved, 520µl of a mixture of 100% ethanol and 3M NaOAc (ph7.0) at a ratio of 25:1 was added and mixed in order to precipitate the DNA as Na salt.

Centrifugation was repeated as before for five minutes at maximum speed, the liquid tipped off and a further spin of a few seconds used to dry the sides of the tube before the final liquid was removed by pipette. This left a gelatinous, transparent pellet of DNA at the base of the tube, with was dissolved in 300µl of TE, this time without the RNase.

EZNA columns were then used to purify the samples by removing any unwanted polyphenols and polysaccharides that might interfere with the PCR. (Omega Bio-Tek., 2013).

150 µl of the supplied CXD Buffer and 300 µl of 100% ethanol was added to each sample and vortexed to obtain 750 µl of a homogenous mixture. After inserting a HiBind DNA Mini Column into a 2mL Collection Tube, I then added 100 µl of 3M NaOH and let it sit at room temperature for four minutes, before centrifuging column and tube at maximum speed for 1 minute. Once this was complete I added each of the samples to a column and centrifuged at 10,000 x g for one minute, afterwards discarding the collection tube and liquid.

Having placed the column into a new 2mL collection tube, I then added 700 µl of the supplied DNA Wash Buffer, which I had previously diluted with 100% ethanol. After centrifuging again for one minute at 10,000 x g, I threw away the liquid, but retained the tube, repeating the step by adding another 700 µl of DNA Wash Buffer and centrifuging again at the same speed.

This time, after discarding the liquid, I centrifuged the empty columns in their collection tubes at maximum speed for two minutes. This dried the column membranes to remove any remaining ethanol that might otherwise disrupt the rest of the process.

Finally I removed the tubes to 1.5mL Eppendorf tubes and added 50 μl of Elution Buffer, pre-heated to 65°C, to each column, incubating them in a water bath for five minutes, also at 65°C, before centrifuging at 10,000 x *g* for one minute. I repeated these steps one more time in new Eppendorf tubes and compared the two samples for each specimen using a Nanodrop Spectometer to assess DNA purity and yield.

The *nrITS* region was amplified using primers ITS 4 (5' – TCCTCCGCTTATTGATATGC) and ITS 5 (5' – GGAAGTAAAAGTCGTAACAAGG) (White et al., 1990), the *TrnL-F* spacer using primers e (5' - GGTTCAAGTCCCTCTATCCC) and f (5' - ATI'TGAACTGGTGACACGAG) (Taberlet et al., 1991), and *ndhF* using primers ndhF84f (5' – TCTTCGCCGTATAGTGGGTTTTTC) and ndhF713r (5' – ATCRGGTAACCATACATGAAGRGG) (Datwyler & Weiblen, 2004).

For nrITS and TrnL-F I amplified the Genomic DNA using the MJ Research PTC 200 PCR machine, with an initial 30s at 94°C, followed by 34 cycles of 5s at 94°C, 10s at 55°C, and 40s at 72°C, and finally 2min at 72°C. For Ndhf, the thermal cycling was performed in 25 cycles of 94°C for 30s, 48°C for 60s, 68°C for 90 s, and a final extension at 72°C for 7 min.

The results of the PCR were assessed for success on an 1% agarose gel with ethidium bromide, and samples with clear, single bands of correct length sequenced in both directions. Any with less clear bands were purified using Exosapit before being sent for sequencing.

Phylogenetic Analyses

The complementary DNA Sequences were assembled in Geneious v. 9 using the deNovo assemble function and then checked manually. I then aligned the sequences using Mafft v.7 (Kazutaka and Standley, 2013), with Bioedit v.7.2.6.1 (Hall, 1999) for manual adjustments. The nrITS, TrnL-F, NdhF and combined matrices were each analysed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI).

Maximum Parsimony (MP) analyses were run in PAUP v.4.0a (Swofford, 1998), using heuristic searches with all characters treated as unordered and of equal weight, and gaps treated as missing data. Starting trees were generated by random Stepwise Addition, and 1000 replicates were run with random sequence addition, tree bisection-reconnection (TBR) branch swapping, zero length branches collapsed, and Multrees on. A single tree was held at each step and no topological constraints were in effect. A bootstrap analysis was run with 1000 replicates and Multrees off, and used to assess clade support within the MP trees. Consensus trees were generated using the Strict, Semi-strict and Majority Rule consensus models.

Maximum Likelihood was run in RAxML v.8 (Stamatakis, 2014) with random starting trees (-d), a random seed value for parsimony inferences of 12345 (-p 12345), rapid hill-climbing (-f d), 1000 alternate runs (-# 1000), using the default setting of the General-Time-Reversible (GTR) model allowing a different rate of evolution for each of the six substitution sites, plus Gamma distribution to estimate distribution rates across sites (-m GTRGAMMA). 1000 (-# 1000) non-pararmetric bootstraps (-b 12345) were run using a random seed value for parsimony inferences of 12345 (-p 12345) and the General Time Reversible CAT model (-m GTRCAT). Bipartition information was then drawn on the most likely tree (-f b) (Goolsby, 2017) .

For the BI analyses a model test was conducted on each dataset using the Akaike Information Criteria in jModelTest 2 (Darriba et al. 2012) to select a suitable model of sequence evolution for the data. The nrITS and TrnL-F datasets were assessed both partitioned and as a whole. BI was run in MrBayes v.3.2.6 (Ronquist et al., 2012) with one cold and three incrementally heated Markov chain Monte Carlo (MCMC) chains run 5000,000 times or until the average deviation of split frequencies was below 0.01. Trees were sampled every 1000 generations and the first 10% were discarded as burnin, with Posterior Probabilities constructed from the remaining trees.

Resulting trees were visualised and edited in iTOL (Letunic & Bork, 2007) and prepared for figures in Adobe Illustrator CC 2017.

2.2 Molecular Results

The overall success rate for amplification and sequencing of samples was low, with 53% for *nrITS*, 31% for *ndhF*, and 35% for *TrnL-F* (See Appendix 1 for table of results). This was likely a result of the difficulty of extracting good quality DNA from herbarium specimens (Sarkinen et al. 2012). Interestingly, they also found that only fragments with fewer than 300bp were easily amplifiable, however I was able to get good quality extractions and amplifications of between 600 and 1000bp for the samples of mine that did work. This appears to reinforce their hypothsis that success rates when working with Herbarium DNA can be strongly influenced by taxon specific factors such as leaf thickness, texture and chemistry.

All analyses of all sampled regions were unanimous in the delimitation of five clearly defined clades, while the two Hawaiian taxa were resolved together, but either as part of the outgroup *Laportea*, or as a sixth clade in the ingroup, sister to all the rest. The results were also consistent in the resolution of a monophyletic clade containing *U. laciniata* and *U.baccifera*, and the separation of the remaining *Urera* between a second Latin American clade and an African one. The results thus corroborate the earlier work of Wu et al. (2013) and Kim et al. (2015), which had suggested polyphyly within the genus *Urera*.

Analysis of the *nrITS* region gave a more detailed resolution at the terminals, but the plastid and nuclear analyses were otherwise congruent in their identification of the clades. The relationship between the five main clades differed between the parsimony and model-based analyses, but support values were low for both, and the topology at that level is probably therefore best treated as a polytome. There was also clear evidence of polyphyly and paraphyly at the species level throughout the different clades, with more work required to unpick these relationships, some of which are already hinted at in the literature.

TrnL-F

Maximum Parsimony analysis of the *TrnL-F* region yielded 540 most parsimonious trees with a score of 117. The resulting strict consensus tree produced a monophyletic ingroup of five clades. The outgroup *Laportea* was resolved as non-monophyletic, with *U. glabra* and *T. latifolia* nested within it. Each of the five ingroup clades was well supported by bootstrap analysis, with 83% or higher for each, but the relationship between clades was an unresolved polytome. Within each clade, no real clarity was gained with regards relationships between the species. The ML analysis generated its most likely tree in run 255 with a likelihood value of -2124.7. This produced an almost identical topology to that found in the MP analysis with bootstrap values of at least 85% for each of the five ingroup clades, and *T. latifolia* and *U. glabra* once again nested within the outgroup *Laportea*. The Bayesian analysis likewise produced the same topology, with a Posterior Probability of 1 for each of the five clades, once again on a polytome and with *U. glabra* and *T. latifolia* nested within *Laportea*.

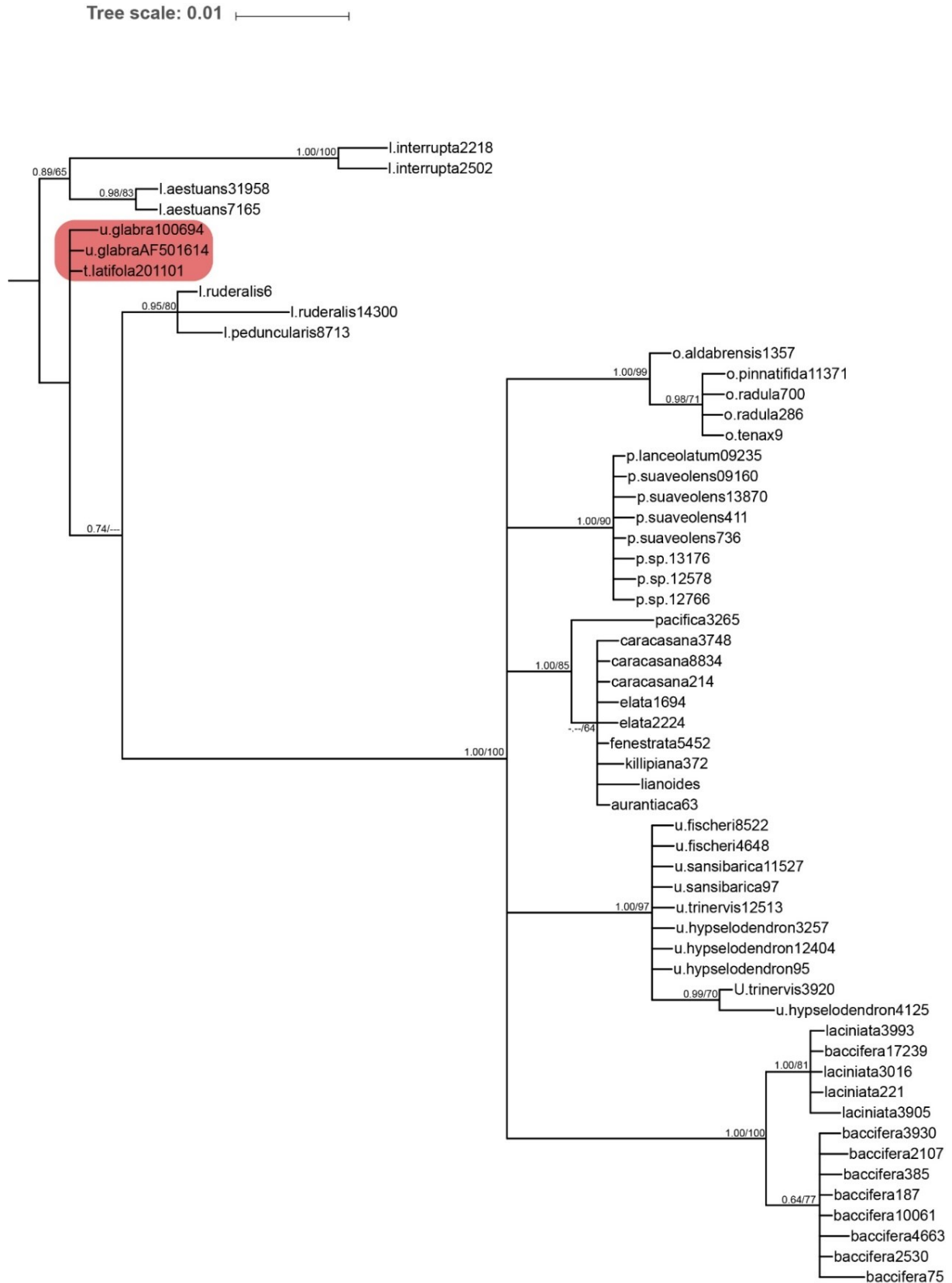Fig. 3 Bayesian tree for TrnL-F region with Posterior Probabilities and ML Bootstrap values, showing five clear ingroup clades, and U. glabra and T. latifolia within the outgroup Laportea
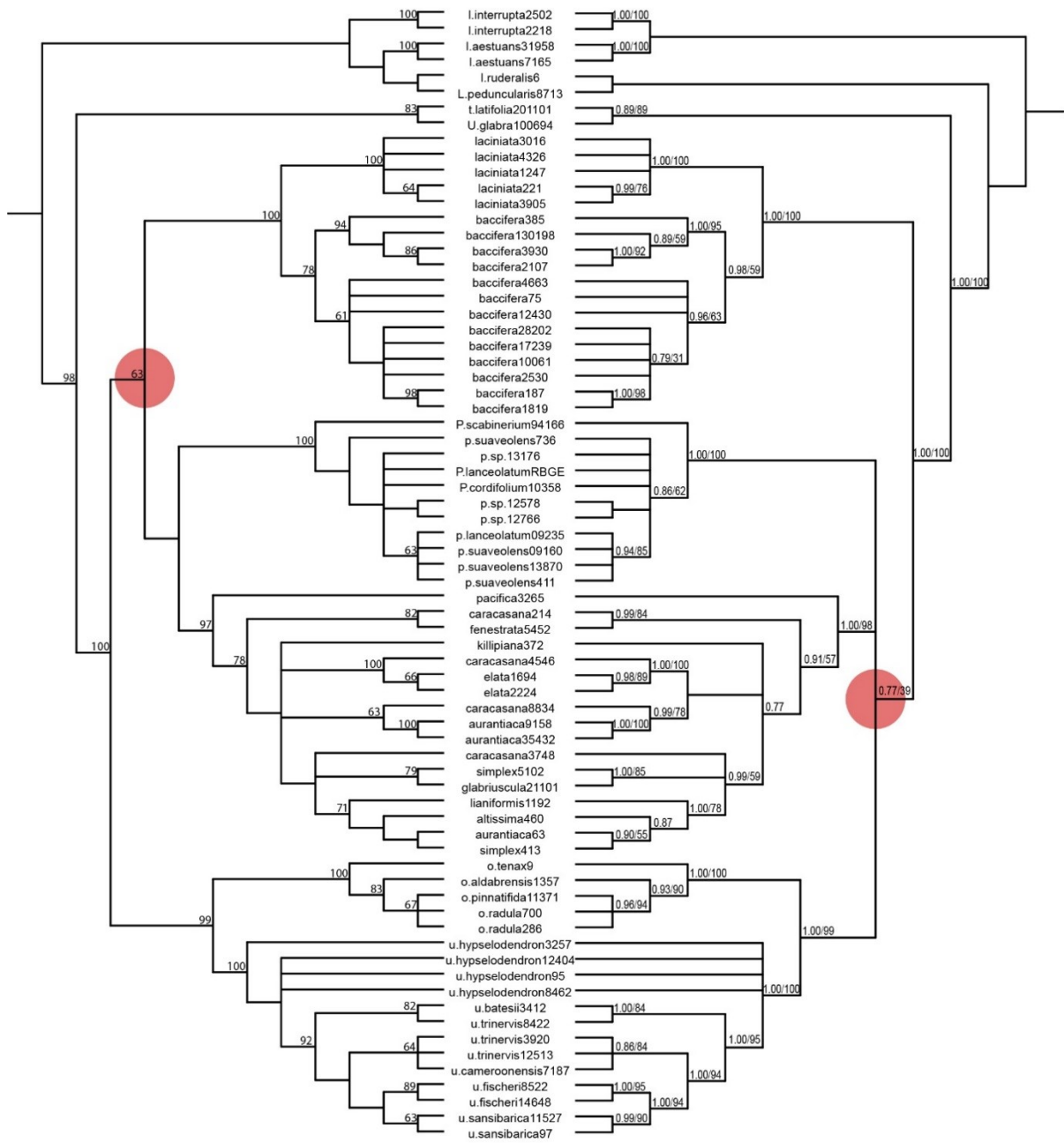
Fig. 4 MP & MB trees for nrITS region with Posterior Probabilities and Bootstrap values, showing six clear ingroup clades, and monophyletic outgroup Laportea, but with different topology within between the clades.

nrITS

For the *nrITS* region, MP analysis produced 96 trees with a score of 604. This time the outgroup was resolved as monophyletic, with *T. latifolia* and *U. glabra* joining the ingroup as a sixth clade, sister to the other five, and bootstrap support for each of these clades was 97% or higher. The *nrITS* data was also able to provide a slightly more resolved topology within and between the clades. The Strict consensus suggests the two African clades are monophyletic and sister to the rest of *Urera*, plus *Poikilospermum*. However bootstrap support for this was low at 63%. In the most likely tree, generated in run 428 of the ML analysis with a score of -4027.7, the same six ingroup clades (including *T. latifolia* and *U. glabra*) were resolved, but this time with *U. laciniata* and *U. baccifera* as sister to the other two *Urera* clades, *Poikilospermum* and *Obetia*. Once again, the bootstrap support for this arrangement remains low though, at 35%, and the same topology appears in the Bayesian analysis, with the positioning of *U. baccifera* and *U. laciniata* between the Hawaiian clade and the rest receiving a similarly poor posterior probability of 0.7.

Within *U. baccifera* there appears to be fairly strong evidence of at least two clades, and with two specimens in particular (Taylor 187 BM and Sandoval 1819 MO) forming a clade paraphyletic within the rest. A simillar pairing is visible in *U. laciniata* between Huaman 221 BM and Monteagudo 3905 BM. Within the rest of Latin American *Urera*, *U. pacifica* Steinmann. is resolved as sister to the rest of the clade, though the bootstrap support is only 78%. *U. caracasana*, which is described by Steinmann (2005) as a typical "dustbin species" appears in multiple positions across the clade, highlighting issues of taxonomy and identification within the Neotropical species. Within the African *Urera* clade, *U. hypselodendron*. comes out as sister to the other species, while the specimens of *U. trinervis* appear to group either with *U. batesii* Rendle. or *U. cameroonensis*. Specimens identified as *P. lanceolatum* (Trécul) Merr. and *P. suaveolens* (Blume) Merr. appear together in two separate groups, signaling a similar problem in this clade.

ndhF

A shortage of data, resulting from the difficulty of amplifying DNA from herbarium material and the absence of relevant sequences from previous studies on Genbank meant that the *ndhF* region was not of use in further clarifying the work here (not shown).

Combined

Analysis of a combined matrix containing the *TrnL-F* and *nrITS* data had no significant impact on clarifying the relative positions of, and relationships between the clades. However it did generate some increased support values for relationships at the terminals.

The resulting six clades are annotated on the combined tree opposite.
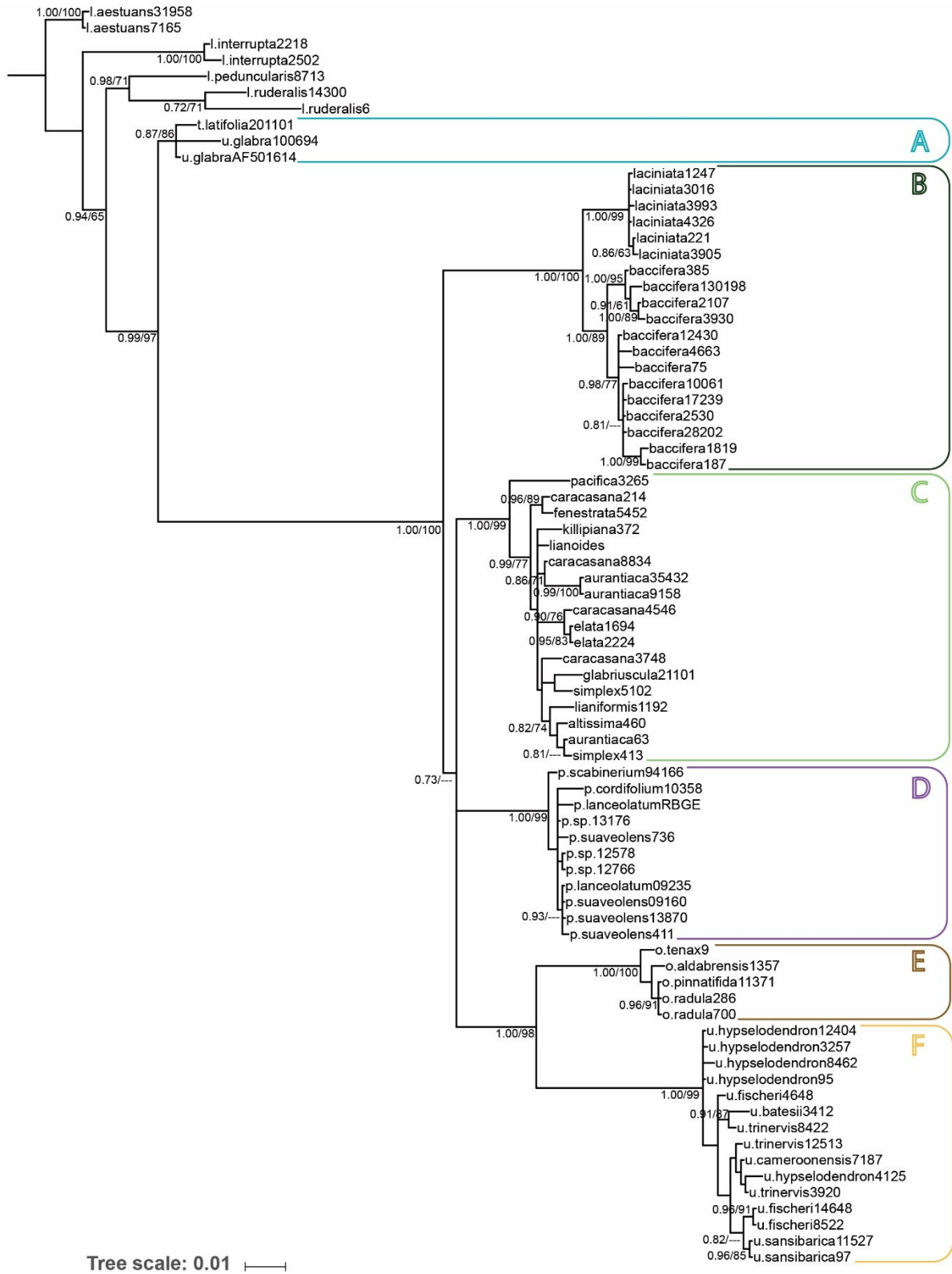
Fig. 5 MB consensus tree for combined nrITS & TrnL-F region with Posterior Probabilities/ML Bootstrap values, showing six clear ingroup clades annotated..

3. MORPHOLOGICAL ANALYSES

3.1 MORPHOLOGICAL METHODOLOGY

Use of Morphology in combination with Molecular results

Scotland et al. (2003) advocated rigorous and critical study of relatively few morphological characters in the context of a molecular phylogeny as the best way to integrate the two types of data. Under this methodology each character is examined for congruence with relevant nodes on the molecular tree leading to reciprocal illumination between the two datasets. Hawkins et al. (1997) showed that Characters and Character States should be distinguished and treated separately, and that there are thus two stages involved in the process of forming a hypothesis of primary homology: firstly comparative study of organismal variation is used to define characters, and then the characters are partitioned and coded as different states assigned to the analysed taxa.

In order to define potentially useful macro-morphological characters for the delimitation of the clades recovered in my phylogeny, an initial review of the literature, including protologues and regional Flora treatments was compared to observations from herbarium specimens at K, BM, MO, E. The observed characters were subsequently partitioned into states based on the variation displayed across the taxa. Finally, the resulting matrix was mapped onto the combined molecular tree for use in delimiting the taxa and clades within morphological circumscriptions.

Microscopy

Female flower and fruit morphology include potentially phylogentically informative characters, and their small size necessitated the use of the light microscope and Scanning Electron Microscope (SEM) for their analysis. Samples were collected from Herbarium material at BM, E & K. Where possible, specimens that had been successfully sampled for DNA were chosen (See Appnedix 3). In some instances this was not possible, in which case morphologically similar samples were studied. In particular, I was keen to find material at all stages of flower development. At least one representative species was chosen for each of the clades produced in my molecular analysis.

Samples were rehydrated in a solution of 10% Aerosol OT in 6:1 solution with acetone and left for 12-24 hours, before being washed three times in 70% Ethanol (Ayensu, 1967). They were then stored in 70% Ethanol, before being dissected under a ZEISS Stemi 2000-C stereo-microscope and photographed using the AxioCam MRc 5 (ZEISS) digital camera, in preparation for critical point drying (CPD) and analysis under the SEM. In preparation for use of the SEM, samples were dried using a Critical Point Drier (CPD). This dries the material, while maintaining the structure. Six samples at a time were placed in separate slots in the CPD carrying basket and taken through an ethanol-acetone series of dehydration as follows:

15 minutes in 70% Ethanol

10 minutes in 95% Ethanol

5 minutes in 100% Ethanol (dried with a molecular sieve)

2x5 minutes in 100% Acetone

Samples were then immediately transferred to the Emitech K850 CPD, precooled to around 4°C using liquid CO_2. Ten flushes of liquid CO2 were used to replace the Acetone, before the temperature was raised to around 35°C. The Critical Point was reached at around 31°C and a pressure of 1100 bar, and at this point the pressure in the chamber was slowly reduced through bleeding. Once the chamber was empty, the samples were transferred in their carrying basket to a jar with desiccated silica gel to maintain the lack of moisture.

The specimens were each mounted onto metal stubs using adhesive carbon discs, under the ZEISS Stemi SV 6 stereo-microscope, before being sputter-coated with Platinum for two minutes in the Emitech K575X Sputter Coater. This creates a surface that can conduct the electrons in the SEM. Extra bursts of 30 seconds were used where necessary, to ensure sufficient coating and reduce charging in the SEM. Up to 8 sputter-coated stubs at a time were mounted on a stage and placed in the Leo Supra 55VP (Zeiss) SEM for imaging. Adobe Photoshop CC 2017 was used to adjust, compile and colour the images.

Identification of potentially informative characters

Wu et al. (2015) used Ancestral Character State Reconstruction (ACSR) to assess the utility of 19 characters in gaining more systematic resolution and a better understanding of character evolution across the family Urticaceae. However, they did not evaluate their chosen characters for homology. While they identified synapomorphies for some clades, they found that diagnostic value was undermined both by reversals within those clades and homoplasy between clades, though their analysis was undermined by their failure to assess characters for homology. They found that most of the characters traditionally used in morphological classifications in Urticaceae were not useful at higher taxonomic levels, leading to disagreements on the placement of certain genera between molecular and morphological classifcations. Specifically they found that the clade containing the polyphyletic *Urera* was variable for 12 of the 19 characters they assessed, and other than biogeography they could not find definitive characters to justify breaking *Urera* into monophyletic units.

This assessment fits with Kim et al.'s (2015) analysis of the tribe Urticeae, in which they used ACSR of eight morphological characters assessed for homology including: habit; leaf arrangement, number and position of stipules, shape of achenes, presence or absence of bulbils, presence or absence of stinging hairs, and female tepal number; all of which they selected on the basis of their utility for inferring relationships at the genus and species level. They too found relatively few synapomorphies to fit the clades identified in their phylogenetic analysis, though they did find a woody habit to be a synapomorphy for the clade containing *Poikilospermum, Urera* and *Obetia*. They were also able to reconstruct the ancestral condition in the tribe as herbaceous, with alternate leaves, a single intrapetiolar stipule, asymmetrical achenes, no bulbils, and four free female tepals. On this basis interpetiolar stipules, symmetrical achenes, and tubular female perianths are the derived state, and offer potentially informative characters, which were included in my analyses.

However neither of these studies included sufficient samples of *Urera* and its sister genera to make valuable judgements on synapomorphies at this level, and Wu et al (2015) state that such a task would require more focused morphological work at the genus level. In addition their characters frequently lacked sufficient variety of states or logical construction to account for the variation described in the literature and observed in specimens.

Weddell's (1852) descriptions remain the most detailed morphological analysis of the taxa in this clade. He circumscribed *Urera* as having: 4-5parted male flowers, 4merous female flowers with larger interior lobes, stigmas that are either capitate-penicillate or lanceolate-filiform, an accrescent calyx nearly always berry-like and concealing the achene, a woody habit, axillary two-nerved stipules, and stems either unarmed or armed with stinging hairs or spines. From this it was concluded that male flower merosity, stigma morphology, fruit morphology, and the presence and morphology of stinging hairs to be likely to be variable enough characters to be phylogenetically informative.

Kim et al.'s (2015) designation of the clade as woody within an otherwise herbaceous Urticeae, highlighted this as a character worthy of analysis, and Bonsen and ter Walle's (1984) analysis of wood anatomy in the Urticaceae also supported that assessment.

Monro & Rodrigues (2009) stated that the macro-morphological characters most often used for the delimitation of species within *Urera* are leaf shape, leaf margin, morphology of inflorescence, distribution and morphology of trichomes, fruit size and colour, cystolith shape, and stigma shape. Steinmann (2005) agrees, particularly on the utility of the presence and distribution of urticating hairs, which he defined as "relatively long, stiff, straight, translucent, swollen-based and highly stinging trichomes." He also highlights whether the receptacle or the perianth becomes fleshy in fruit and whether the female flower is erect or bent away in relation to the pedicel, but these appear to be of greater utility at the interspecific rank.

Finally, Friis (1985) highlighted a suite of both vegetative and floral characters that can be used in combination to separate the African from the Neotropical species of *Urera*. These are habit, leaf morphology and degree of perianth fusion.

Based on the above, the morphological characters evaluated here were: Lifeform/habit; woodiness; adventitious roots; morphology and distribution of stinging hairs; leaf lamina shape; leaf margin; stipule fusion; cystolith morphology; inflorescence morphology; merism of male flowers; stigma morphology; relative size and degree of fusion in female tepals; extent of tepal inflation in fruit; and achene morphology.

As stated by Wu et al. (29015) biogeography was one of the few consistently useful characters for the delimitation of the members of this clade in their analysis. On this basis, two Biogeographic characters were analysed: distribution and ecology.

Review of character utility, primary homology & character conflation

In their assessment of Maddison's (1993) "Red tail-Blue Tail-No tail" problem, Hawkins et al. (1997) argued that the most theoretically robust approach is to code two separate characters: one relating to the presence or absence, and the second to the colour. This avoids conflation of characters that are not homologous. Unfortunately, the terminology used in describing Urticaceae taxa often fails to meet this approach, and it was thus necessary to assess each character, in the light of this advice and reassign characters where necessary.

Lifeform/habit

When discussing the lifeform or habit of a plant, terms such as tree, shrub, liana or herb are frequently used, but this approach fails to adequately capture the diversity of growth habits found, since the terms are subjective and contain a number of conflated characters. From an assessment of the literature, herbarium specimens, and collection notes, I found that life form would be better

divided into two characters useful for the delimitation of the different monophyletic groups. These characters are axis support, and presence or absence of wood.

Axis support refers to how the main stems are supported, and can be divided between those taxa taxa that are self-supporting; those with a scrambling or scandent habit that are partially non self-supporting, but not adpressed to them; and those with an adpressed habit, which are entirely non self-supporting . I denoted these character states as self-supporting, partially self-supporting, and not self-supporting.

Woodiness

Previous studies (e.g. Wu et al., 2015; Kim et al., 2015) have limited the character of woodiness to the presence or absence of woody tissue and two resulting states, herbaceous (no woody tissue) or woody. This approach delimits our taxa as a woody clade within the otherwise herbaceous Urticeae tribe (Kim et al, 2015). However, I found the extent of woodiness in the taxa to be more variable than a simple presence and absence binary state. Since most herbarium specimens are composed of branchlets or twigs, it can be difficult to assess the woodiness of the main stem, which is not often noted in detail by the collector. The branchlets of most specimens examined remain at least partially hollow or pithy at 5-10mm in diameter; however the degree of woodiness surrounding this differs between the taxa. *U. laciniata* and *U. baccifera* lack any evidence of wood in their branchlets, and as a consequence are flattened in the collection process, even up to 20mm in diameter. The main stem in these species is apparently also essentially entirely pithy rather than being composed of true wood (Monro, pers. comm.). The branchlets of the remaining taxa with the exception of *Obetia*, by comparison, while often hollow in the centre at around 10mm in diameter, always show some evidence of wood. As a consequence they do not collapse when pressed. In contrast, *Obetia* possesses a pachycaul stem, with soft, fibrous, pithy wood throughout (Friis 1993), and the branchlets are partially collapsed by pressing. Drawing on the above observations I categorized habit

and woodiness as: branchlets without evidence of wood; branchlets with soft, pithy wood; or branchlets with presence of wood.

Adventitious Roots

Linked to a non self-supporting habit is the presence or absence of adventitious roots and other outgrowths on the stem. It is likely that these developments are linked to an adpressed-climbing habit as is the case in other climbing plants such as Ivy (*Hedera sp.*). Adventitious roots were observed in both *Poikilospermum* and the majority of African members of *Urera*, but not in any of the other clades. In *U. sansibarica/fischeri*, distinctive hook-like outgrowths termed "protruberances" by Friis (1985) were observed, which appear to be homologous to adventitious roots. These are, however, often covered in bulbed hairs which suggests that they may be better considered as shoots rather than roots, but their function in a climbing habit appears to be simillar. The states used are therefore: present, absent, or replaced with outgrowths.

Morphology and distribution of stinging hairs

Members of the Urticeae are united, with exceptions, by their possession of "urticating" hairs, which often cause a stinging pain on contact with skin (Kim et al. 2015). These hairs are defined by Steinmann (2005) as: "relatively long, stiff, straight, translucent, swollen-based and highly stinging trichomes", though degree of sting is not a good character, since it is somewhat subjective. They can be found in varying densities, sizes and positions, but are entirely absent from two clades in our phylogeny. In addition*, U. laciniata* and *U. baccifera* possess bulbed hairs reaching >3mm in length and becoming lignified, so as to resemble spines. They possess these spines alongside the more common smaller bulbed hairs, and the underlying morphology may well be the same, but the enlarged spines are significantly different enough to be considered as a separate character. As such there are two characters: presence or absence of bulbed hairs <2mm long; and presence or absence of bulbed spines >3mm in length and often lignified.

Leaf lamina shape

Leaf lamina in Urticeae are frequently prominently lobed (Friis, 1993), but amongst the taxa anlaysed here that is the case only in a few species. The remainder of taxa possess leaves that are variously ovate, lanceolate, obovate or oblong; however, the variation does not appear to provide any taxonomic characters at this rank. There also appears to be a dichotomy between leaves with a clearly pinnate venation, and those that are basally trinerved with the lowest pair of secondary veins more prominent than the rest and often reaching almost to the tip of the lamina.

Leaf margin

Morphology of leaf margins is another character where traditional terminology such as serrate (asymmetrical, acute and angled towards the tip), dentate (symmetrically acute) or crenate (symmetric and rounded) is difficult to code as such as it conflates more than one character. This can be combatted by focusing on size, defined by spacing or degree of incision of teeth. Some taxa had entire margins, lacking teeth entirely and so there are two characters: margin, with states toothed or entire; and tooth spacing, with the states teeth >10mm apart, or teeth <10mm apart.

Stipule morphology

The ancestral state of stipules in the tribe Urticeae has been reconstructed as likely to be intrapetiolar and partially fused (Kim et al. 2015) and this is mostly the case within the clade studied here, bu they can also be intrapetiolar, but completely fused, or free and interpetiolar. In some taxa the stipules approach amplexicaul and almost completely envelop the stem. There are therefore three separate characters: stipule position (intrapetiolar or interpetiolar); stipule fusion (partially fused, completely fused, or completely free); and percentage amplexicaul (<50% or >50%).

Cystolith morphology

While cystolith morphology appeared initially to be potentially informative, variation within groups and even within species or individuals meant that this was not the case. Cystoliths can be punctiform, fusiform, or elongated, oblong or linear, but this may be more a result of size – being punctiform when they first form and elongating with age. They are often arranged in patterns such as parallel along the nerves on the underside of leaf lamina, or radially around hairs on the upper surface, but not in any taxonomically informative way at this rank. I therefore decided to abandon cystoliths in my analysis.

Inflorescence morphology

Friis (1993) described the inflorescences in Urticaceae as essentially cymose panicles, often with dichasial branching, and this is what I observed in the taxa sampled here. Terms such as panicle and cyme did not provide taxonomic information, but the forms observed included some taxa with loose asymmetrical panicles with an unordered, indeterminate branching pattern emanating from a main axis, that more or less tapers towards the apex; while others had loose symmetrical compound cymes with variable, yet ordered levels of dichotomous branching that is determinate and always symmetrical. Within the second group, some had inflorescences where the individual flowers are so densely clustered as to form spherical glomerules. The characters are therefore: development determinate or indeterminate; branching pattern symmetrical or asymmetrical; and internal branches visible or not.

Merism of male flowers

The number of flower parts in male flowers proved to be of limited taxonomic use, as it varied within groups and even within species and individuals, and it was thus abandoned for this analysis.

Stigma morphology

Urticaceae stigmas are covered in fine flattened filiform hairs; an adaptation to wind pollination for trapping pollen in the air (Ronse de Craene, 2012). Chen (1985) described eleven different stigma morphologies found in Urticaceae, explaining that this character is extremely diverse across the family and important in the classification of genera. According to his categories, the stigmas found within the taxa analysed here are (i) capitate-penicillate: essentially sessile on top of the carpel with the hairs in a variously tight or loose pom-pom or brush-like structure; (ii) ligulate: elongated, tongue-shaped and slightly curved forwards, with the base wider than the obtuse apex, and the short stigmatic hairs arranged only along the abaxial surface; (iii) oblong: shortly elongated with papillose hairs all the way round; or filiform: long and narrow, with a short style and hairs all the way round; or (iv) spathulate: with an elongated stem widening from the base into a broad, rounded apex with stigmatic hairs on one side. These descriptions are conflated and so I split them into two seperate characters: stigma extent sessile or elongated, and hair distribution symmetrical or asymmetrical.

Relative size and degree of fusion in female tepals

All the taxa analysed have an essentially 4-merous perianth in female flowers, derived from the Calyx (Dong et al., unpublished), with the inner tepals smaller and outer tepals larger. To date the degree of fusion has been treated simplistically and it comprises a spectrum, which I classify as three states: completely free; basally fused (lobed); or tubular and almost completely fused (toothed).

Accrescent and inflated tepals in fruit (see below) appear to have caused confusion in the past, in that the tepals in mature flowers can appear basally fused, but from my analysis of herbarium specimens, rehydrated fruit under the light microscope and SEM imaging this appears to be the result of either postgenital fusion or inflation emanating from the base of the tepals or the receptacle rather than a reflection of flower structure.

Extent of tepal inflation in fruit

Fleshy tepals are widely documented on labels, collection notes and in the literature, but can be difficult to assess from herbarium specimens. I was able to rehydrate smaller fruits of the Latin American and African *Urera*, but for the larger fruits found in *U. baccifera* it was not possible and they remained flattened. From my observations, I recognized two characters: enlarging of the tepals in fruit, and fleshiness in fruit. Tepals can enlarge in fruit or not, and fleshiness can be tepals fleshy, tepals dry, or pedicel fleshy instead.

Achene morphology:

The fruit itself is an achene which can be greater or less than 2mm in length. There are four discrete achene shapes in evidence: Tear-shaped (lachrymiform) with a round base and tapering apex reflexed to one side, ~1.5x as long as wide; lenticular, almost completely circular, equally as long as wide; ovoid/almond-shaped (amygdaliform) with a wide base and tapering unreflexed apex, ~1.5x as long as wide (oblong); or oblong-elliptical, widest in the middle and ~2x as long as wide. All of these shapes share a rounded base and it is thus the apex morphology that provides the characters. The apex can be enlarged or not, and when enlarged its shape can be straight or curved. The surface of the achene can be variously smooth, granular, ridged or verrucose, but without apparent taxonomic signal at this rank.

Biogeography:

Distribution data were collected from the literature, herbarium labels and GBIF (GBIF.org (29th February 2016) GBIF Occurrence Download http://doi.org/10.15468/dl.ywhpmz). Definitions for states were taken from the WWF Global 200 biogeogrpahic realms (Olson & Dinerstein, 2002). Ecology was divided into two characters states, wet or dry, and data was once again collected from literature and herbarium labels.

Table 2. Morphological/Biogeographic Characters and Character States

CHARACTER	States
Branchlet Woodiness	Wood absent; Wood Pithy; Wood Present
Stem Support	Self-supporting; Intermediate; Not self-supporting
Adventitious Roots	Present; Absent; Replaced with Stem Outgrowths
Bulbed Hairs	Present; Absent
Bulbed Spines	Present; Absent
Leaf Outline	Simple; Lobed
Leaf Venation	Basally Tri-nerved; Pinnate
Leaf Margin	Toothed; Entire
Margin Teeth Spacing	>5mm; <5mm; N/A
Stipule Position	Intrapetiolar; Interpetiolar
Stipule fusion	Parrailly Fused; Fully Fused; Free
% Stipule Amplexicaul	<50%; >50%; N/A
Inflorescence Development	Indeterminate; Determinate
Inflorescence Branch Pattern	Symmetrical; Asymmetrical
Inflorescence Internal Branching	Visible; Not Visible; N/A
Stigma Extension	Extended; Sessile
Stigma Hair Distribution	Asymmetrical; Symmetrical
Female Perianth Fusion	Free; Partially Fused; Fully Fused
Perianth Size in Fruit	Enlarged; Not Enlarged
Perianth Fleshiness in Fruit	Fleshy; Dry; Pedicel Fleshy Only; N/A
Achene Apex Elongation	Elongated; Not Elongated
Achene Apex Shape	Curved; Straight; N/A
Distribution	Hawaii; Neotropic; Afrotropic; Indo-Malay & Australasia
Ecology	Wet; Dry

Combined phylogenetic analysis & Ancestral Character State Reconstruction

The Twenty-two morphological characters and two biogeographic ones were coded into a matrix as discrete, and binary or multistate. They were then combined with the molecular data and re-analysed under the same Bayesian parameters outlined in the Molecular Methods section, in an attempt to gain improved resolution of the relationships between clades.

Thirteen Characters from the matrix were then used to undertake an Ancestral Character State Reconstruction, in order to explore evolutionary trends within the clade and whether possession of the ancestral state correlates with either of the contrasting topological hypotheses from the molecular analyses (see fig. 5). The characters analysed were branchlet woodiness; stem support; presence or absence of bulbed hairs; leaf venation; stipule fusion; inflorescence development; inflorescence branching; stigma elongation; Stigma hair arrangement; perianth fusion; perianth fleshiness in fruit; achene apex morphology; and distribution.

I chose to follow the advice of (Royer-Carenzi, & Didier 2016), Cunningham et al. (1998) and Pagel (1999) that Maximum Likelihood is preferable to Maximum Parsimony for Ancestral Character State Reconstruction, since it considers relative branch lengths in its analysis and by assessing all potential reconstructions, provides relative probabilities of each character state at each node. The characters were traced onto the combined molecular tree in Mesquite v. 3.2 (Maddison & Maddison 2017) using ML models.

3.2 MORPHOLOGICAL RESULTS

From the 24 characters identified with taxonomically informative variation between the six clades revealed by the molecular analyses, synapomorphies were identified for three of the clades (B,D & E), and unique suites of characters for all six. See Appendix 2 for table of results per species

Clade Morphology

Stem Support

Two clades contained exclusively non self-supporting taxa. Members of clade F are lianas climbing in trees or over rocks (Friis 1985) and those of Clade D tend to be hemi-epiphytic climbers or scramblers (Chew) adpressed to their host. Members of the other clades are all self-supporting with the occasional shift to an intermediate, more scandent habit, as found in *U. lianiformis* and some individuals of *U. baccifera*.

Branchlet Woodiness

Clade B is the only clade entirely lacking wood in its branchlets, while soft pithy wood is found only in the branchlets of clade E. The remaining clades all have woody branchlets.

Adevntitious roots

Adventitious roots are found in all members of clade D, and all those of clade F with the exception of *U. fischeri* and *U. sansibarica*, which instead possess stem outgrowths covered in stinging hairs. None of the other clades contain taxa with adventitious roots.

Bulbed hairs & spines

Bulbed hairs are entirely lacking in all members of clades A and D, and some members of clade C. The remaining clades all possess bulbed hairs throughout. Bulbed spines of at least 3mm in length and becoming lignified are a synapomorphy for clade B.

Leaf outline & venation

Most members of clade E and one member of clade B possess leaves with a lobed outline. The rest all possess simple leaves, without lobes. Pinnate venation is found in clades A and D. All other clades have a basally tri-nerved venation pattern, with the lowest pair of secondary nerves more prominent than the rest and frequently reaching almost to the leaf apex.

Leaf margin

Entire margins in are found in all taxa in clade D and one member of clade F. All other clades possess varying degrees of toothed margins. The teeth of clade B are noticeably larger than those found in other clades, at least 10mm apart at the apex.

Stipule position, degree of fusion, and amplexicauly

Free, interpetiolar stipules are a synapomorphy for Clade E, all other clades having stipules that are intrapetiolar and either partially or completely fused. Clades A & D possess stipules that are almost completely amplexicaul, enveloping more than 50% of the stem.

Inflorescence development, branching pattern and visibility

Determinate, symmetrically branching inflorescences are found in clades A, C and D. Clades B, E and F all instead possess indeterminate, asymmetrical ones. Branching so dense as to obscure inner branches is found in most members of clade D and half of clade A.

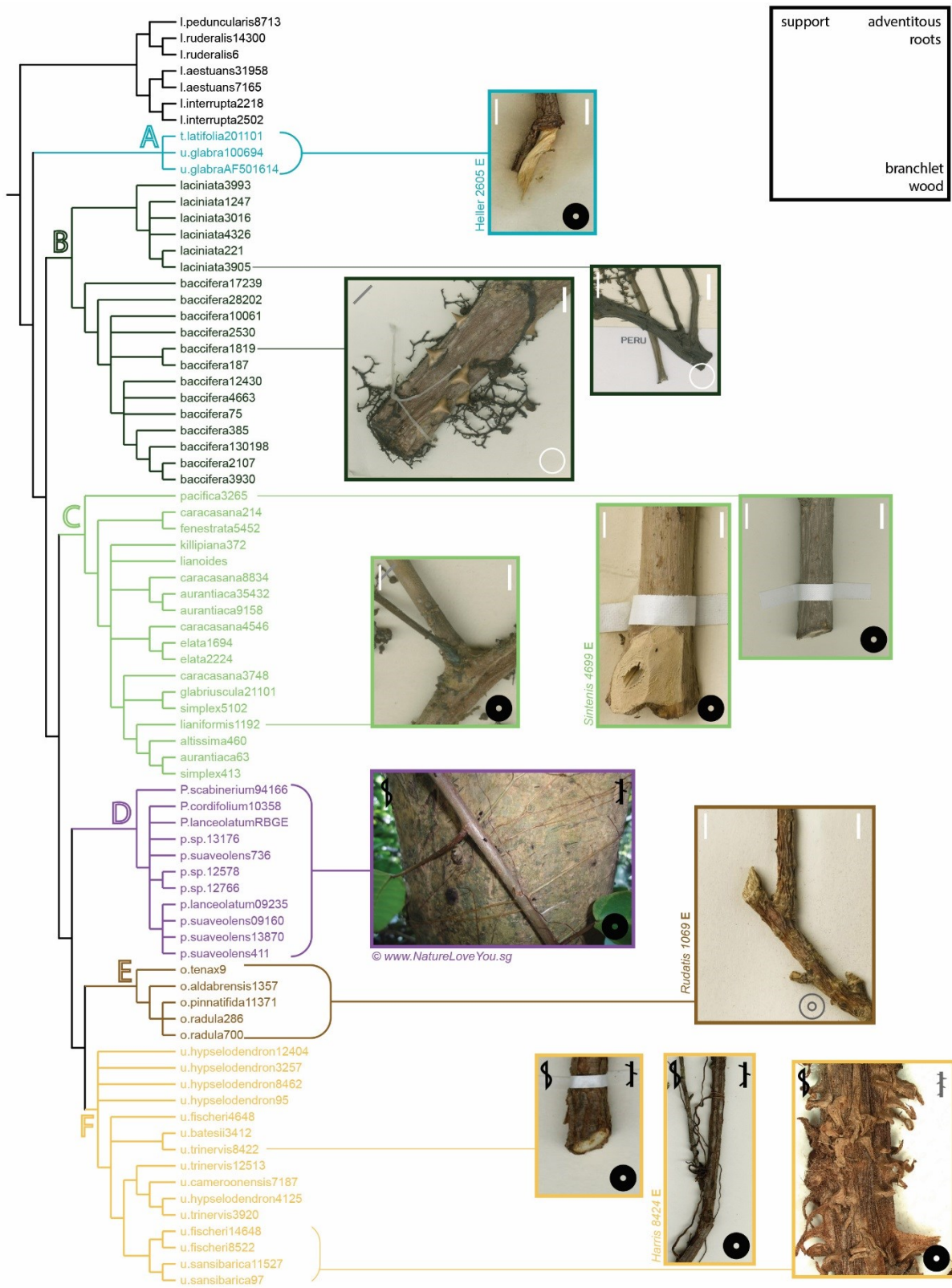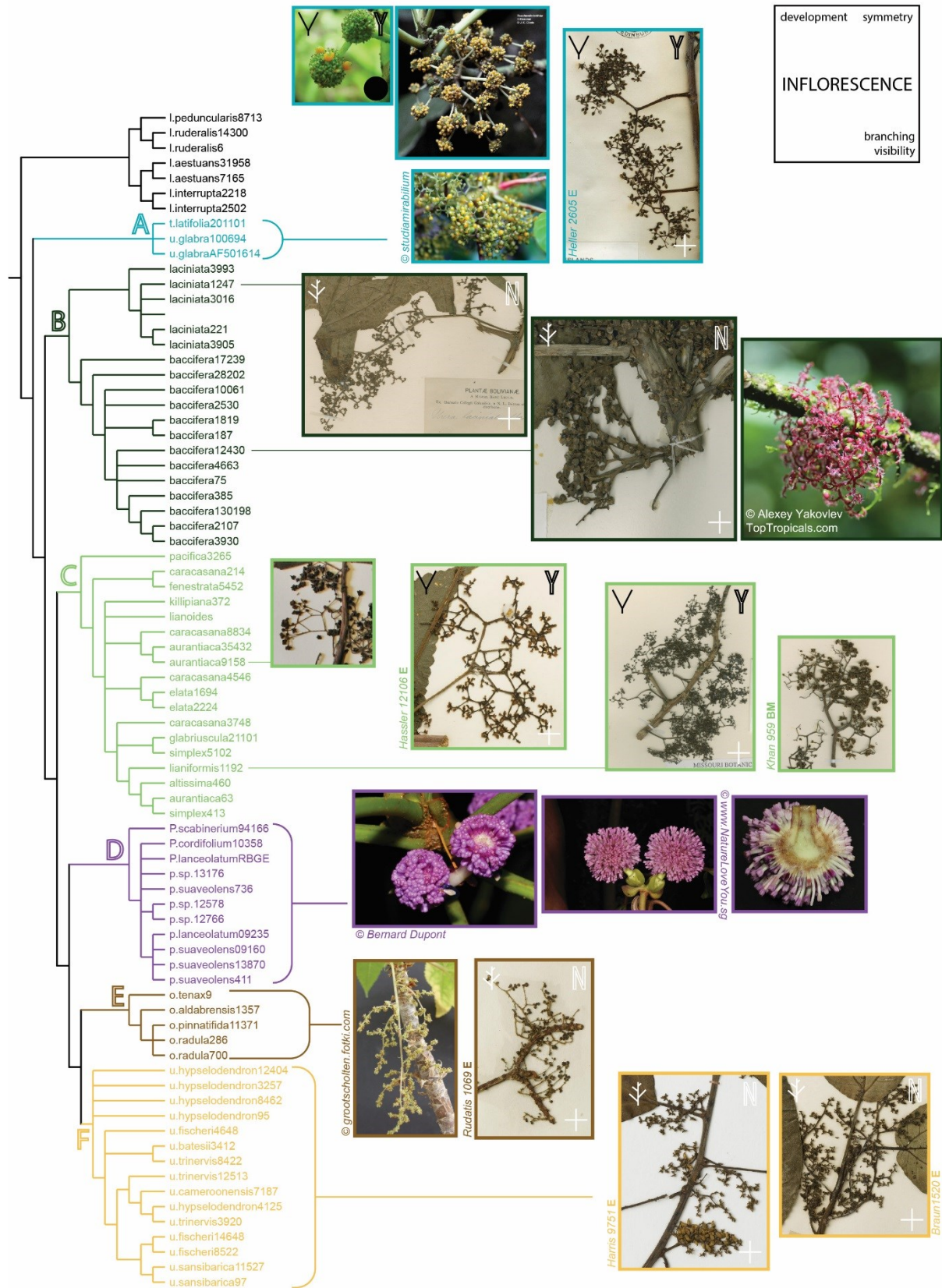*Fig. 6 Stem support and woodiness*

Fig. 7 Inflorescence morphology

Stigma extension and symmetry

Stigmas are sessile on top of the carpel in all members of clades C and F, and one member of clade B. They are extended in all other clades. The stigmatic hairs are found on only one side of the stigma in all of clades A, D and E and one member of clade B. All other taxa have hairs symmetrically arranged all the way round the stigma.

Perianth fusion

All members of clade F with the exception of *U. hypselodendron* possess a female perianth almost entirely fused, with four small teeth. Clade D is predominantly composed of taxa with a female perianth fused only at the base, but some members have entirely fused perianths as in clade F. Clade A has partially fused perianths, and clades B, C and E have completely free female perianths.

Perianth size and fleshiness in fruit

Tepals are accrescent and become larger in fruit in members of all clades, with the exception of *U. laciniata* in clade B, and *U. pacifica* in Clade C. Tepals become fleshy in fruit in all clades with the exception of clade D, and *U. pacifica* in clade C. In *U. pacifica* the pedicel and receptacle immediately below the flower become fleshy instead.

Achene length, apex extension and apex shape

Achenes are no longer than 2mm in clades A & C. In all other clades they are at least 2.5mm in length. Achene apexes are extended in all members of all clades except clade C, where most members possess achenes with a blunt, unextended apex, resulting in an almost circular shape. Of the taxa with extended achene apexes, those in clades A, B and E possess apexes that are curved, while those of clades D and F are straight.

Fig. 8 Female flowers under Scanning Electron Microscope

KEY TO FIGURES

Exceptions

Clade states

Fig. 9 morphology by clade

Clade biogeopgraphy/ecology

Distribution

Clade A is found exclusively in Hawaii; clades B and C in the Neoptropics; clade D in Indo-Malaya & Australasia; and clades E and F in the Afrotropics.

Fig. 10 Distribution by clade. Map from taken from Olson & Dinerstein (2002)
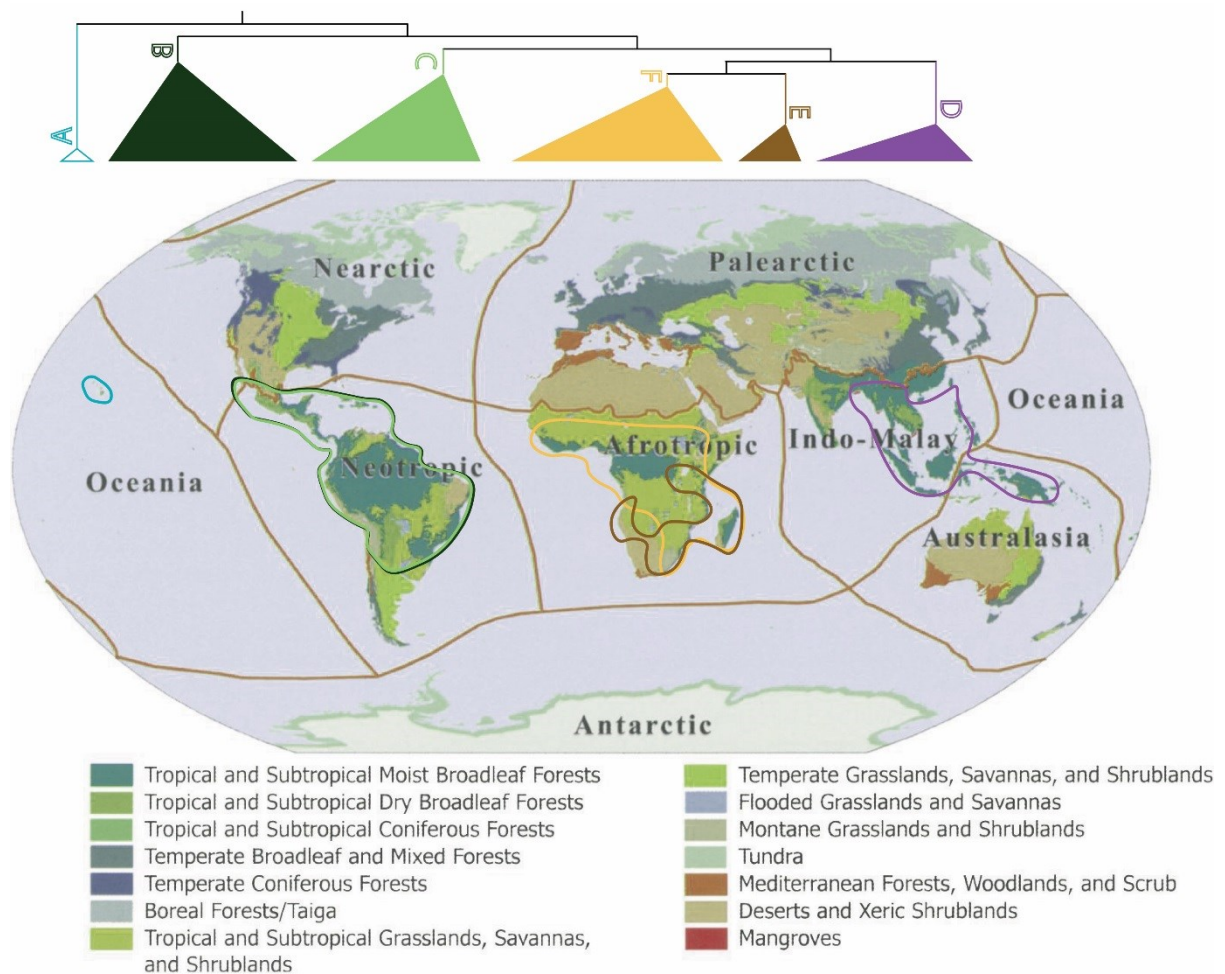
Ecology

All clades with the exception of clade E are adapted to a wet ecology. One further exception to this is

U. baccifera, which can be found in wet and seasonally dry regions of Latin America.

Combined Molecular+Morphological phylogeny

The Bayesian analysis of the combined Molecular and Morphological matrices provided no extra

resolution to the topology of the tree and the relationships between the six clades. It instead

resolved the five main clades on a single polytome.

Fig. 11 BI consensus tree for combined molecular and morphology analysis
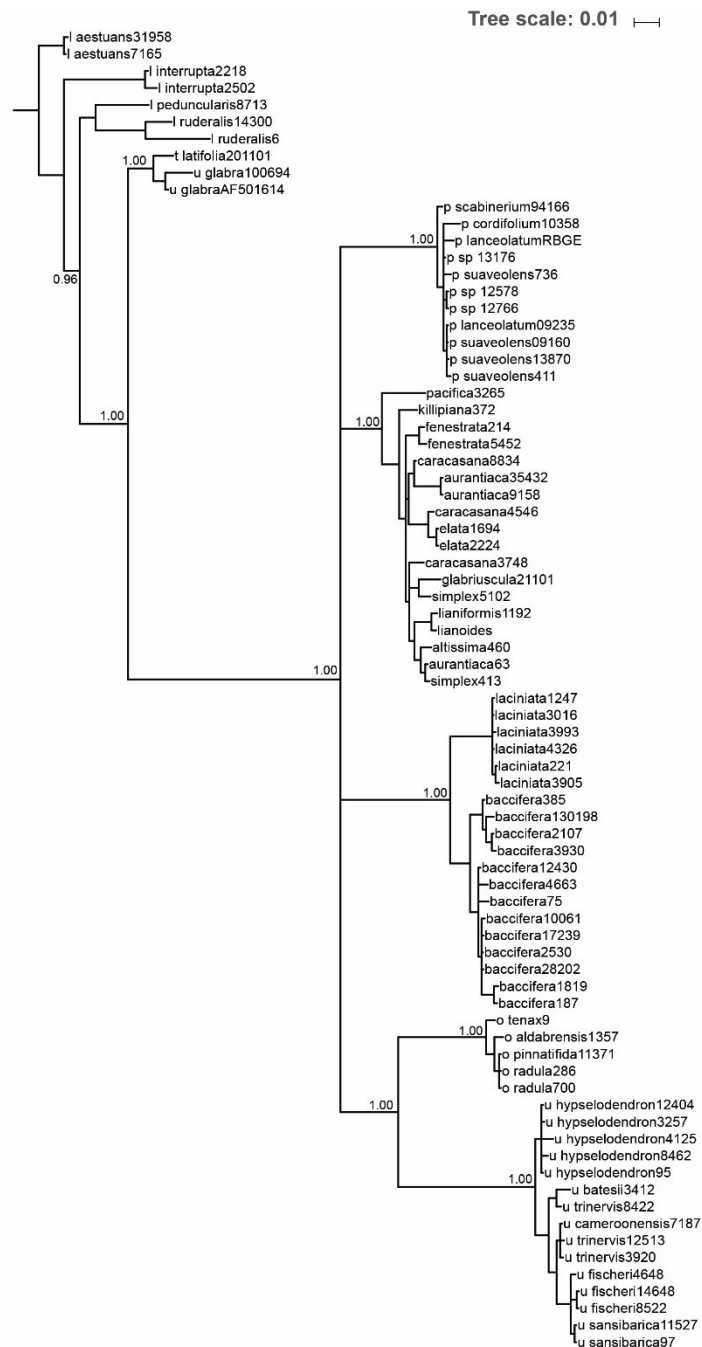
Ancestral Character State Reconstruction

Results from the ACSR for the entire clade are reported in the table below

Table 3. ACSR results

CHARACTER	Ancestral State (Likelihood)
Branchlet woodiness	Woody (94%)
Stem Support	self-supporting (87%)
Presence of bulbed hairs	Absent (77%)
Leaf Venation	Basally tri-nerved (52%)
Stipule fusion	Fused (70%)
Inflorescence development	Determinate (83%)
Inflorescence Branching	Visible (100%)
Stigma elongation	Extended (100%)
Stigma hairs	Asymmetrical (100%)
Perianth fusion	Free (50%), [48% partially fused]
Perianth fleshiness	Fleshy (92%)
Achene apex	Curved (100%)
Distribution	- (all equally likely)

4. Discussion

4.1 Congruence of Molecular and Morphological/Biogeographic data

The results of the molecular phylogenetic analyses support the work of earlier studies in identifying a set of polyphyletic and paraphyletic relationships in *Urera*, and there is a unanimous level of congruence in the delimitation of five clear clades, each with 85% support or higher, corresponding to three separate groups of *Urera*, plus one corresponding to *Poikilospermum* and another to *Obetia*. Despite the position of the Hawaiian taxa being less clear in the analysis of the *TrnL-F* region, appearing to be nested within the outgroup *Laportea*, the *nrITS* and combined analyses resolve it as a sixth clade between the outgroup and the five other clades, with 100% and 97% support respectively.

Various degrees of congruence with these six clades can be found with respect to the 25 characters utilised in the morphological and biogeographic analysis. No one character exhibits a different state in all six, but in combination, they provide a suite of characters that can be used to circumscribe each, and morphological synapomorphies exist for half of them.

Clade B (Urera) is the most readily identified from its morphology of the six, with three morphological synapomorphies: a lack of wood in branchlets, bulbed spines longer than 3mm, and leaf teeth at least 10mm apart. Clade E (Obetia) is also easily distinguished by its pithy-wooded branchlets, lobed leaves, and free, interpetiolar stipules.

Clade F (African Urera /Scepocarpus) is not self-supporting, almost always possessing adventitious roots and a completely fused, 4-dentate perianth. It can be distinguished from clade D on the basis of its asymmetrical, indeterminate inflorescences and possession of bulbed hairs, as well as its distribution in tropical Africa as opposed to Asia.

Clade C (Urera II) possesses an achene smaller than 2mm in length and almost circular in outline, lacking an extended apex. It can also be distinguished from its fellow Neotropical clade (B) by its lack

of bulbed spines greater than 3mm in length, and its possession of woody branchlets, and symmetrical, determinate inflorescences.

Perhaps the most similar clades are A (Touchardia) and D (Poikilospermum), with both groups entirely lacking bulbed hairs and possessing symmetrical, determinate inflorescences often so dense as to become spherical glomerules. The most obvious difference is their distributions in Hawaii and Indo-Malaya & Australasia respectively, but clade E is also not self-supporting and has adventitious roots, while clade A is a self-supporting shrub, lacking them. Clade C also has an entire margin and its achenes are longer than 2mm in length and straight-apexed, whereas clade A has achenes that are less than 2mm long with a curved apex.

4.2 Possible evolutionary trends

A not self-supporting habit appears to be a derived state and is found only in clades D and F, where it is common to all taxa. This state also appears to be linked to the development of adventitious roots, which are found throughout both clades, with the exception of *U. fischeri* and *U. sansibarica* in clade F. The intermediate state of support is found in a few members of clades B and C and could perhaps be a result of a response to similar ecological conditions and the transition to a not self-supporting, liana habit.

Bonsen and Ter Walle (1984) studied the wood anatomy of 21 Urticaceae genera including members of *Poikilospermum*, Touchardia, and all three clades of Urera, though not Obetia. They found unlignified elements in all taxa, which they suggested represent a specialized feature found in the Urticales only within Urticaceae. The presesence of these unlignified elements in the secondary xylem of the climbing species gives rise to a distinctive pattern that they suggest may be an adaptation for increased mechanical strength. This led to them to hypothesise that the ancestor of this group is likely to have been herbaceous, an idea confirmed by Kim et al. (2015). Based on their results they also proposed a close relationship of *Poikilospermum* to *Urera* and *Touchardia* in their "Group B", describing the wood anatomy as extremely similar. Such detailed analysis was beyond

the scope of this study, but the pattern uncovered in branchlet woodiness did offer variation between our six clades. Clade B was the only one to lack any evidence of wood in its branchlets, and clade E the only one to possess soft, pithy wood. The former state may indicate an ancestral condition in the transition to woodiness throughout branchlets found in the other clades. The latter is likely to be an adaptation to the arid conditions in which *Obetia* grows (Burston et al., 1997).

Bulbed spines are a synapomorphy for clade B and have earned them the colloquial name Chichicaste (Monro & Rodrigues 2009) and "Cow-Itch", as well as their utility in cattle-fencing, which Burger (1977) states may be partly responsible for their distribution. Clades A and D are the only clades to entirely lack bulbed-hairs. Wu et al. (2015) hypothesise that this may be linked to a loss following long-distance radiation events; in Hawaii as a result of the absence of herbaceous predators, and in the case of *Poikilospermum* as a result of the loss of key genes due to a founder effect and genetic bottleneck. These hypotheses lack any evidence and are had to test, and it is also the case that many members of clade C only possess a limited number of stinging hairs, either sparsely distributed or in a few places such as on the inflorescence or the petiole, or they can even lack them entirely. It is certainly an intriguing loss given that stinging hairs are present throughout the rest of the Urticeae, where they appear to provide valuable protection from herbivorous predators (and collectors! (Burger 1977)) .

Leaf lamina in Urticeae are frequently prominently lobed (Friis, 1993), but amongst the taxa anlaysed here that is the case only in *U. laciniata* and all of clade E. The remainder of taxa possess leaves that are variously ovate, lanceolate, obovate or oblong; however, the variation does not appear to provide any taxonomic characters at this rank, though Friis (1985) states that African members of *Urera* (clade F) may possess a more homogenous leaf morphology than those of Latin America (clades B & C). The transition to a simple leaf outline may in turn be linked to a pinnate nervation as found in clades A and D. Meanwhile, an entire leaf margin is found only in clade D, and a margin with teeth more than 5mm apart is characteristic of clade B. It seems likely therefore that

across the clade there is a transition from lobed, basally tri-nerved, toothed leaves to simple, pinnately nerved ones with entire margins.

In all clades except clade E, stipules are intrapetiolar and at least partially fused, as is the ancestral state in Urticeae (Kim et al. 2015). Clade E by contrast, have free interpetiolar stipules, which appear to be the derived state. The stipules of clades A and E are almost amplexicaul, enveloping nearly the entire stem. We appear to see two separate transitions therefore from an intrapetiolar, partially-fused pair of stipules to one group with free interpetiolar stipules and one with completely fused stipules almost enveloping the stem.

Similarly the development of the determinate and symmetrical inflorescence that unite clades A, C and D appears to be derived from the loose, indeterminate, and asymmetrically branched panicles possessed by the outgroup, wider tribe and clades B, E and F. In both A and D the internal branching can be so shortened and dense as to create spherical glomerules of flowers, which may well be a further derivation. Weddell (1869) used determinate cymes and indeterminate panicles to divide *Urera* into putative, unnamed sections.

A sessile, penicillate-capitate stigma was listed by Weddell (1856) as the definitive state in *Urera*, though he also noted the exception found in *U. laciniata*, which he stated is more similar to *Laportea*. His assertion is borne out by the results here, with clades B (excluding *U. laciniata*), C and F all possessing sessile stigmas with symmetrically arranged stigmatic hairs. Clades A, D and E all have extended stigmas, though the hairs can be either symmetrically arranged, or asymmetrically as in *U. laciniata* and *Laportea*. In some specimens of *U. baccifera*, the stigma can appear slightly elongated, and it is possible that it represents an intermediate state between that of *U. laciniata* and those of the taxa with sessile stigmas.

Clades B, C and E share completely free perianths, which is an ancestral state of the tribe (Kim et al. 2015). A completely fused perianth tube, with only the teeth remaining free is found in all members

of clade F, with the exception of *U. hypselodendron*, which is sister to the other species sampled in the phylogeny, perhaps supporting a completely fused perianth as the derived state. This condition led Bentham (1880) to propose a sub-generic section for species with fused perianths, and Friis (1985) to first suggest the division of Urera between the Neotropical and Palaeotropical species. The perianth in clade D can be either partially or fully fused, though only taxa with the partially fused state were sampled for molecular analysis here. Clade A's perianths are also partially fused.

In the past a perianth becoming fleshy in fruit has been used as one of the defining features of the genus (e.g. Gaudich, 1830; Weddell 1856), however there are exceptions and variations across the taxa sampled here. In clade B for example, *U. laciniata*'s perianth remains dry and fails to inflate, while in *U. pacifica* the pedicel inflates and becomes fleshy instead of the perianth, which remains dry and unenlarged. *U. pacifica* is sister to the rest of the taxa sampled here and as with the lack of fusion in the perianth of *U. hypselodendron*, this may represent a transitional state from dry to fleshy tepals. The perianths of clade D all appear to inflate and become fleshy in fruit, but some have the additional adaptation of a mucilaginous inner layer, which expels the achene. Clade E shows the most radically different morphology, with tepals that are persistent and accrescent, growing in size after fertilization to completely surround and obscure the achene, but remaining dry and papery. All of these adaptations share a common application in the dispersal of fruits, and while the development of a fleshy perianth also occurs in other Uritcaceae taxa (Friis, 1993) it is not seen in *Laportea* or any other taxa closely related to *Urera*. The fleshy fruits are likely to be attractive to birds, but there is also evidence that they are collected by ants (Dutra et al., 2006). The dry and enlarged perianths of *Obetia* fruits on the other hand seem well adapted to a wind dispersal strategy, and could well be linked to the arid environments in which the plants are found.

Differences in the shape of the achene across the different taxa showed a relatively good correlation to the clades identified in the phylogeny. All taxa possess a single unilocular achene containing a single seed. The achene is slightly compressed with a ridge running along the edge, which may be

the result of an ancestral fusion of two ovaries and subsequent pseudomonomery (Ronse de Craene 2012). Clades A and C both have noticeably smaller achenes than the other clades, consistently less than 2mm in length; and Clade C is unique in that most of its species possess a nearly circular, lenticular achene, lacking an extended apex below the sessile stigma. Once again, *U. pacifica* is the exception to this rule. Of the taxa with extended achene apexes, clades A, B, and E, plus *U. pacifica* share one that is curved over, while those of D and F are straight-sided. It seems possible that the extension and curvature of the achene apex are related to the position and extension of the stigma. In this scenario *U. baccifera* and *U. pacifica* might represent a transitional state between the extended achene apex and stigma found in *U. laciniata* and *Laportea*, and the sessile stigmas of clades C and F (Friis, pers. comm.). However, where Obetia and *Poikilospermum* might fit with this hypothesis is hard to say.

The results were concordant with those of Wu et al. (2015), in that biogeography was one of the most consistently useful characters in the delimitation of these clades. Clades B and C overlap in the Neotropics, as do E and F in Tropical Southern and Eastern Africa. The latter two appear to occupy different ecological niches, with *Obetia* having adapted to more arid conditions, which is also reflected in morphological adaptations such as their pachycaul stem, wind-dispersed fruits and seasonal deciduousness. Analysis of the African Flora (Pokorny et al., 2015) has shown that fluctuations of climate aridity in the Miocene and Pliocene in particular have led to the diversification of a number of xeric specialist taxa, and this may account for the divergence of the African *Urera* and *Obetia*.

Clades B and C on the other hand both appear to occupy similarly wet tropical ecologies in the Neotropics, though *U. baccifera* is sometimes found in more arid Seasonally Dry Tropical Forest. There is some evidence that clade C is more tolerant of undisturbed habitats however (Monro, pers. comm.), and this may in turn explain the markedly less fearsome bulbed hairs and total lack of spines that are found in clade B.

On the basis of the above observations, it was hoped that the combined morphological and molecular phylogenetic analysis and the ACSR might thus help provide some resolution to the pattern and order of divergence of the six clades. However, results of the combined analysis provided no extra resolution, instead supporting the molecular analyses in resolving a polytome.

For clade B, a number of character states appear to resemble those found in the outgroup and ancestral to the wider tribe, matching the poorly supported hypothesis of the model-based molecular analyses. Among these are a lack of wood in branchlets, a self-supporting habit, possession of stinging hairs; an extended and reflexed achene apex; partially fused intrapetiolar stipules; an indeterminate, assymetrical inflorescence; and a perianth lacking any fusion. The lobed leaves, dry uninflated perianth, and elongated, one-sided stigma of *U. laciniata* also closely match *Laportea* as was noted by Weddell (1856). However, the presence of the Hawaiian taxa (clade A) as sister to the other clades, combined with their morphology more closely matching that of clade D means that this pattern was not reflected in the results of the ACSR. This and the lack of resolution in the clade make accurate reconstruction of the ancestral state for the clade difficult. Of the characters analysed for the ACSR, the results are split roughly in half between their support for these two scenarios, providing no extra clarity.

The suggestion that the Hawaiian taxa and *Poikilospermum* represent the ancestral state for the clade, with clades B, C, E and F representing a string of secondary reversals is certainly possible. However, the syndromes affecting the morphology of island endemics are widely documented (e.g. Bohle et al., 1996, Bowen & van Vuren, 1997) and a similar problem affects attempts to reconstruct the ancestral distribution of Begonia for example (Wendy et al. 2004).

The lack of woody branchlets in clade B could be interpreted as part of a transition from the herbaceous state in the rest of the tribe to the woody one found in all other taxa in this clade. However the ACSR constructs the ancestral state of the entire clade as 94% likely to be woody.

Island woodiness is a well-known phenomenon first suggested by Alfred Russell Wallace (Bohle et al., 1996), and could plausibly explain the condition being present in *Touchardia*. Similarly, absence of bulbed hairs is reconstructed as 77% likely in the ACSR, despite the fact that presence of them is a synapomorphy and the ancestral state for the tribe (Kim et al. 2015). This scenario would involve the redevelopment of stinging hairs across all five of our other clades with the exception of *Poikilospermum*. Once again, the loss of defences against herbivory by island endemics has often been observed (Bowen & van Vuren, 1997), and this result should be treated accordingly. Of course, this does not rule out Hawaii as the origin of the woody or sting-less states in our clade, with a subsequent dispersal with numerous reversals to the rest of the tropics. The biogeography results of the ACSR are equivocal and it seems equally plausible that they could either have arrived from or dispersed to Hawaii from Central America or Indo-Malaya & Australasia (Baldwin & Wagner, 2010). Kim et al. (2015) state that the *Laportea* clade sister to our taxa are native to Central America, but other literature sources and its current distribution seem to contradict this. Hawaii is a relatively recent group of volcanic islands, and further work to provide a dating of the phylogeny may be able to resolve this question.

It is clear then that further analysis is required to clarify some of the evolutionary trends in this group of taxa. The most valuable addition would be a more resolved phylogeny with wider sampling in African *Urera* and *Poikilospermum,* and additional gene regions hopefully providing greater clarity about the order of divergence between the six clades. In the meantime, combining the data here with that of Kim et al. (2015) and conducting the ACSR across the whole tribe may also provide some improved clarity.

4.3 Taxonomic Treatment

Species concepts are a matter of frequent and often heated debate amongst taxonomists (de Quieroz, 2007), but the rank of genus appears to receive less attention and many genera have persisted largely intact since the introduction of the binomial system by Linnaeus in 1751. The Linnaean system is a hierarchical one, leading to a recurrent tension between ease of communication and creation of a "natural" classification that is evolutionarily representative. (Stevens 2002). His intention in designing it, as well of that of Bentham's was one of data management as much as classification (Stevens, 2002) and that is often at odds with modern ideas of monophyly.

In their discussion of paraphyly in the genus *Hibiscus,* Pfeil and Crisp (2005) outlined three criteria for the circumscription of genera: firstly that they are monophyletic, secondly that they can be defined by robust clades, and thirdly that their creation requires the fewest possible nomenclatural changes. To these, Monro (2006a) added the further conditions that two or more molecular regions be used in the phylogenetic analysis, and that the clades be morphologically identifiable with the use of a hand lens. However, as Brummitt (2002) points out in his discussion of how best to reconcile the Linnaean system with evolutionary hierarchy, paraphyly in itself does not necessitate the creation of new taxa.

It is clear from the molecular analysis that the genus *Urera* is polyphyletic and divided in four, and that it is paraphyletic with *Obetia* and possibly *Poikilospermum* contained within it. The morphological analysis shows that while there do not exist individual synapomorphies for each and every clade, they can usefully be divided by a suite of morphological and biogeographic characters.

Under these conditions therefore, the question becomes how this diversity might best be represented taxonomically. The options available include:

(i)   Maintaining the status quo with minor adjustments. In this scenario, *U. glabra* would

join *Touchardia*, but the remaining genera would be left intact, with *Obetia* and

potentially *Poikilospermum* nested within a paraphyletic *Urera*.

(ii)   Sinking all taxa into a broader *Urera.* Here *Obetia* and *Poikilospermum* would be

included in a more broadly defined *Urera*. *Touchardia* could either be included or remain

separate.

(iii)   Splitting *Urera* in three. This would result in six separate genera, one for each of the

clades, with *U. glabra* once again part of *Touchardia*.

Scenario (i) requires the fewest nomenclatural changes and thus the least disruption, but since none

of these plants is a major horticultural or agricultural crop, and it is not a widely studied group, that

in and of itself is not significant justification.

Were the molecular results entirely unexpected from the morphology-based work of the past, then

the presence of paraphyly alone would not necessarily justify altering the taxonomy of a genus that

has lasted largely intact since its publication almost two hundred years ago. Brummitt (2002) cites as

an example of this, the family *Cactaceae* and its position nested within a paraphyletic, but

morphologically natural and easily recognisable *Portulacaceae.* However, from the very first

treatments of *Urera*, multiple authors have expressed doubts about the unity of the genus, and its

longevity is arguably merely a reflection on the lack of a holistic analysis of the taxa since the time of

Weddell. In his later works, Weddell (1856; 1869) had created unnamed divisions within the genera

based primarily on inflorescence form and distribution, two characters I have shown here to be

useful in the delimitation of the clades. His subsequent decision to create the new genus from Africa

with a fused perianth, *Scepocarpus*, but not to place any of the existing African *Urera* within it, is

somewhat puzzling. That decision was compounded when Bentham (1880) sank the monotypic

Scepocarpus manii Wedd. into *Urera*, despite having observed that the two African species he

analysed, *U. oblongifolia* Benth. and *U. obovate* Benth., also possessed a fused perianth differing significantly from the rest of the genus and thus deserved a separate section from other *Urera* (Bentham, 1849). In picking up on these decisions and adding that the African taxa's liana habit and consistent leaf morphology also separated them from the Neotropical ones, Friis (1985) stated that the only things stopping him from proposing a formal taxonomy to represent these differences were the unfused perianth of *U. hypselodendron*, the isolated position of *U. glabra* in Hawaii and the lanceolate stigma of *U. laciniata*. As illustrated above, these three issues have now been resolved by the phylogenetic work carried out here. Friis (1985) noted that the leaves and habit of *U. hypselodendron* were similar to those of other African species, but that its perianth was free and unequal as in the neotropical ones. The position of this species as sister to the others sampled suggests its perianth could represent an ancestral condition. The same seems possible for the stigma in *U. laciniata*, given its putative proximity to the outgroup, while *U. glabra*, is unequivocally resolved within *Touchardia*.

The paraphyly observed here is therefore different to that described by Brummitt (2002) for Portulacaceae in that *Urera* already possessed questions about the naturalness of its circumscription. If taxonomic changes thus appear preferable, Scenario (ii) is probably the next simplest of the three, but would require a broader circumscription for *Urera*. The presence of fleshy perianths in fruits would not make sense for current *Obetia*, though this could be altered instead to "enlarged". It should also be noted that *U. laciniata* and *U. pacifica* already fail to meet this criterion as it is. Stinging hairs and perianth fusion are also characters that would need a more flexible definition. Interestingly in Kim et al.'s (2013) discussion of the paraphyletic position of *Hesperocnide* Torrey within *Urtica* L., they cite *Urera* as an example of a genus where fused and female perianths coexist, and go on to propose that *Hesperocnide* be sunk into *Urtica*, rendering it monophyletic. What Brummitt would make of this decision is not recorded. As we have seen, the variation of characters between these six clades is often somewhat of a continuum with evidence for a progression from herbaceous to woody, or self-supporting to liana and free to fused 4-parted female

perianths for example, so its inclusion within one broad genus is not entirely outlandish. Is such a broadly circumscribed genus desirable though, when it could readily be avoided? Especially given the past conjecture on the divisions within *Urera*.

Perhaps more importantly than historical precedent or levels of taxonomic disruption, scenario (iii) better illuminates the diversity in morphology between the taxa and more easily enables analysis of evolutionary trends including a shift to woodiness, a climbing habit, loss of stings, dispersal events and adaptation to drought. As shown, distinct biogeographic and ecological trends are also in evidence here, but *Urera* has often been defined as pantropical, with its apparent absence from Asia frequently overlooked or ignored. Meanwhile, *Obetia*'s transition to an arid habitat is rare in the Urticaceae, which may be the result of wider trends in the African Flora, and marks it apart from all the other taxa here.

In the past, generalisations about *Urera* have created confusion, for example with regards habit and wood structure (e.g. Kim et al. 2013, Wu et al. 2105, Bonsen & ter Walle, 1983), and while this could perhaps be avoided by well-defined sections within a single genus, *Obetia* and *Poikilospermum* are readily identifiable genera that do not appear to make sense as sections of *Urera*, from which they can be immediately differentiated. Perhaps this is what Linnaeus was referring to when he wrote "if the genera are confused, all is confusion, necessarily." (Linnaeus, 1751; Stevens, 2002). Including the morphologically, biogeographically, and ecologically distinct *Obetia* and *Poikilospermum* within *Urera* would thus further exacerbate these problems, and it is my judgement that the diversity is therefore better reflected and exposed to future analysis by keeping the groups separate.

Given that this study has shown a high level of congruence between molecular, morphological and biogeographic signals, and with historical doubts about the circumscription of *Urera*, I therefore suggest that the taxonomy be resolved as follows:

Touchardia Gaudich., Voy. Monde Bonite, Bot., Atlas: Tab. 82 (1844); St John, Phytologia 63: 183-184; Wagner, Manual of the Flowering Plants of Hawaii. 2: 1310-1312 (1990).

Generotype: *T. latifolia* Gaudich., C. Gaudichard 402, Iles Sandwich [Hawaii] 1830?, lectotype P!.

Short, upright shrubs, with wood throughout branchlets. Stems lacking adventitious roots. Entirely lacking bulbed hairs or spines. Leaves simple, pinnately nerved. Margins entire. Stipules intrapetiolar, fully fused and two-keeled, almost completely amplexicaul. Inflorescences dense, symmetrically branched determinate cymes, the inner branches occasionally so short as to resemble spherical glomerules. Male flowers 4- or 5-merous. Female flowers with four basally fused tepals. Stigma extended with hairs asymmetrically arranged on one side. Tepals in fruit enlarged and fleshy. Achenes slightly compressed, less than 2mm in length, the apex extended and reflexed.

Wet forests on Hawaii. Includes Hawaiian taxa assigned to Urera (e.g. U. glabra)

Urera Gaudich., Voy. Uranie: 496. (1826[1830])

Generotype (designated by Britton & Wilson, 1924): *U. baccifera* (L.) Gaudich. ex Wedd., Plumier, P. Amer.: tab. 260. 1760, lectotype (designated by de Rooij [1975: 302]); Fawcett 7177, Stony Hill, Jamaica 1898, epitype BM!.

Upright or scandent shrubs to small trees, lacking wood in branchlets. Stems without adventitious roots. Possessing bulbed hairs and large, bulbed spines around 3-10mm in length. Leaves simple or deeply lobed, the lowermost secondary nerves larger than the rest and reaching almost to the apex. Margins coarsely dentate, with teeth at least 10mm apart. Stipules intrapetiolar, partially to fully fused and two-keeled, less than 50% amplexicaul. Inflorescences lax, asymmetrically branched, indeterminate panicles. Male flowers 5-merous. Female flowers with 4 free tepals, the lateral pair largest. Stigma sessile with hairs symmetrically arranged, or elongated with hairs asymmetric, only

on one side. Tepals in fruit enlarged and fleshy, or not enlarged and dry. Achenes slightly

compressed, at least 2mm in length, with an extended, reflexed apex.

Disturbed or undisturbed, wet or dry forests across the Neotropics.

Latin American Urera B (Clade C) [If published I will propose the name *Urellia* as a diminunitive of

Urera. The vernacular name "Chichicaste", derived from a Nahuat word meaning "to vibrate"

(Monor & Rodrigues, 2009) was considered, but it is most commonly applied to *U. baccifera*, which

is the generotype for *Urera*.]

As a generotype I will select *U. alceifolia* (Poir.) Gaudich. ex Wedd.. Although this name is currently

considered a synonym of *U. caracasana,* my analysis here has shown that species to be polyphyletic,

and *U. alceifolia* was the only member of this group named by Gaudichaud (1830) when he originally

proposed *Urera*. It was lectotypified by de Rooij (1975) as follows: Martin s.n., French Guyana,

Cayenne. lectotype, P!

Upright or scandent shrubs to small trees, with wood throughout branchlets. Stems without

adventitious roots. Occasionally possessing small bulbed hairs, but lacking large, bulbed spines.

Leaves simple, the lowermost secondary nerves larger than the rest and reaching almost to the apex.

Margins finely dentate, with teeth less than 5mm apart. Stipules intrapetiolar, partially to fully fused

and two-keeled, less than 50% amplexicaul. Inflorescences lax, symmetrically branched determinate

cymes. Male flowers 4- or 5-merous. Female flowers with 4 free tepals, the lateral pair largest.

Stigma sessile with hairs symmetrically arranged. Tepals in fruit enlarged and fleshy; rarely not

enlarged and dry, the pedicel becoming fleshy instead. Achenes slightly compressed, less than 2mm

in length, often with a blunt apex almost completely circular in outline; rarely with an extended,

reflexed apex.

Disturbed or undisturbed, wet forests across the Neotropics.

Poikilospermum *Zipp. ex Miq.,* Ann. Mus. Bot. Ludg.-Bat. 1: 203 (1864); Wedd., DC., Prodr. 16, 1: 235 (1869); Baillon, Nat. Hist. Pl. B: 539 (1874); Benth. & Hook. f., Gen. Pl. 3. 389 (1880); Engl. in Engl. & Prantl, Nat. Pflanzenfam. 3: 114 (1889); Merr. Contr. Arnold Arbor. 8: 47 (1934); Backer, Bek. Fl. Java 6: 53 (1948), Chew,

Generotype: *P. amboinense* Zipp. ex Miq., Zippelius sp. Fem., Amboina (lost?).

Concephalus Bl. Bidjr. 483 (1825), non Necker (1790).

Climbers attached to other plants or rocks, with wood throughout branchlets. Stems with adventitious roots. Entirely lacking bulbed hairs or spines. Leaves simple, pinnately nerved. Margins entire. Stipules intrapetiolar, fully fused and two-keeled, almost completely amplexicaul. Inflorescences dense, symmetrically branched determinate cymes, the inner branches often so short as to resemble spherical glomerules. Male flowers 2- or 4-merous. Female flowers with 4 basally or fully fused tepals. Stigma extended with hairs symmetrically or asymmetrically arranged. Tepals in fruit enlarged and fleshy, occasionally with in an inner mucilaginous layer. Achenes slightly compressed, more than 2mm in length, the apex extended and straight-sided, not reflexed.

Beside streams and rivers in wet forests of N.E. India, S. China, Mainland S.E. Asia, and Malaysia.

Obetia *Gaudich.*, Voy. Monde Bonite, Bot., Atlas: Tab. 82 (1844); Wedd., Monogr. Urt.: 106 (1856) & in DC., Prodr. 16, 1: 69 (1869); Benth. & Hook. f., Gen. Pl. 3. 382 (1880); Engl. in Engl. & Prantl, Nat. Pflanzenfam. 3, 1: 106 (1888); Hutch., Gen. Pl. 2: 182 (1967). Friis,

Generotype: *O. ficifolia* (Savigny) Gaud., P. Commerson s.n., Reunion ("Bourbon"), holotype P-JUSS, sheet no. 16. 852. http://coldb.mnhn.fr/catalognumber/mnhn/p/p00121691

Upright or scandent shrubs to small trees, with soft, pithy wood in branchlets. Stems without adventitious roots. Possessing small bulbed hairs, but lacking large, bulbed spines. Leaves lobed, the

lowermost secondary nerves larger than the rest and reaching almost to the apex. Margins finely dentate, with teeth less than 5mm apart. Stipules interpetiolar, free, less than 50% amplexicaul. Inflorescences lax, asymmetrically branched indeterminate cymes. Male flowers 5-merous. Female flowers with four free tepals, the lateral pair largest. Stigma elongated with hairs symmetrically arranged on all sides. Tepals in fruit enlarged, dry and papery. Achenes slightly compressed, greater than 2mm in length, with an extended, reflexed apex.

Arid environments in South and East Africa, Madagascar, Aldabra and the Mascarene Islands.

Scepocarpus Wedd., Prodr. (DC.) 16(1): 98. 1869.

Generotype: *S. manni* Wedd., Mann 146, Fernando Po 1860, K!

Climbers attached to other plants or rocks, with wood throughout branchlets. Stems with adventitious roots. Possessing small bulbed hairs, but lacking large, bulbed spines. Leaves simple, the lowermost secondary nerves larger than the rest and reaching almost to the apex. Margins finely dentate, with teeth less than 5mm apart, rarely entire. Stipules intrapetiolar, partially to fully fused and two-keeled, less than 50% amplexicaul. Inflorescences lax, asymmetrically branched indeterminate cymes. Male flowers 4- or 5-merous. Female flowers with fully fused tubular perianth, often with four teeth; rarely with four free tepals, the lateral pair largest. Stigma sessile with hairs symmetrically arranged. Tepals in fruit enlarged and fleshy. Achenes slightly compressed, more than 2mm in length, the apex extended and straight-sided, not reflexed.

Forests across Tropical Africa and Madagascar.

5. Conclusions

This study provides the most comprehensively sampled phylogeny of *Urera* undertaken to date, and confirms its polyphyletic and paraphyletic structure. All the clades were well supported and circumscribed by readily observed morphological characters, but the exact topology of divergence between them will require further investigation through increased specimen and region sampling. The suspicions of Friis (1985) that the Neotropical and African taxa should perhaps be separated were also upheld, and the previously unsampled *U. laciniata* was resolved in a clade with *U. baccifera* as a further division within the Neotropics. These two species are the only remaining members of a monophyletic *Urera*, but evidence of polyphyly, plus morphological and habitat variability at the species level suggests there may be grounds for further division. It is suggested that the six clades be separated into individual genera, requiring the resurrection of Weddell's (1869) *Scepocarpus* and the creation of a new name for the remainder of the Neotropical species. *Obetia* and *Poikilospermum* remain unchanged and intact, and *U. glabra* joins *Touchardia*, also endemic to Hawaii. Biogeographic distribution remains one of the strongest characters in the identification of these clades, and its role in the divergence and development of this group warrants further investigation.

References:

Álvarez, I., & Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution*, *29*(3): 417–434. http://doi.org/10.1016/S1055-7903(03)00208-2

Ayensu, E. S. (1967). Aerosol OT Solution—An Effective Softener of Herbarium Specimens for Anatomical Study. *Stain technology*. 42: 155-6. 10.3109/10520296709115000.

Baldwin, B. G., & Wagner, W. L. (2010). Hawaiian angiosperm radiations of North American origin. *Annals of Botany*, *105*(6), 849–879. http://doi.org/10.1093/aob/mcq052

Bentham, G. (1849) Urera In; Hooker, W. J., Vogel, T., Webb, P. B., Hooker, J. D., & Bentham, G. *Niger flora; or, An enumeration of the plants of western tropical Africa*, p. 587. London, Hippolyte Bailiere

Bentham (1880) Urera in: Bentham, G. & Hooker, J. D. *Genera Plantarum*, vol. 3,1: 459 London, L. Reeve & Co.

Berg, C.C., (1978) Cecropiaceae a New Family of the Urticales. *Taxon*, 27(1): 39–44.

Bonsen, K.J. & ter Welle, B.J. H. (1984) Systematic wood anatomy and affinities of the Urticaceae. *Bot. Jahrb. Syst.* 105: 49-71

Borla, V., Bush, G., Chave, J., Clement, W., Feldcamp, J., Goodwin, S., Wood, E. (2004) The Origin of the Fig : Phylogenetic ndhF Sequences. *American Journal of Botany*, 91(5): 767–777. http://doi.org/10.3732/ajb.91.5.767

Bowen, L., Vuren, D. Van, Bowen, L., & Vuren, D. V. A. N. (1997). Insular Endemic Plants Lack Defenses Against Herbivores *Conservation Biology*, 11(5): 1249–1254.

Britton, N. L. & P. Wilson. (1924) Botany of Porto Rico and the Virgin Islands. *Sci. Surv. Porto Rico & Virgin Islands* 5: 243.

Brummitt, R. K. (2002) How to chop up a tree. *Taxon*, 51: 31-41

Burger, W. (1977) Urera. In: *Flora Costaricensis. Fieldiana, Bot.* 40: 276–280.

Burston, C., Martin, R., Walker, C., (1997). Obetia ficifolia - A Pachycaul Stinging Nettle, *British Cactus & Succulent Journal 15*(1), 2–6.

Chen, C.J. (1985) *Sphaerotylos* C. J. Chen. A remarkable new genus of Urticaceae from China with notes on stigmas of the family. *Acta phytotaxon* 23(6):444–56.

Chew, W.-L. (1963) Florae Malesianae Precursores - XXXIV A Revision of the Genus Poikilospermum (Urticaceae) *Gardens' bulletin, Singapore*, v. 20,1: 1-103

Cunningham, C. W., Omland, K. E. & Oakley, T. H. (1998) Reconstructing ancestral character states: a critical appraisal *Tree* vol.13: 361-366

Darriba D, Taboada GL, Doallo R, Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8), 772.

De Queiroz, K. (2007) Species Concepts & Species Delimitation *Syst. Biol.* 56(6): 879-886

De Rooij, M. J. M. (1975) Urera. In: J. Lanjouw & A. L. Stoffers (eds.), *Flora of Suriname*, Vol. 5(1). E. J. Brill, Leiden.

Dong, J. (2016). *Morphological Variation and Floral Development of Major Clades in Urticaceae -A Focus on The Female Flowers.* Msc Thesis. Royal Botanic Gardens Edinburgh

Doyle, J.J.; Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, v.19, p.11-15.

Dutra, H.P., Freitas, A.V.L., & Oliveira, P.S. (2006). Dual ant attraction in the Neotropical shrub Urera baccifera (Urticaceae): The role of ant visitation to pearl bodies and fruits in herbivore deterrence and leaf longevity. *Functional Ecology* 20: 252 - 260. 10.1111/j.1365-2435.2006.01096.x.

Feliner, G. N., & Rosselló, J. A. (2007). Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution*, *44*(2), 911–919. http://doi.org/10.1016/j.ympev.2007.01.013

Friis, I. (1983) A synopsis of the genus Obetia (Urticaceae) *Kew Bulletin*, vol 38, s. 221-228.

Friis, I. (1985) The genus Urera (Urticaceae) in eastern tropical Africa. *Nord. J. Bot*. 5: 547-553

Friis, I. (1989) The Urticaceae: a systematic review. In P. Crane, ed. Evolution, systematics, and Fossil History of the Hamamelidae, Vol.2. Oxford: Clarendon Press, pp. 285–308.

Friis, I., (1993). Urticaceae. In: Kubitzki, K. Rohwer, J. G., Bittrich, V. (eds.), The Families & *Genera of Vascular Plants*, Vol. 2: 612-639 Springer-Verlag, Berlin

Gaudichaud-Beaupre, C. (1830) Urera. In*: Voyage autour du Monde, entrepris par ordre du roi,. . . execute sur les corvettes de S.M. l'Uranie et la Physicienne. . . par M. Louis de Freycinet. Botanique.* Pp. 496–497 Pillet-aine, Paris.

Goolsby, E. W. (2017). Rapid maximum likelihood ancestral state reconstruction of continuous characters: A rerooting-free algorithm. *Ecology and Evolution*, *7*(8), 2791–2797. http://doi.org/10.1002/ece3.2837

Hadiah, J. T., Conn, B. J., & Quinn, C. J. (2008). Infra-familial phylogeny of Urticaceae, using chloroplast sequence data. *Australian Systematic Botany*, *21*(5), 375–385. http://doi.org/10.1071/SB08041

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT *Nucleic Acids Symposium Series*, Vol. 41: 95-98

Hawkins, J. A., Hughes, C. E., Hughes, C. E., Scotland, R. W., & Scotland, R. W. (1997). Primary Homology Assessment, Characters and Character States. *Cladistics*, *13*(3): 275–283. http://doi.org/10.1111/j.1096-0031.1997.tb00320.x

Katoh, K., Standley, D. M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability, *Molecular Biology and Evolution* Vol. 30,4,1: 772–780

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647-1649.

Keay, R. W. J. (1958) *Flora of West Tropical Africa,* 2. Ed. 1, 2: 297-828 London, Crown Agents

Kim, C., Deng, T., Chase, M., Zhang, D.-G. D., Nie, Z.-L. Z., & Sun, H. (2015). Generic phylogeny and character evolution in Urticeae (Urticaceae) inferred from nuclear and plastid DNA regions. *Taxon*, 64: 65–78. http://doi.org/10.12705/641.20

Kim, K. J., & Jansen, R. K. (1995). ndhF sequence evolution and the major clades in the sunflower family. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(October), 10379–10383. http://doi.org/10.1073/pnas.92.22.10379

Letunic, I. & Bork, P. (2007). Interactive Tree Of Life (iTOL): An online tool for phylogenetic tree display and annotation. *Bioinformatics* 23: 127-8.

Linnaeus, C. (1751) *Philosophia botanica, in qua explicantur fundamenta botanica cum definitionibus partium, exemplis terminorum, observationibus rariorum, adjectis figuris aeneis.* G. Kiesewetter, Stockholm.

Maddison, W. P. and D.R. Maddison. (2017) *Mesquite: a modular system for evolutionary analysis.* Version 3.2 http://mesquiteproject.org

Monro, A.K. (2006a) *Taxonomy of Pilea (Urticaceae)* PhD Dissertation, Oxford Brookes University, Oxford

Monro, A.K., (2006b). The Revision of Species-Rich Genera : A Phylogenetic Framework for the Strategic Revision of Pilea (Urticaceae) Based on cpDNA , nrDNA , and Morphology. American Journal of Botany, 93(3): 426–441.

Monro, A. K., & Rodríguez, A. (2009). Three new species and a nomenclatural synopsis of Urera (Urticaceae) from Mesoamerica. *Annals of the Missouri Botanical Garden*, *96*(2), 268–285. http://doi.org/10.3417/2006211

NCBI Resource Coordinators. (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 44: D7–D19. http://doi.org/10.1093/nar/gkv1290

Neyland, R., & Urbatsch, L. E. (1996). The ndhF chloroplast gene detected in all vascular plant divisions. *Planta*, *200*(2), 273–277.

Olson, D. M. & Dinerstein, E. (2002) The Global 200: Priority Ecoregions for Global Conservation *Annals of the Missouri Botanical Garden* Vol. 89, No. 2: 199-224

Omega Bio-Tek. (2013). E.Z.N.A.® HP Plant DNA Mini Kit user manual, (May), 1–24. Retrieved from http://omegabiotek.com/store/wp-content/uploads/2013/04/D2485-HP-Plant-DNA-Mini-Kit-Combo-Omega.pdf

Pagel, M. (1999) The Maximum Likelihood Approach to Reconstructing Ancestral Character States of Discrete Characters on Phylogenies *Syst.Biol*. **48**(3):612–622

Pfeil B. E., Crisp M. D. (2005) What to do with Hibiscus? A proposed nomenclatural resolution for a large and well known genus of Malvaceae and comments on paraphyly. Australian Systematic Botany 18, 49-60. https://doi.org/10.1071/SB04024

Pires, J. C., Graham, S. W., Mcpherson, M. A., Prince, L. M., Patterson, T. B., Rai, H. S., Roalson, E. H., Evans, T. M., Hahn, W. J., Millam, K. C., Meerow, A. W., Molvray, M., Kores, P. J., O'brien, H. E., Hall, J. C., Kress, W. J., & Sytsma, K. J. (2006) Phylogenetic relationships of monocots based on the highly informative plastid gene ndhF: evidence for widespread concerted convergence, pp. 28–51. In: J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], *Monocots: comparative biology and evolution (excluding Poales). Rancho Santa Ana Botanic Garden, Claremont, California*, USA.

Rendle, A. B. (1917) Urticaceae in: Prian, D., (ed.), *Flora of Tropical Africa* 6, 2: 240-306, London. L. Reeve & Co.

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

Ronse De Craene, L.P., (2012) *Floral Diagrams. An aid to Understanding Flower Morphology and Evolution*, University Press, Cambridge.

Royer-Carenzi, M., & Didier, G. (2016). A comparison of ancestral state reconstruction methods for quantitative characters. *Journal of Theoretical Biology*, *404*, 126–142. http://doi.org/10.1016/j.jtbi.2016.05.029

Särkinen, T., Staats, M., Richardson, J. E., Cowan, R. S., & Bakker, F. T. (2012). How to Open the Treasure Chest? Optimising DNA Extraction from Herbarium Specimens. *PLoS ONE*, *7*(8), 1–9. http://doi.org/10.1371/journal.pone.0043808

Scotland, R. W., Olmstead, R. G., & Bennett, J. R. (2003) Phylogeny reconstruction: the role of morphology. *Systematic Biology*, *52*(4), 539–548. http://doi.org/10.1080/10635150390223613

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies, *Bioinformatics*, Vol 30, 9: 1312–1313,

Standley, P. C. & Steyermark, J. A. (1952) Urera. In: *Flora of Guatemala. Fieldiana, Bot.* 3: 424–428.

Steinmann, V. W. (2005) Four new neotropical species and a new combination of Urera (Urticaceae). *Acta Bot. Mex*. 71: 19–43.

Stevens, P. F. (2002). Why Do We Name Organisms ? Some Reminders from the past Author(s) *Taxon* 51: 11-26

Swofford, D. L. (1998) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5), 1105–1109. http://doi.org/10.1007/BF00037152

Wagner, W. L., Herbst, D. R., & Sohmer, S.H. (1990) *Manual of the flowering plants of Hawaiʻi.* 2 University of Hawaii Press & Bishop Museum Press, Honolulu.

Weddell, H.A., (1852) Addition a la flore de Amerique du Sud. *Annales des Sciences Naturelles; Botanique, sér.* 3, 18: 193-232

Weddell, H.A., (1854) Revue de La Famille des Urticées. *Annales des Sciences Naturelles; Botanique,* sér. 4,1: 173-212

Weddell, H. A. (1856) *Monographie de la Familles des Urticées, Tome IX*, 592. Paris, Museum d'Histoire Naturelle

Weddell, H.A., (1869) Urticaceae. In: De Candolle *Prodromus systematis naturalis regni begetabilis, Vol. 16,1*: 32–235/64. Masson, Paris.

Wu, Z. Y., Monro, A. K., Milne, R. I., Wang, H., Yi, T. S., Liu, J., & Li, D. Z. (2013). Molecular phylogeny of the nettle family (Urticaceae) inferred from multiple loci of three genomes and extensive generic sampling. *Molecular Phylogenetics and Evolution*, *69*(3), 814–827. http://doi.org/10.1016/j.ympev.2013.06.022

Appendix 1. PCR success

Species	Collector	Number	Country	Herbarium	ITS	Ndhf	trnL-F
L. aestuans	P. M. Peterson	7165	Panama, Bocas del Toro	US	KM586464		KM586636
L. aestuans	Albertina & Molina	31958	Honduras, Rio Lindo	US	KM586465		KM586637
L. interrupta	Festo & Luke	2502	Kenya	EA	KM586445		KM586617
L. interrupta	Festo	2218	Tanzania	EA	KM586446		KM586618
L. ruderalis	Hunt	6	Micronesia	US	KM586461		KM586633
L. ruderalis	Steve&Wood	14300	Guam	US	KM586462		KM586634
O. aldabrensis	Renvoize	1357	Seychelles	US	KM586460		KM586632
O. pinnatifida	Greenway	11371	Tanzania	EA	KM586449		KM586621
O. radula	Deng	700	Kenya	KUN	KM586431		KM586603
O. radula	Ezrom et al.	286	Tanznia	EA	KM586451		KM586623
O. tenax	Botha	9	S Africa	K	KF137886		KF138367
O. tenax		17316		K			
P. cf. scabrinerium	Wilkie	94166		E	✓		
P. cf. suaveolens	Puan Ching	51390		E			
P. cordifolium	Sinclair & Kadim	10358		E	✓		
P. lanceolatum	WuZY-09235		CHINA, YUNNAN	KUN	KF137912		KF138396
P. lanceolatum				E	✓		
P. sp.	Sun	13176	Laos	KUN	KM586453		KM586625
P. sp.	Sun	12578	Laos	KUN	KM586454		KM586626
P. sp.	Sun	12766	Laos	KUN	KM586455		KM586627
P. suaveolens	GBOWS736		CHINA, YUNNAN	KUN	KF137913		KF138397
P. suaveolens	WuZY-09160		CHINA, YUNNAN	KUN	KF137914		KF138398
P. suaveolens	Sun	13870	Yunnan	KUN	KM586456		KM586628
P. suaveolens	Deng	411	Yunnan	KUN	KC284964		KC285016
U. pacifica	Steinman	3265	Mexico	BM	✓	✓	✓
T. glabra		100694	Hawaii	KUN	KF1379930		KF138416
T. latifolia		8716			✓		
T. latifolia	Jffrey201101		Hawaii	KUN	KF137927		KF138412
U. altissima	A. Lliully et al.	460	Bolivia, Chuquisaca	K	✓		✓
U. aurantiaca	E. Zardini & T. Tilleria	35423	Paraguay, Central	K	✓	✓	✓
U. aurantiaca	E. Zardini et al.	9158	Paraguay, Paraguari	K			
U. aurantiaca/caracasana?	I. Loza et al.	63	Bolivia, La Paz	K	✓	✓	✓
U. baccifera	Sandoval	1819	El Salvador	MO	✓	✓	✓
U. baccifera	Cayola	2530	Bolivia	BM	✓		✓
U. baccifera	S. Beck & K. Bach	23206	Bolivia, La Paz	K		✓	
U. baccifera	B. A. Krukoff	10061	Bolivia, La Paz	K	✓	✓	✓
U. baccifera	D. C. Daly et al.	11980	Brazil, Acre	K			
U. baccifera	Berg et al.	FUEL 1301-98	Brazil, Londrina	K			
U. baccifera	D. Zappi et al.	2107	Brazil, Minos Gerais	K	✓		✓
U. baccifera	Taylor	187	Costa Rica	MO	✓	✓	✓
U. baccifera	M. Rios et al.	75	Ecuador, Pichincha	K	✓	✓	✓
U. baccifera	Howard	18799	Grenada	BM			
U. baccifera	Croat	12430	Panama	MO	✓		
U. baccifera	R. Vasquez et al.	28202	Peru, Pasco	K			
U. baccifera	T. D. Pennington & A. Daza	17239	Peru, San Martin	K	✓	✓	✓
U. baccifera	J.H. Kirkbride,	3930	Brazil, Salinas	US	KM586469.1		KM586641
U. baccifera	R. Wasum,	385	Brazil, Rio Grande do Sul	US	KM586468		KM586640
U. baccifera	Monro	4663	Panama, bocas del toro	BM	KF137928		KF138414
U. cameroonensis	Leeuenberg	7187		K	✓		
U. caracasana	Whitefoord	4546	Dominica	BM			
U. caracasana		3748	Mexico, Jalisco	US	KM586467.1		KM586639.1
U. caracasana	Wood, J. R. I.	8834	Bolivia, Chuquisaa	K	KF137929.1		KF138415
U. caracasana	Monro	4346	Panama, BOCAS DEL TORO	BM	KF138413		KF138248
U. elata	Adams	11496	Jamaica	BM			
U. elata	Fosberg	42858	Jamaica	BM			
U. elata	B. Hansen et al.,	1694	Mexico, Chiapas	US	KM586471		KM586643
U. fenestrata	Monro	5452	Costa Rica	K	✓	✓	✓
U. fenestrata	Hampshire	214	Panama	BM	✓	✓	✓
U. fischeri	Faden	70/942		K			
U. fischeri	Faden & Beenlje	85/22	Kenya	EA	KM586427		KM586599
U. fischeri	Brenan et al.	14648	Kenya	EA	KM586443		KM586615
U. glabriuscula	Rosas	1383	Mexico	BM			
U. glabriuscula	Calonico	21101	Mexico	BM	✓		
U. guanacastensis	Rivera	1152	Costa Rica	BM			
U. hypselodendron	Friis & Lawson	5404		K			
U. hypselodendron		8462		K	✓		
U. hypselodendron	Friis et al.	4125	Ethiopia	C			KF138417
U. hypselodendron	beenlije	3257	Kenya	EA	KM586430		KM586602
U. hypselodendron	Greenway	12404	Tanzania	EA	KM586439		KM586611
U. hypselodendron	Musila et al.	95	Kenya	EA	KM586450		KM586622
U. killipiana	Serviu	372	Mexico	BM	✓	✓	✓
U. laciniata	Araujo et al.	3016	Bolivia	BM	✓	✓	✓
U. laciniata	Monteagudo et al.	3905	Peru	BM	✓	✓	
U. laciniata	Macia et al.	4326	Bolivia	BM	✓		
U. laciniata	Miller	655	Ecuador	BM			
U. laciniata	Huaman et al.	221	Peru	BM	✓	✓	✓
U. laciniata	Ticona et al.	149	Bolivia	BM			
U. laciniata	Bang	1247	Bolivia	BM			
U. laciniata	Acosta	1907	Costa Rica	MO			
U. laciniata	de Nevers	6488	Panama	MO			
U. laciniata	Campos	3932	Peru	BM		✓	
U. laciniata	A.K. Monro	3993		BM			DQ179367.1
U. lianiformis	Nelson	2926A	Honduras	BM			
U. lianiformis	Solano	6825	Costa Rica?	BM	KF138570.1		KF138418.1
U. lianiformis?	M. Timana	1192	Peru	BM	✓		✓
U. sansibarica	Luke	11527	Tanzania	EA	KM586428		KM586600
U. sansibarica	MDE	97	Kenya	EA	KM586444		KM586616
U. simplex	Rueda	1098	Ecuador	BM			
U. simplex	Aulestin	413	Ecuador	BM	✓	✓	✓
U. simplex	Monro	5102	Panama	BM			
U. simplex	W.H. Lewis et al.	2224	Panama, Los Santos	US	KM586470		KM586642
U. trinervis	Harris	8422	Gabon	K	✓		-
U. trinervis	Friis et al.	3920	Ethiopia	C	KF137932		KF138421
U. trinervis	Luke	12513	Maniema	EA	KM586440		KM586612
U. verrucosa	Rosas	576	Mexico	BM			
U. verrucosa	Khan et al.	959	Costa Rica	BM			
Percentage Successfully Sequenced					53%	31%	35%

Appendix 2. Morphology by species

taxa	hydrology	distribution	stem support	wood in branchlets	adentitious roots	bulbed hairs	bulbed spines (>3mm long)	lamina outline	lamina venation	lamina margin	stipule position	stipule fusion	stipule coverage	Inflorescence development	Inflorescence symmetry	Inflorescence branches	stigma form	stigma hair arrangement	female tepal fusion	tepal extent in fruit	tepal fleshy in fruit	achene size	achene apex	achene apex disposition
T. latifolia		Hawaii	self-supporting	wood present	absent	absent	absent	simple	basally tri-nerved	toothed	intrapetiolar	fused	>50%	determinate	symmetrical	not visible	elongated	asymmetrical	partially fused, 4-lobed	enlarged	fleshy	<2mm	extended	reflexed
U. glabra		Hawaii	self-supporting	wood present	absent	absent	absent	simple	basally tri-nerved	toothed	intrapetiolar	fused	>50%	determinate	symmetrical	visible	elongated	asymmetrical	partially fused, 4-lobed	enlarged	fleshy	<2mm	extended	reflexed
U. laciniata		Neotropics	self-supporting	wood absent	absent	present	present	lobed	basally tri-nerved	toothed	intrapetiolar	partially fused /fused	<50%	indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	not enlarged	dry	>2mm	extended	reflexed
U. baccifera		Neotropics	self-supporting /intermediate	wood absent	absent	present	present	simple	basally tri-nerved	toothed	intrapetiolar	partially fused /fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	>2mm	extended	reflexed
U. altissima		Paraguay & S. Brasil	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. aurantiaca		Bolivia, Paraguay & S. Brasil	self-supporting	wood present	absent	?	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. caracasana		Neotropics	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. elata		Carribbean & Mexico	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. fenestrata		Costa Rica, Panama	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused /fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. glabriuscula		Mexico & Guatemala	self-supporting	wood present	absent	absent	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. guanacastensis		Costa Rica	self-supporting	wood present	absent	absent	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused /fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. killipiana		Mexico & Guatemala	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused /fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. llaniformis		Mexico, Central America, Peru & Bolivia	intermediate	wood present	absent	?	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. pacifica		Mexico	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	not enlarged	dry	>2mm	extended	reflexed
U. simplex		Neotropics	self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
U. verrucosa		Mexico & Central America	self-supporting	wood present	absent	absent	absent	simple	basally tri-nerved	toothed	intrapetiolar	fused	<50%	determinate	symmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	<2mm	blunt	
P. scabinerium		Borneo	not self-supporting	wood present	present	absent	absent	simple	pinnate	entire	intrapetiolar	fused	>50%	determinate	symmetrical	not visible	elongated	symmetrical	fused at base, 4-lobed	enlarged	fleshy	>2mm	extended	straight
P. suaveolens		India, Mainland S. E. Asia, Malaysia	not self-supporting	wood present	present	absent	absent	simple	pinnate	entire	intrapetiolar	fused	>50%	determinate	symmetrical	not visible	elongated	symmetrical	fused at base, 4-lobed	enlarged	fleshy	>2mm	extended	straight
P. cordifolium		Western Malaysia	not self-supporting	wood present	present	absent	absent	simple	pinnate	entire	intrapetiolar	fused	>50%	determinate	symmetrical	not visible	elongated	symmetrical	fused at base, 4-lobed	enlarged	fleshy	>2mm	extended	straight
P. lanceolatum		India, Myanmar & China	not self-supporting	wood present	present	absent	absent	simple	pinnate	entire	intrapetiolar	fused	>50%	determinate	symmetrical	not visible	elongated	symmetrical	fused at base, 4-lobed	enlarged	fleshy	>2mm	extended	straight
O. aldabrensis		Aldabra	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	toothed	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
O. ficifolia		Mascarene Islands	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	toothed	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
O. madagascariensis		Madagascar	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	toothed	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
O. radula		Madagascar & East Africa	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	toothed	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
O. carruthersiana		Angola & Namibia	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	entire	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
O. tenax		South Africa, Botswana, Namibia & Zimbabwe	self-supporting	wood pithy	absent	present	absent	lobed	basally tri-nerved	toothed	interpetiolar	free		indeterminate	asymmetrical	visible	elongated	asymmetrical	free to base	enlarged	dry	>2mm	extended	reflexed
U. batesii			not self-supporting	wood present	present	present	absent	simple	basally tri-nerved	entire	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	free to base	enlarged	fleshy	>2mm	extended	straight
U. fischeri			not self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight
U. sansibarica		Kenya & Tanzania	not self-supporting	wood present	present	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight
U. cameroonensis		Tropical Africa	not self-supporting	wood present	absent	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight
U. trinervis		Tropical Africa	not self-supporting	wood present	present	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight
U. hypselodendron		Central & East Africa	not self-supporting	wood present	present	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight
U. manii		Tropical Africa	not self-supporting	wood present	present	present	absent	simple	basally tri-nerved	toothed	intrapetiolar	partially fused	<50%	indeterminate	asymmetrical	visible	sessile	symmetrical	completely fused, rim 4-dentate	enlarged	fleshy	>2mm	extended	straight

Appendix 3. Material used in SEM analysis

- 77 -

Species	Collector	Number	Herbarium
U. laciniata	Monteagudo et al.	3905	BM
U. laciniata	Bang	1247	BM
U. baccifera	Cayola	2530	BM
U. baccifera	Croat	12430	MO
U. baccifera	T. D. Pennington & A. Daza	17239	K
U. baccifera	Herrera	9995	BM
U. aurantiaca	E. Zardini & T. Tilleria	35423	K
U. caracasana	Whitefoord	4546	BM
U. lianiformis	M. Timana	1192	BM
U. pacifica	Steinman	3265	BM
U. simplex	Aulestin	413	BM
P. cordifolium	Sinclair & Kadim	10358	E
P. cf. suaveolens	Puan Ching	51390	E
O. tenax		17316	K
U. cameroonensis	Leeuenberg	7187	K
U. trinervis	Harris	8422	K
U. hypselodendron	Reekmans	8462	K
T. latifolia	Degener	8716	K

- 77 -