Molecular Phylogenetics and Evolution 158 (2021) 107008

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Combination of Sanger and target-enrichment markers supports revised generic delimitation in the problematic 'Urera clade' of the nettle family (Urticaceae)☆

Tom Wells [a,b,*], Olivier Maurin [c], Steven Dodsworth [c,d], Ib Friis [e], Robyn Cowan [c], Niroshini Epitawalage [c], Grace Brewer [c], Felix Forest [c], William J. Baker [c], Alexandre K. Monro [f]

[a] The Royal Botanic Garden Edinburgh, EH3 5NZ, United Kingdom
[b] Oxford University, Department of Plant Sciences, OX1 3RB, United Kingdom
[c] The Royal Botanic Gardens, Kew, Plant and Fungal Tree of Life (PAFTOL) Team, TW9 3AE, United Kingdom
[d] School of Life Sciences, University of Bedfordshire, Luton LU1 3JU, United Kingdom
[e] Natural History Museum of Denmark, Universitetparken 15, DK-2100 Copenhagen Ø, Demark
[f] The Royal Botanic Gardens, Kew, Identification & Naming Department, TW9 3AE, United Kingdom

ARTICLE INFO

Keywords:
Angiosperms353
Generic delimitation
Lianas
Secondary woodiness

ABSTRACT

Urera Gaudich, *s.l.* is a pantropical genus comprising c. 35 species of trees, shrubs, and vines. It has a long history of taxonomic uncertainty, and is repeatedly recovered as polyphyletic within a poorly resolved complex of genera in the Urticeae tribe of the nettle family (Urticaceae). To provide generic delimitations concordant with evolutionary history, we use increased taxonomic and genomic sampling to investigate phylogenetic relationships among *Urera* and associated genera. A cost-effective two-tier genome-sampling approach provides good phylogenetic resolution by using (i) a taxon-dense sample of Sanger sequence data from two barcoding regions to recover clades of putative generic rank, and (ii) a genome-dense sample of target-enrichment data for a subset of representative species from each well-supported clade to resolve relationships among them. The results confirm the polyphyly of *Urera s.l.* with respect to the morphologically distinct genera *Obetia*, *Poikilospermum* and *Touchardia*. Afrotropic members of *Urera s.l.* are recovered in a clade sister to the xerophytic African shrubs *Obetia*; and Hawaiian ones with *Touchardia*, also from Hawaii. Combined with distinctive morphological differences between Neotropical and African members of *Urera s.l.*, these results lead us to resurrect the previously synonymised name *Scepocarpus* Wedd. for the latter. The new species epiphet *Touchardia oahuensis* T.Wells & A.K. Monro is offered as a replacement name for *Touchardia glabra* non H.St.John, and subgenera are created within *Urera s.s.* to account for the two morphologically distinct Neotropical clades. This new classification minimises taxonomic and nomenclatural disruption, while more accurately reflecting evolutionary relationships within the group.

1. Introduction

The nettle family Urticaceae comprises 53 genera grouped into four tribes and encompassing c. 2150 species of herb, shrub, tree and vine distributed throughout the tropical and temperate regions of the world. Of the four tribes, the Urticeae contains c. 10 genera (Friis, 1989a), among which both Wu et al. (2013) and Kim et al. (2015) recovered extensive polyphyly focussed around the genus *Urera* Gaudich..

Urera was first described by Gaudichaud-Beaupré (1830) to account for several specimens of *Urtica*-like plants from the Neotropics and South East Asia that bore fruits whose perianths become fleshy upon ripening, and for which *Urera baccifera* (L.) Gaudich. ex Wedd. was later selected as the genus type (Britton and Wilson, 1924). *Urera* is represented by 148 published epithets (The Plant List, 2019), of which c. 35 represent morphologically distinct species, including shrubs, trees or lianas, growing in tropical Africa, Madagascar, the Neotropics and Hawaii

☆ Main Funding provided by The Royal Botanic Garden Edinburgh, as part of MSc programme, with High-Throughput Sequencing undertaken as part of the Plant and Fungal Tree of Life project funded by the Calleva Foundation and the Sackler Trust.
* Corresponding author.
E-mail address: tom.wells@plants.ox.ac.uk (T. Wells).

https://doi.org/10.1016/j.ympev.2020.107008
Received 9 October 2019; Received in revised form 20 October 2020; Accepted 29 October 2020
Available online 5 November 2020

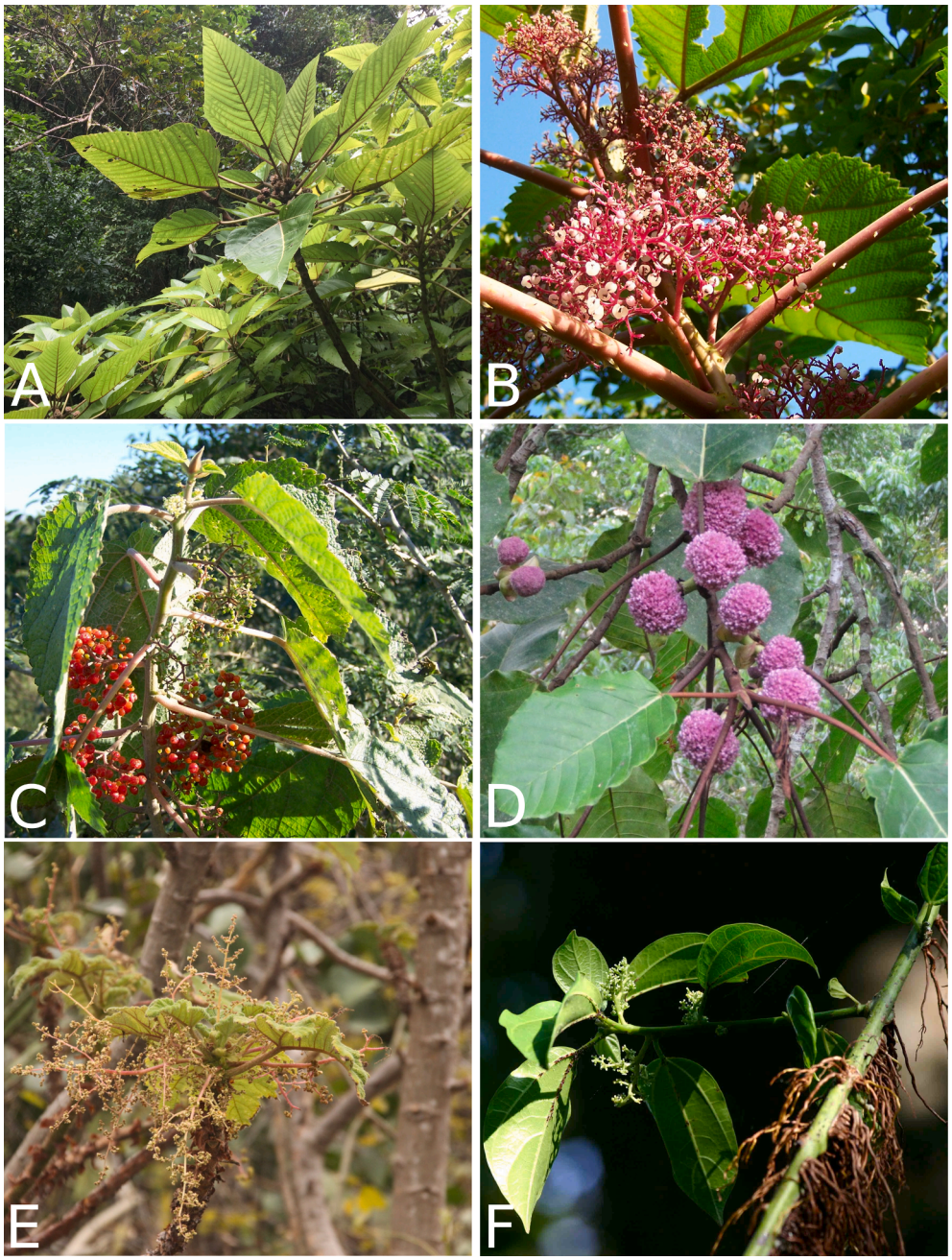

Fig. 1. Examples of the taxa in the "Urera clade" based on the analyses of Wu et al. (2013) & Kim et al. (2015), showing differences in lifeform, leaf venation and margin, inflorescence branching patterns, and fruit fleshiness. A: *Touchardia latifolia* (Photo by Jason Cantley); B: *Urera baccifera* (Photo by Ulf Dreschel); C: *Urera aurantiaca* (Photo by Ulf Dreschel); D: *Poikilospermum sp*. (Photo by Dixie Damrel); E: *Obetia sp*. (Photo by Christian Kunath); F: *Urera trinervis* (Photo by Bart Wursten).

(Friis, 1993). It is characterised by female flowers with a 4-parted perianth that becomes fleshy and brightly coloured in fruit, as well as by the presence of bulbous, stinging hairs on the stems, leaves and inflorescences. In addition to being recovered as polyphyletic based on DNA sequence data, *Urera* also possesses surprising morphological diversity within the context of the Urticaceae. Most notably this includes the wide variation in life-form noted above, as well as differing stem appendages (adventitious roots, lignified spines or extended fleshy protuberances), inflorescence symmetry (dichotomous cymes known as dichasia, or irregularly branching indeterminate panicles), and stigma morphology (sessile or stalked on apex of the ovary).

In his monograph of the Urticaceae, Weddell (1856) proposed an informal subgeneric classification for *Urera* based on the presence of a capitate or ligulate stigma (§1 Stigma rotundatum, §2 Stigma lanceolatum) and the symmetry and branching of the inflorescence (A – Cymae manifeste dichotomae, B – Cymae haud dichotomae (paniculiformes)). Later, in what was to be the last comprehensive review of the genus, Weddell (1869) created the monospecific African genus *Scepocarpus* Wedd. to account for fruiting material of a plant collected in Equatorial Guinea by Gustav Mann. Weddell's description of *Scepocarpus* is identical to that for *Urera*, with the exception of the female perianth, which is described as being fused and tubular, as opposed to free for most of its length and four-lobed. Weddell appears to have overlooked, however, the fact that he had provided descriptions of three African species of *Urera* that also had a tubular female perianth: *Urera obovata* Benth., *U. acuminata* (Poir.) Gaudich. ex Decne and *U. cameroonensis* Wedd. Rather than transferring these species to *Scepocarpus*, Bentham (1880) later instead placed *Scepocarpus* in synonymy with *Urera*, and it is as such that they have been treated ever since.

More recent taxonomic work on *Urera* has relied on regional floristic treatments and broad family synopses (Wagner et al., 1999; Friis, 1982, 1985, 1989a, 1993, 2018; Letouzey, 1967, 1968; Monro, 2015; Monro &

Rodríguez, 2009; Steinmann, 2005; Wagner) without an in-depth revision of the genus across its entire range. Friis (1989b) noted Weddell's creation of *Scepocarpus*, and suggested that splitting *Urera* deserved serious consideration, based primarily on the presence of free perianth parts in the female flowers of the Neotropical species, versus the largely fused perianth parts of those from Africa, but concluded that further analysis was required before doing so.

In the phylogenetic analyses of Kim et al. (2015) and Wu et al. (2013), three currently recognised genera were found to be nested within the traditional circumscription of *Urera*: the African xerophytic trees and shrubs, *Obetia* Gaudich. (7 spp.), the Hawaiian tree, *Touchardia* Gaudich. (1 sp.), and the South East Asian hemi-epiphytes and vines, *Poikilospermum* Zippelius ex Miquel (c. 20 spp.) (Fig. 1). The relationships among these four genera, however, remained poorly resolved in these analyses, which used only a limited number of Sanger markers (Kim et al. 2015: *nrITS, rbcL, TrnL-F;* Wu et al. 2013: *nrITS, rbcL, trnL–trnF, rpll4–rps8–infA–rpl36, matK, 18S, matR*). To date, no attempt has been made to resolve them with greater taxon or genome sampling, or to translate phylogenetic hypotheses into taxonomic and nomenclatural actions. Hereon, we refer to the taxa within the monophyletic grouping that contains a polyphyletic *Urera*, as the '*Urera* clade'.

Ensuring that genera are monophyletic provides classifications that better reflect evolutionary history and can provide a framework for answering broader scientific questions, but it is also essential that species can be assigned to these genera easily and unambiguously so as to remove barriers to identification and species discovery. We therefore aimed to improve the phylogenetic resolution among putative genera in the '*Urera* clade', and where strongly supported, to revise the generic delimitation in accordance with morphological and biogeographic data.

2. Materials & methods

We expanded the taxon and genome sampling used for the phylogenies of Wu et al. (2013) and Kim et al. (2015), and further combined the results with a detailed review of the morphology. We applied a two-tier genome sampling approach, where (i) a taxon-dense sample of Sanger sequence data from one nuclear and one chloroplast barcoding region was used to recover monophyletic clades of putative generic rank, and (ii) a genome-dense sample of target-enrichment data for a subsample of representatives from each well-supported clade from the Sanger data was used to resolve relationships among them. Morphological and biogeographic data were then gathered and projected onto this resolved topology, assessed for homology and used to delimit genera. Potential genera needed to be both monophyletic and morphologically diagnosable in the field or herbarium using basic scientific equipment (10x hand lens) and a minimum of cost. Clades that could not be diagnosed morphologically were not, therefore, considered as potential genera.

2.1. Choice of markers for phylogenetic analyses

For the initial taxon-rich analysis we sampled the non-coding internal transcribed spacer of ribosomal DNA (*nrITS*), and plastome (*trnL-F*) regions used by Wu et al. (2013) and Kim et al. (2015). In our second analysis we sequenced 353 low-copy genes from the nuclear genome for representative species of each grouping identified in the analyses of *nrITS* and *trnL-F* sequence data as monophyletic. For the selection of nuclear genes, we used an available set of angiosperm-wide baits (Angiosperms353; Johnson et al., 2018) developed as part of the Plant and Fungal Trees of Life (PAFTOL) initiative at the Royal Botanic Gardens, Kew (Eiserhardt et al., 2018).

2.2. Taxon sampling

2.2.1. Sampling for Sanger sequencing

We used published phylogenies of the Urticaceae (Hadiah et al., 2008; Monro, 2006; Wu et al., 2013) and Urticeae (Kim et al., 2015), and regional taxonomic studies (Chew, 1964; Friis, 1983, 1985; Letouzey, 1968; Monro & Rodríguez, 2009; Wagner et al., 1999) to design our sampling framework. Kim et al. (2015) found strong support for *Laportea* Clade II (*Laportea aestuans* (L.) Chew, *L. interrupta* (L.) Chew, *L. ruderalis* (G.Forst.) Chew) as sister to the '*Urera* clade', and on this basis these taxa were included as our outgroup.

Table 1
Taxon sampling for nrITS & trnL-F sequencing (including Genbank samples). 1: Kim et al. 2015. 2: Monro & Rodríguez, 2009. 3: Chew 1964. 4: Friis, 1989a. 5: Wagner et al. 1999.

Genus	Distribution	No. species sampled	Total species number	% of species	No. of accessions
Urera II & III[1]	Central & South America	13	16[2]	81%	40
Poikilospermum	South East Asia	4	20[3]	20%	11
Urera I[1]	Africa & Madagascar	5	20[4]	25%	14
Touchardia	Hawaii	1	1[5]	100%	1
Obetia	Africa & Madagascar	4	8[4]	50%	5
All genera		27	65	42%	71

Ingroup taxa included those genera whose recognition made *Urera* polyphyletic according to Wu et al. (2013, Clade 3F) and Kim et al. (2015, Clade E). These were *Obetia*, *Poikilospermum*, and *Touchardia*, and each was sampled from across its geographic range (Table 1). This resulted in 71 accessions for a total of 27 species (Table S2). Of these 71 accessions, 34 were sampled for this study by the authors, while 37 others from previous studies (Kim et al., 2015; Wu et al., 2013) were downloaded from GenBank (Benson et al., 2005). Material sampled for this study was obtained from herbarium specimens at BM, E and K for which we were confident of the determinations. We verified their determinations by comparing against specimens cited in the original species description (protologue), and using type collections and images on Global Plants (https://plants.jstor.org/, accessed 2017).

2.2.2. Sampling for targeted sequencing

Using the phylogenies generated from our Sanger sequencing data, we selected a subset of representative taxa for each strongly supported clade (Table S3). We selected *Laportea cuspidata* and *L. canadensis* as outgroups, representatives of Clades *Laportea I* and *II* (Kim et al., 2015), and *Pilea cadierii* as representative of the sister tribe to the Urticeae, the Elatostemeae (Kim et al., 2015). A member of the two subgenera of *Poikilospermum* were included in the targeted sequencing analysis as they were distinctive in morphology and distribution (Chew, 1964), which could indicate a non-monophyletic grouping.

2.3. Extraction, amplification and analysis of DNA sequence data

2.3.1. Sanger DNA extraction, amplification & sequencing

DNA was isolated from fragments of herbarium specimens using a modified CTAB protocol (Doyle & Doyle 1987), and further purified using an E.Z.N.A.® HP Plant DNA Mini Kit (Omega Bio-tek Inc., Norcoss, GA.). This combination of CTAB + silica binding has been shown to generate high yields and purity of DNA and the highest rates of PCR success when working with herbarium specimens (Särkinen, et al., 2012). The *nrITS* region was amplified using primers ITS 4 (5′ – TCCTCCGCTTATTGATATGC) and ITS 5 (5′ – GGAAGTAAAAGTCGTAACAAGG) (White et al., 1990). The *trnL-F* region using primers e (5′ - GGTTCAAGTCCCTCTATCCC) and f (5′ - ATI'TGAACTGGTGACACGAG) (Taberlet et al., 1991). Genomic DNA for each sample was amplified using 2.5 μl dNTPs, 2.5 μl 10X Buffer, 1.25 μl $MgCl_2$, 0.75 μl of each primer, 0.2 μl Polymerase Taq, and 2 μl of template with 15 μl H_2O, and the following PCR cycling conditions: an initial 30 s at 94 °C, followed

by 34 cycles of 5 s at 94 °C, 10 s at 55 °C, and 40 s at 72 °C, and finally 2 min at 72 °C. The results of the PCR were assessed on a 1% agarose gel with ethidium bromide, and samples with clear, single bands of correct length were sequenced in both directions at Edinburgh Genomics.

2.3.2. Target-enrichment DNA extraction & library preparation

Targeted sequencing data was generated as part of the Plant and Fungal Trees of Life (PAFTOL) project at RBG Kew. DNA extractions were performed using a modified CTAB protocol (Doyle and Doyle, 1987) and purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). Purified DNA extracts were run on a 1.5% agarose gel to assess the average fragment size. Samples with very low concentration (not visible on a 1.5% agarose gel), were assessed on a 4200 TapeStation System using Genomic DNA ScreenTapes (Agilent Technologies, Santa Clara, CA, USA). The quality of the DNA was evaluated based on agarose gel and TapeStation images, and were then quantified using a Qubit® 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). DNA samples with the average fragment sizes above 350 bp were sonicated using a Covaris M220 Focused-ultrasonicator with Covaris microTUBES AFA Fiber Pre-Slit Snap-Cap (Covaris, Woburn, MA, USA) with varied shearing times depending on the DNA fragment size profile, to obtain an average of 350-bp insert sizes. Dual-indexed libraries for Illumina® sequencing were prepared using the DNA NEBNext® Ultra II Library Prep Kit at half the recommended volume, with Dual Index Primers Set 1, NEBNext® Multiplex Oligos for Illumina® (New England BioLabs, Ipswich, MA, USA). Quality of libraries were evaluated on an Agilent Technologies 4200 TapeStation System using High Sensitivity D1000 ScreenTape (Agilent Technologies, Santa Clara, CA, USA). Libraries were subsequently quantified using a Qubit® 3.0 Fluorometer. Equimolar (10 nM) libraries were pooled and a total of 1 μg DNA in each pool was enriched using the target capture kit Angiosperms-353 v1, Catalog #308196; (Johnson et al., 2018) following the manufacturer's protocol v4 (4.0; http://www.arborbiosci.com/mybaits-manual). Hybridizations were performed at 65 °C for 28–32 hrs in a Hybex™ Microsample Incubator and using red Chill-out™ Liquid Wax (Bio-Rad, Hercules, CA, USA) to prevent evaporation. Enriched products were amplified from the bait-bound templates, with KAPA HiFi 2X HotStart ReadyMix PCR Kit (Roche, Basel, Switzerland) for 10 cycles. PCR products were then cleaned using the QIAquick PCR purification kit (Qiagen). Products were quantified with a Qubit™ 3.0 Fluorometer and in some cases reamplified (and repurified) a second time between 3 and 8 cycles due to low DNA concentrations. Final products were run on an Agilent Technologies 4200 TapeStation System using High Sensitivity D1000 ScreenTape to assess quality and average fragment size. Two pools of 25 hybridised libraries each, were multiplexed together and sequenced on an Illumina MiSeq with v2 (300-cycles, 150 bp paired-end reads) and v3 (600-cycles, 300 bp paired-end reads) chemistry (Illumina, San Diego, CA, USA) at the Royal Botanic Gardens, Kew.

2.3.3. Sanger sequence alignment and analysis

Forward and reverse sequences were assembled in Geneious v. 9 using the deNovo assemble function and then checked manually. Assembled sequences were aligned using Mafft v.7 (Katoh and Standley, 2013) and the resulting *nrITS*, *trnL-F* and concatenated matrices were each analysed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI).

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 10,000 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 algorithms.

ML analyses were run in RAxML v.8 (Stamatakis, 2014) with random starting trees (-d), a random seed value for parsimony inferences of 12,345 (-p 12345), and rapid bootstrapping and ML tree scoring in a single run (-f a), and using the default setting of the General-Time-Reversible model with Gamma distribution (-m GTRGAMMA). One thousand (-# 1000) non-parametric bootstrap replicates (-b 12345) were applied using a random seed value for parsimony inferences of 12345 (-p 12345) and the General Time Reversible CAT model (-m GTRCAT).

BI analyses were run following a model test to select a suitable model of sequence evolution for the data using the Akaike Information Criteria in jModelTest 2 (Darriba et al., 2015) (See TableS2). The *nrITS* and *trnL-F* datasets were assessed as partitions 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 5,000,000 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 burn-in, with posterior probabilities constructed from the remaining trees.

2.3.4. Computational data processing and phylogenomic analyses of the targeted sequencing data

Raw reads of the sequencing output (.fastq files) were trimmed using Trimmomatic (Bolger et al., 2014) to remove reads with a quality score below 30 and reads that had any 4-bp window below 30, retaining reads with at least 36 bp (LEADING:30 TRAILING:30 SLIDING WINDOW:4:30 MINLEN:36). Paired reads were combined with unpaired reads (singlets left after quality filtering) and used to recover target sequences using HybPiper version 1.3 (Johnson et al., 2016) using a target file available at https://github.com/mossmatters/Angiosperms353. Reads were mapped to de-gapped medoid sequences using BLASTX (Camacho et al., 2009). Each gene was *de novo* assembled using SPAdes (Bankevich et al., 2012). Coding sequences were extracted using Exonerate (Slater and Birney, 2005). Non-coding sequences (i.e., introns and UTRs) flanking the coding sequences were recovered using the script intronerate.py available with HybPiper. Gene matrices were aligned separately using MAFFT V7 (mafft-7.419-gcc_fc6.x86) using the slower but more accurate settings "–localpair –maxiterate 1000" with the option to generate reverse complement sequences to align them together with the remaining sequences based on 6mer counting (–adjustdirectionaccurately). Matrices were subsequently trimmed, using phyutility (Smith and Dunn, 2008) to delete sites that are missing at least 80% data (-clean 0.8). Gene trees from trimmed matrices were generated using RAxML (Stamatakis, 2014) and a species tree statistically consistent with coalescence was estimated using ASTRAL-II (Mirarab and Warnow, 2015). Extensive branch annotations were generated using ASTRAL-II and the function Full Annotation (-t 2), which provides different measurements for branch annotations such as Quartet support, Alternative posteriors, and Alternative quartet topologies. Multilocus bootstrapping (MLBS) was also run separately for the Astral species tree.

2.4. Selection & assessment of morphological characters

Morphological characters of potential use for delimiting genera were selected through a review of Weddell's (1856) descriptions in his monograph, as well as species protologues and regional Flora treatments (e.g. Chew, 1964; Friis, 1982, 1983, 1985, 1989b; Letouzey, 1967, 1968; Monro & Rodríguez, 2009; Steinmann, 2017; Wagner et al., 1999). Observations from herbarium specimens at BM, E, and K were used to verify the utility of these characters, and to look for others not previously identified in the literature. Once identified, characters were partitioned into states based on the variation displayed across the taxa and assessed for homology following Hawkins et al. (1997). These character states were then compared for congruence with the topology of the targeted sequencing species tree, with the aim of assessing the

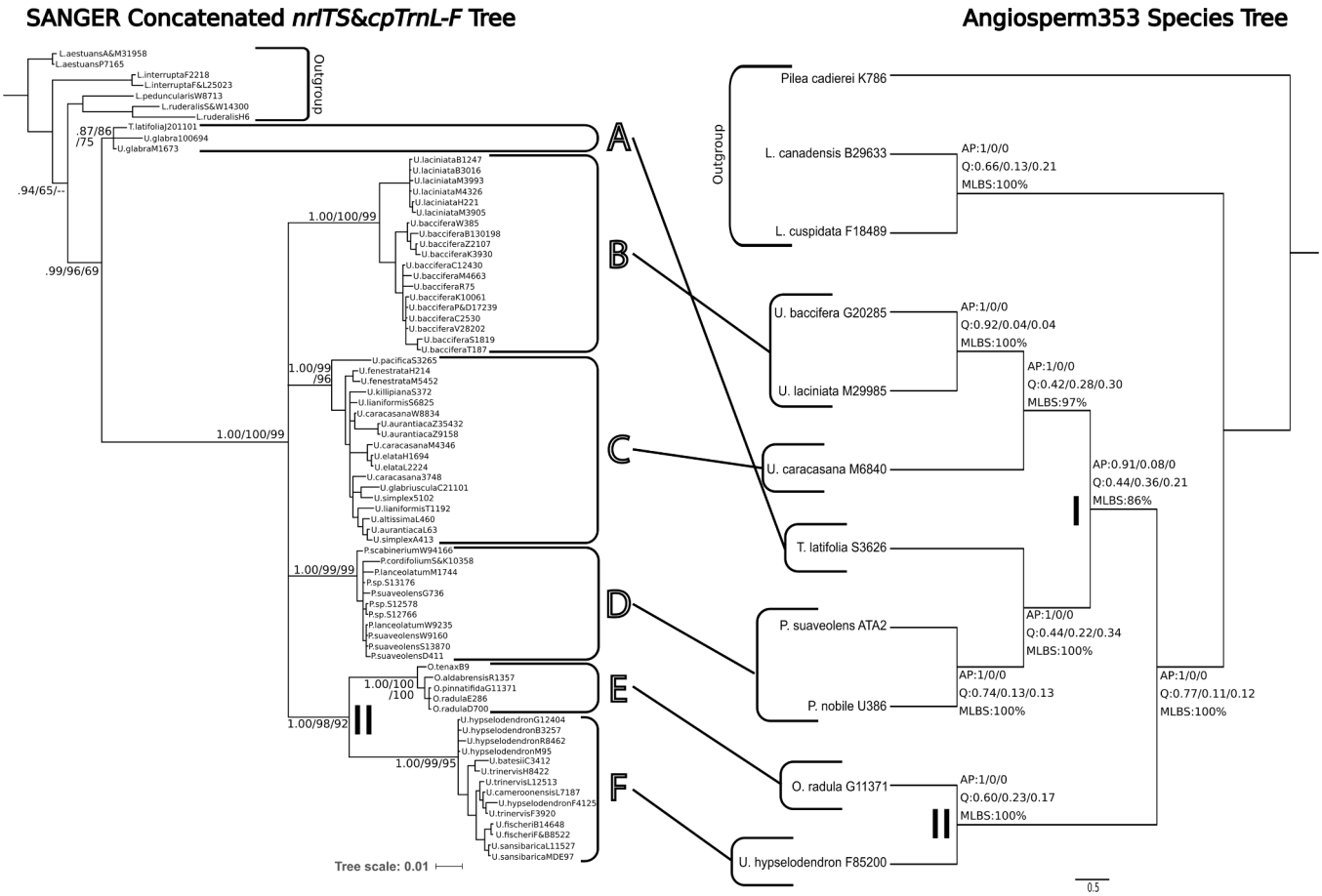

Fig. 2. Comparison of phylogenies generated from Sanger and targeted sequencing data, contrasting lack of resolution among well-supported clades in Sanger data analyses, and a well-supported and resolved topology for the targeted sequencing data. Left: Sanger data analysed using Bayesian (BI), Maximum Likelihood (ML) and Parsimony (MP) methods - support values: BI(Posterior Probabilities)/ML(Bootstrap)/MP(Bootstrap) plotted on the Bayesian majority rule consensus tree. Right: Species Tree generated in ASTRAL-II. Support values at nodes: Alternative Posteriors (AP); Quartet Scores (Q); and Multi-locus Bootstrapping (MLBS).

diagnosability of each recovered clade.

2.5. Biogeography

Biogeography has consistently been shown to be an important factor in diversification within Urticaceae (e.g. Wu et al., 2013), and for this reason we included geographic distribution as a character in our analysis. We used Ecozones as defined by Olson & Dinerstein (2002) and gathered distribution data from specimen labels, floristic treatments and online data aggregators (GBIF.org, accessed 16th May 2019). Distributions were then compared to the targeted sequencing species tree.

3. Results

3.1. Phylogenetic analyses of Sanger sequence data

3.1.1. Analyses of nrITS DNA sequence data

Sequence data for the *nrITS* region was assembled and aligned for 72 accessions (29 new to this study; 66 ingroup, 6 outgroup), and the resulting alignment comprised 618 base pairs, 274 of which were variable, 239 of which were parsimony informative (Table S1). When analysed with MP, ML or BI, eight strongly supported ingroup nodes were recovered. All analyses of *nrITS* data recovered a strongly supported monophyletic ingroup, with the Hawaiian taxa recovered as a moderately supported clade (A) (Fig. S1; MP:83%, ML:89%, BI:0.89) sister to the rest of ingroup taxa clades (Fig. S1: B, C, D, E, F: MP:>97%, ML:>98%, BI:1.00). Relationships among the six ingroup clades however, were largely poorly supported (Fig. S1). Nonetheless, all our analyses of *nrITS* data were consistent in recovering the two Afrotropical clades, E and F, as sister to each other with strong support (MP:99%; ML:99%; BI:1.00).

3.1.2. Analyses of trnL-F DNA sequence data

Sequence data for the *trnL-F* region was assembled and aligned for 56 accessions (19 new to this study; 46 ingroup, 10 outgroup), and the resulting alignment comprised 1034 base pairs, 104 of which were variable, 80 of which were parsimony informative. When analysed using MP, ML or BI, six strongly supported ingroup nodes were recovered (Fig. S1). All analyses of *trnL-F* data recovered the majority of the ingroup taxa as a strongly supported clade comprising a polytomy of five moderately to strongly supported clades (Fig. S1; MP:>83%, ML:>85%, BI:1.00). Relationships among these clades were again poorly supported. The Hawaiian taxa *Urera glabra* Wedd. and *Touchardia latifolia* Gaud. (clade A in *nrITS* tree), however, were recovered in an unresolved polytomy with the outgroup taxa (Fig. S1).

3.1.3. Conflict between data sets, and analyses of combined nrITS-trnL-F data

Our analyses of *nrITS* and *trnL-F* agreed with respect to the monophyly of clades B to F (Fig. S1), but differed with respect to the resolution of Clade A (Fig. S1). The recovery of Clade A as monophyletic and sister to rest of the ingroup taxa based on analyses of the *nrITS* data was strongly supported (MP:98%; ML:100%; BI:0.99), whilst its recovery as a part of a paraphyletic grade in analyses of *trnL-F* data was weakly

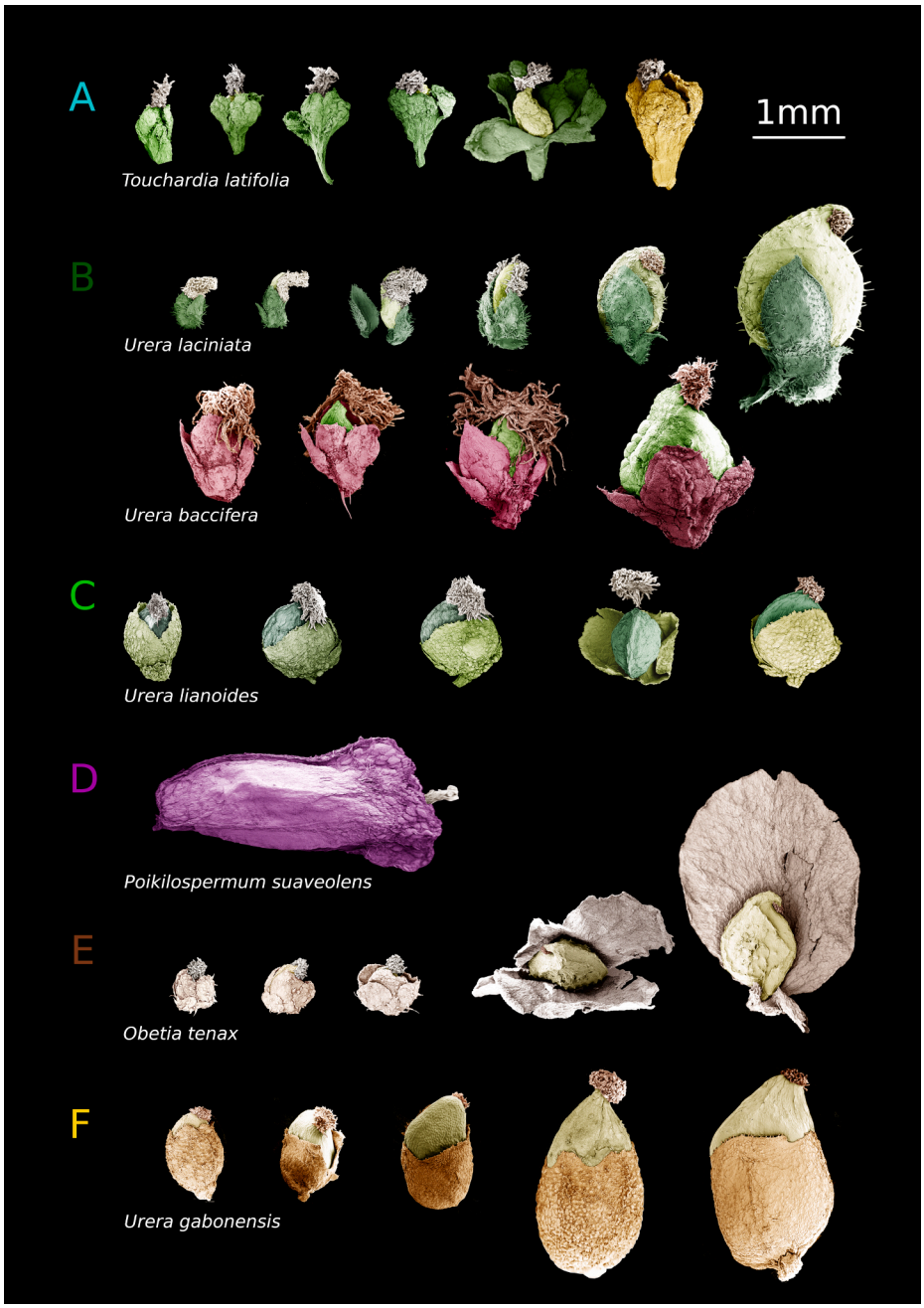

Fig. 3. SEM images of flowers at various stages of development in representative taxa for each clade within the '*Urera* clade'. Flowers rehydrated and then desiccated from herbarium specimens before imaging. Colours added in Adobe Photoshop. Specimens: *T. latifolia* – Degener, 8716, Hawaii [K]; U. *laciniata* - Monteagudo et al. 3905 [BM]; *U. baccifera* - Pennington & Daza 17239, Peru [K]; *U. lianoides* – Timana 1192, Peru [BM]; *P. suaveolens* – Yii 51390, Malaysia [E]; *O. tenax* - s.n. 17,316 [K]; *U. gabonensis* – Harris 8422, Gabon [E].

supported (MP:28%; ML:45%; BI:0.74). Since none of the conflicts between topologies were strongly supported (Fig. S1), we therefore concatenated these two matrices for a combined analysis.

3.1.4. *Concatenated nrITS and trnL-F analyses*

The concatenated alignment comprised sequence data for 80 accessions (73 ingroup, 7 outgroup). The alignment contained 1652 base pairs, 378 of which varied, 319 of which were parsimony informative. The proportion of gaps and undetermined characters in the alignment was 42.58%. When analysed using MP, ML or BI, eight strongly supported ingroup nodes were recovered. The combined analyses of the *trnL-F* and *nrITS* data recovered all of the ingroup taxa within six clades that together formed a clade with moderate to high support (Fig. 2; MP:>75%, ML:>86%; BI:>0.87). As in the *nrITS* analysis, Clade A, comprising the Hawaiian ingroup taxa *Urera glabra* and *Touchardia latifolia,* was recovered as sister to all other ingroup taxa (Fig. 2; MP:69%, ML:96%, BI:0.99). Clades B–F were recovered within a polytomy, each with strong support. Clade B comprises all accessions of *Urera laciniata* Wedd. and *U. baccifera;* and Clade C comprises the remaining accessions of *Urera* from the Neotropics. Clade D comprises all Indo-Malay and Australasian accessions of the ingroup taxa and members of the genus *Poikilospermum*. Clade E comprises all Afrotropic accessions of the genus *Obetia*, and Clade F comprises all Afrotropic accessions of *Urera*. Clades E and F formed a strongly supported Afrotropic clade, as was the case in the *nrITS* analyses (Fig. 2; MP:92%, ML:98%, BI:1.00).

3.2. *Phylogenetic analysis of targeted sequencing data*

Among the ingroup taxa, a minimum of 328 out of 353 targeted loci were successfully mapped, the majority of which were 150% covered (Table S4). The analysis of the resulting gene trees in ASTRAL-II recovered a species tree broadly concordant with that generated from

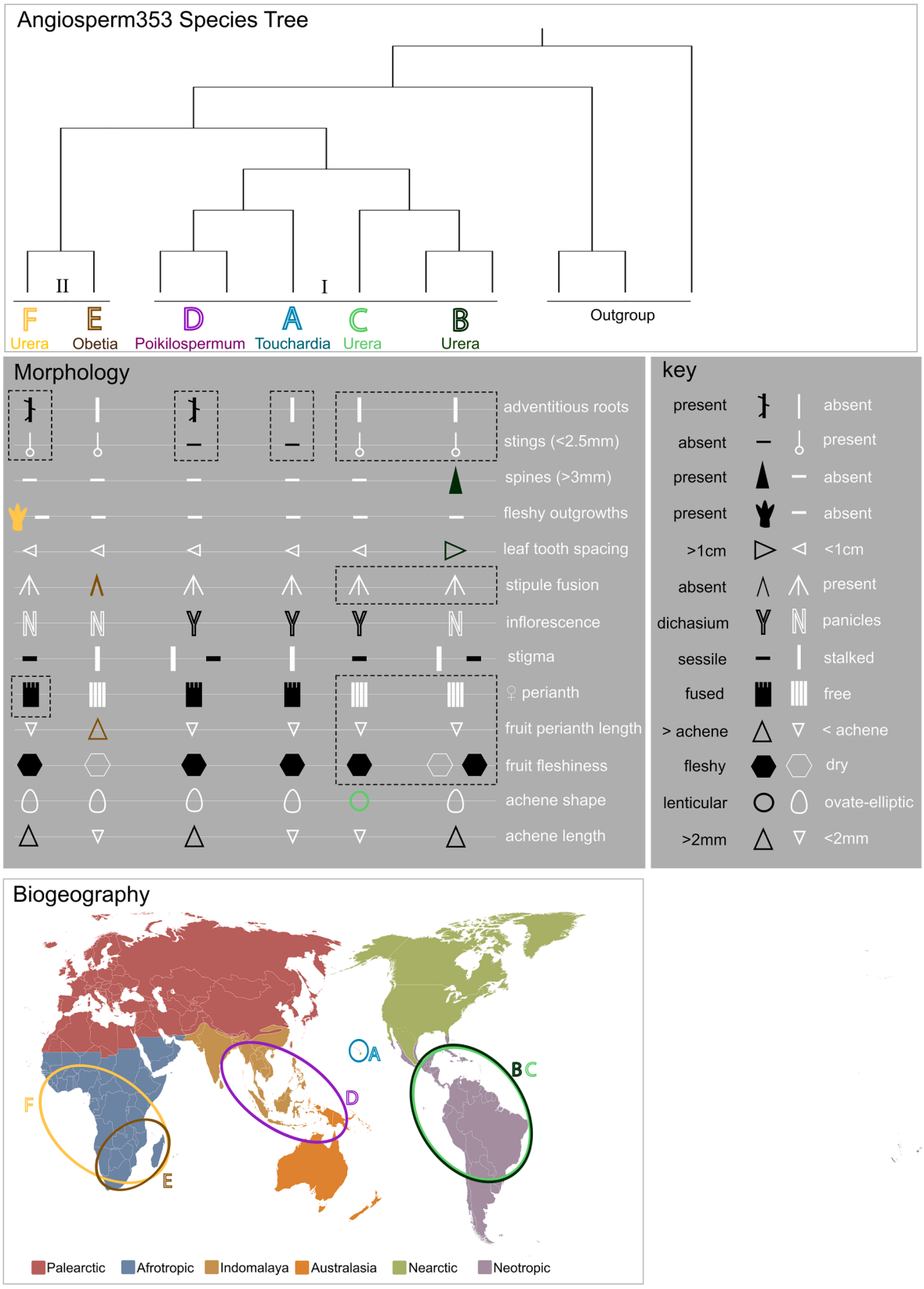

Fig. 4. Comparison of morphological and biogeographic characters with resolved targeted sequencing Species Tree. Showing for each of the six ingroup clades: character states for 12 morphological characters, and a summary of distributions overlaid on a map of Ecozones according to Olson & Dinnerstein (2002). Coloured morphological character states show autapomorphies for the relevant clade; dashed boxes show diagnostic combinations.

Table 2
Phylogenetically informative morphological characters and character states identified.

CHARACTER	STATES
Adventitious Roots	Present; absent
Bulbous stinging hairs < 2.5 mm	Present; absent
Bulbous stinging spines > 2.5 mm, often lignified at base	Present; absent
Stem / petiole with fleshy outgrowths	Present; absent
Leaf dentation spacing	1 cm; <0.5 cm
Stipule Fusion	Fused; not fused
Inflorescence branching pattern	Dichotomous cymes; irregular panicles
Stigma presentation	Stalked; sessile
Female perianth fusion	Fused; free
Perianth length in fruit	Exceeding achene length; not exceeding achene length
Perianth / inflorescence fleshiness in fruit	Fleshy; not fleshy (dry)
Achene outline shape	Elliptic-ovate (length > width); lenticular (length = width)
Achene length	<2 mm long; >2 mm long

the Sanger-sequencing data, but with two points of difference (Fig. 2). The first difference is the position of *Touchardia* (Clade A) sister to *Poikilospermum* (Clade D) in the targeted sequencing species tree, with moderate to strong support (AP:1|0|0; MLBS:100%; Quartets: 44:22:34), as opposed to sister to all other ingroup taxa in the Sanger tree. The second, is a resolved topology for the relationships among the six ingroup clades. In this topology Clade I contains two subclades, one composed of the representative samples for clades B and C (Neotropical *Urera*) sister to each, and the other containing clades A (*Touchardia*) and D (*Poikilospermum*). Clade I is itself sister to Clade II, which contains the representative samples of clades E (*Obetia*) and F (*Scepocarpus*). This topology was moderately to strongly supported in all analyses: the seven ingroup nodes recovered each received 100% support from MLBS; and alternative posterior values of 1|0|0 for all except one node, which received 0.91|0.08|0.00 (Clade I). Quartet scores for the 7 nodes varied from moderate to strong support (42–92%), with a minimum 10% gap to the next best alternative. The lowest quartet scores were within clade I.

3.3. Morphological results

The literature review and analysis of herbarium material identified 13 independent morphological characters partitioned into 24 readily identifiable states, which are phylogenetically informative and can be used individually or in combination to diagnose and delimit the clades A-F recovered in the phylogenetic analyses (Fig. 4; Table 2).

Adventitious roots are present in all taxa in clades D and F (Fig. S2:A, B), and absent from all those in all other clades, even when some species in these clades have a climbing liana-like habit, as is the case for *U. lianoides* A.K.Monro & Al.Rodr. (clade C) and some specimens of *U. baccifera* (clade B). Bulbous, translucent hairs that impart a stinging sensation on contact with the skin are characteristic of taxa in the Urticeae (Kim et al., 2015), and are present to a greater or lesser degree in all taxa in all clades, with the exception of clades A and D where they are entirely absent. Separately, large stinging spines, which are at least 3 mm in length, and often becoming lignified at the base, are present only in clade B (Fig. S2:D,E); absent from all other clades. Dark, fleshy outgrowths bearing multiple stinging hairs (termed "protuberances" by Letouzey (1968)) are variously present on the stems, petioles, leaves and inflorescences of most, though not all individuals in clade F (Fig. S2:C), but entirely absent from all other clades. Leaf margin dentation is noticeably more pronounced in clade B (teeth > 1 cm apart) than in all other clades (<0.5 cm apart); while stipules are partially to completely fused in all clades except E, where they are completely free.

Clades A, C and D all possess dichotomously branching cymose inflorescences (Fig. S2:F), in contrast to clades B, E and F, which have irregularly branching panicles (Fig.S2:E). Internal inflorescence branches are drastically shortened in a number of taxa in clade D, and some members of clades A and C, forming distinctive spherical glomerules of flowers (e.g. Fig. 1:D). This character is not discrete nor consistent across all members of these clades however, so is not of use in delimitation or diagnosis.

Stigma morphology in Urticaceae is highly variable (Wu et al., 2013), but within the Urera clade the stigma is either sessile on the apex of the ovary, or extended on a stalk. Stalked stigmas are found in all members of clades E and A, and some members of D and B, but never in clades C or F, where they are universally sessile and capitate (Fig. 3).

Fusion of the female perianth occurs in all members of clades A and D, and all but two members of clade F (*U. hypselodendron* Wedd. and *U. robusta* A. Chev.). The female perianth remains dry and extends to exceed the length of the achene in fruit only in clade E (Fig. 3). It does not exceed the length of the achene in the remaining clades, instead becoming fleshy in fruit (sometimes along with parts of the inflorescence) in all taxa in these clades, with the exception of one member of clade B (*U. laciniata*). The achene itself is ovate-elliptic (defined as longer than it is wide) in all clades except clade C where it is lenticular (completely round) (Fig. 3). Achenes are>2 mm long in clades B, D and F, and shorter than 2 mm in the other three clades.

The three clades of *Urera* recovered in the phylogenetic analyses (B, C and F) differ in 7 of the 12 morphological characters assessed, sharing only the presence of stinging hairs, fused stipules, a predominantly capitate stigma (except *U. lacinata* in clade B), and a fleshy female perianth in fruit (except *U. lacinata* in clade B), which is shorter than the achene. All five of these character states are also shared with at least one of the other clades. Clades B, C and F differ particularly in terms of stem appendages (presence or absence of adventitious roots, stinging spines, and fleshy outgrowths), but also in terms of degree of leaf margin dentation, inflorescence branching pattern, female perianth fusion, and achene size and shape. These differences are not solely between Neotropical (sister clades B and C) and African (clade F) taxa either. In fact, clades B and C differ in the same number of characters (five) as either does from F. The seven character states shared by clades B and C are: lack of adventitious roots, presence of stinging hairs, absence of fleshy outgrowths, presence of fused stipules, and free female perianths, that are shorter than the achene and fleshy in fruit (except *U. lacianata*, which has dry tepals in fruit). Each of these characters states is also shared with at least one other clade however. Within clade B, *U. laciniata* also possesses distinctive floral morphology, lacking a fleshy perianth in fruit, and having a stalked stigma, but its vegetative morphology and inflorescence form are consistent with that of other taxa in the clade.

The sister clades A and D share the highest number of character states (ten) among any of the higher clades, differing only in terms of presence or absence of adventitious roots, and achene length.

Other higher clades recovered in the targeted sequencing tree do not tend to share many character states. Clade E for example shares only six character states with clade F, none of which are unique to them. The taxa within clade I share four character states, three of which are also shared with clade F (fleshy female perianths in fruit, which are shorter than the achene, and fused stipules), and one with clade E (absence of fleshy stem outgrowths).

3.4. Biogeographic results

Within each of the six clades A-F, all taxa were distributed within only a single biogeographic region, with the exception of clade D, where they are distributed across a contiguous region spanning parts of the Indomalaya and Australasia regions. Sister clades in the targeted sequencing tree also tended to share the same biogeographic regions, with E and F both within the Afrotropic region, and B and C both within the Neotropical one. Taxa in clade A occur only in Hawaii however, which falls in the Nearctic region, but neighbours the regions occupied by sister clade D.

4. Discussion

4.1. Phylogenetic relationships

Our analyses of the Sanger and targeted sequencing data both confirm that *Urera* as currently circumscribed is polyphyletic with respect to the morphologically distinct genera *Obetia*, *Poikilospermum* and *Touchardia*. The Sanger analyses recovered five strongly supported clades (Fig. 2, B-E), and one moderately supported one (Fig. 2, A), but failed to resolve relationships among these clades. The strong support for the clades identified in the Sanger analysis, and the sister relationship between clades E (*Obetia*) and F (African *Urera*), confirms the polyphyly of *Urera* and provides grounds for its re-classification along the lines suggested by past taxonomic works (Friis, 1989b). Further clarity and support are offered by the species tree generated from the gene trees of 306 exons and 315 introns recovered using the Angiosperms353 bait kit. The resolved relationships among the representative samples for all six major clades in this analysis (Fig. 2, A-F) confirms the polyphyly of *Urera s.l.* with respect to *Obetia*, *Poikilospermum* and *Touchardia* with strong or moderate support (MLBS: 100%; AP: >0.91; Quartets: >40%). The lowest levels of quartet support were found in clade I, where the difference between the main and alternative quartet support values for several nodes was only around 10% (Fig. 2). This reflects a degree of discordance among gene tree topologies that can result from a number of processes including incomplete lineage sorting, hybridization or methodological error (Sayyari and Mirarab, 2016). Here it suggests that the topology of clade I is only moderately supported, and that further analysis could involve using higher taxon sampling, and more specific genetic loci for the group. Gene tree discordance is not in itself evidence of topological error however, and AP support remains high even for the discordant nodes.

Crucially for the study of relationships within *Urera s.l.*, support for clade II, which contains clades E (*Obetia*) and F (Afrotropic *Urera*), is consistently high in both Sanger and targeted sequencing analyses. This shows African specimens of *Urera s.l.* are more closely related to their fellow African taxa *Obetia* than they are to Neotropical *Urera* (B and C), which are themselves consistently recovered as two reciprocally monophyletic groups. In the targeted sequencing species tree, the moderately supported position of clades B and C sister to each other in clade I, along with clades A and D, further underlines the support for this separation between African and Neotropical accessions of *Urera s.l.*

The relationship of clade A (Hawaiian taxa) to the other ingroup taxa is the main incongruence between the results from the different phylogenetic datasets, moving from a position sister to all other ingroup taxa in the Sanger analyses to one sister to clade D (*Poikilospermum*) in the targeted sequencing species tree (Fig. 2). Clade A's position in the Sanger tree was not strongly supported however, whereas the additional data provided by the targeted sequencing of 353 nuclear regions gives moderate to strong support to its close relationship to clade D. A high degree of similarity in morphology between clades A and D also appears congruent with this topology, as does their distributions in neighbouring biogeographic regions.

4.2. Morphology & biogeography

Our results consistently suggest strong congruence between morphology, biogeography and phylogenetic signal. Of the six clades recovered, four are associated with morphological autapomorphies (B: presence of stinging spines, and leaf teeth > 1 cm apart; C: lenticular achenes; E: free stipules, and a dry perianth longer than the achene in fruit; & F: fleshy outgrowths). Of the remaining two, clade A can be easily diagnosed by a combination of determinate cymose inflorescence branching and the absence of stinging hairs or adventitious roots; and clade D by the absence of stinging hairs and the presence of adventitious roots. In addition to those floral morphology characters previously used to delimit genera (e.g. Chew, 1964; Friis, 1982, 1983, 1989a; Weddell, 1856, 1869), we identify several phylogenetically informative vegetative characters that support generic delimitation, which dramatically improves diagnosability and suggests a number of potentially interesting evolutionary trends.

4.2.1. Stinging hairs & spines, fleshy protuberances

The bulbous, stinging hairs so often associated with the Urticaceae are in fact restricted to the tribe Urticeae (Kim et al. 2015). They are present in four of the six clades in the '*Urera* clade', accompanied by formidable 3–10 mm long spines in clade B, and dark, fleshy sting-covered protuberances in clade F. Stinging hairs or spines of any form are notably entirely absent from clades D (*Poikilospermum*) and A (*Touchardia*), suggesting a single unique loss of this character within the Urticeae.

4.2.2. Habit, lifeform and secondary woodiness

Previous studies have tended to make broad generalisations about habit and wood structure in *Urera s.l.* when inferring evolutionary trends (e.g. Bonsen and ter Welle, 1984; Kim et al., 2015; Wu et al., 2013). We show that in addition to woody shrubs and small trees (clades A, B, C, & E), the '*Urera* clade' also includes climbing lianas (Clade F), and hemi-epiphytic stranglers (Clade D). The '*Urera* clade' thus contains the only climbing taxa within the Urticaceae, with adventitious roots found exclusively in clades D (African *Urera*) and F (*Poikilopsermum*). While the exact role of these roots is unknown (Chew, 1964), they are likely to be associated with an adpressed climbing habit, or for water or nutrient uptake. A scandent or liana-like habit, albeit without adventitious roots, also occasionally occurs in Clades B (forms of *Urera baccifera*) and C (*U. lianoides*, *U. sinuata*), suggesting multiple independent origins of this life-form strategy within the *Urera* clade.

Taxa in clade B tend to have soft, hollow branches lacking any wood, and even within the main stem the wood tends to be soft and pithy compared to that in clades A, C, D and F for example. This apparent reduction in woodiness in clade B may be a consequence of a shift to more open, nutrient-rich riparian habitats (Monro, 2015), while the development of pachycaul wood in clade F (*Obetia*) has enabled its adaptation to more arid scrub and savannah.

Our results (Fig. 4) thus suggest that a number of separate shifts in woodiness, habit and lifeform have taken place in this clade within the context of a predominantly herbaceous Urticeae tribe (Kim et al., 2015). The drivers and mechanisms involved in secondary woodiness remain only partially understood, but appear to be correlated with ecological changes such as aridity, absence of pollinators, lack of frost, and competition (Kidner et al., 2016; Lens et al., 2009, 2013; Moyers & Rieseberg, 2013; Rowe & Paul-Victor, 2012). Here it seems possible that they may be associated with the colonisation of closed-canopy forest habitats, and subsequent shifts back into more open terrain.

4.2.3. Fleshy fruits

Within the Urticaceae, shifts to closed canopy forest have occurred in all tribes and have often been associated with fleshy fruits or fruit-like inflorescences. A fleshy fruit syndrome derived from inflated and frequently brightly coloured fruiting perianths or parts of the inflorescence occurs in the Urticeae (*Dendrocnide*, *Gyrotaenia*), Boehmerieae (*Cypholophus, Debregeasia, Oreocnide, Neraudia, Pipturus*), and Cecropieae (*Pourouma, Musanga, Myrianthus*). In the Urticeae, fleshy fruits are normally brightly coloured, ranging from white in *U. baccifera* (clade B), to orange, yellow or red in other *Urera s.l.* (clades C & F), bright purple in *Poikilospermum* (clade D), and white or very dark purple to black in *Dendrocnide*. In *Urera baccifera* the peduncle and pedicels also become fleshy and are red, magenta or pale yellow in colour. In contrast, within the Boehmerieae and Elatostemeae, fruits are a duller off-white (except for *Debregeasia* where they are orange), and in the Cecropieae they are pale yellow or dark maroon. Brightly coloured fruits suggest bird and/or primate dispersal (Voigt et al., 2004), and the white ones of *Urera baccifera* have been shown to play a role in plant-ant interactions (Dutra

et al., 2006).

Across the Urticaceae the fleshy fruit syndrome is achieved by the inflation of several non-homologous structures: the peduncle, the pedicel, the bracts or the perianth parts. Within the '*Urera* clade' however, it is always the result of inflation and pigmentation of the perianth, supplemented to a greater or lesser degree by inflation of inflorescence branches. This suggests that fleshy fruits generated through inflation of the perianth parts are a synapomorphy for the '*Urera* clade', but with secondary losses in part of clades B (*U. laciniata*) and all of E (*Obetia*). Bolmgren & Eriksson (2005) proposed the hypothesis that fleshy fruit evolution is driven by habitat, with the strength of frugivore mediated selection for fleshy fruits increasing as recruitment sites become spatially unpredictable and darker. This would suggest that as for woodiness, the colonization of closed canopy forest may have been a key driver for the development of fleshy fruits within the Urticaceae, with reductions and reversals occurring as the landscape becomes more open. For example, in the case of *Obetia* with the aridification of Africa in the Miocene and Pliocene (Pokorny et al., 2015); or in the case of *U. laciniata*, the colonisation of the banks of large rivers. The Urticaceae and the now well-resolved '*Urera* clade' in particular, may therefore represent an ideal study group for testing hypotheses about the drivers and mechanisms that enable biome shifts and associated adaptive evolution.

4.2.4. Biogeography

We also document strong congruence between phylogenetic relationships and geographic distribution (Fig. 4), with sister clades in the targeted sequencing species tree sharing the same biogeographic region (B&C: Neotropic; E&F: Afrotropic) or neighbouring ones (D: Indomalaya/Australasia & A: Hawaii). The latter relationship shows a pattern also documented for the woody plant genus, *Melicope* (Rutaceae, Harbaugh et al., 2009), and appears to support Fosberg's (1948) contention that regions to the West and South of the Hawaiian archipelago contributed the majority of the founder species to its flora.

The ingroup taxa can also be divided into two well-supported clades in the targeted sequencing tree: those from the Neotropics, Indomalaya and Australasia make Clade I, whilst those from the Afrotropics make Clade II, sister to Clade I (Fig. 4). A similar pattern has also been recovered within each of the two species-rich Urticaceae genera: *Pilea* (Monro, 2006) and *Elatostema* (Tseng et al., 2019), and for the family as a whole (Huang et al., 2019; Wu et al., 2013), as well as for other taxa including *Schefflera* (Araliaceae, Li & Wen, 2014). This close relationship between Neotropical and Asia-Pacific taxa has previously been ascribed to a possible Asian origin followed by dispersal through the Bering land bridge from the late Cretaceous to the Neogene (Wen et al., 2016).

4.3. Proposed revision of generic delimitation

A polyphyletic *Urera,* as recovered by our analyses, is in broad agreement with previous phylogenetic studies of the group (Wu et al. 2013; Kim et al. 2015). Some of the patterns of morphology and geography we document within the genus have also been noted in the past and used to suggest the possibility of its division. Weddell (1856, 1869) having first created informal subgeneric classifications and then established the genus *Scepocarpus* for taxa with a fused perianth (later sunk into *Urera* by Bentham), and Friis (1989b) having suggested separating the Neotropical and African species, but stating that a broader analysis was required before any final decision could be made.

Our results provide that broader analysis, combining a resolved phylogeny with a comprehensive comparison of the morphology. The six strongly supported clades we recovered (Fig. 2: A-F) show good congruence with both morphological and geographic distribution data. They are each readily diagnosed and delimited using a small number of morphological character states (Figs. 4 and 5), making their identification in the field or herbarium simple. Our analyses of Sanger sequence data strongly support the monophyly of the six lineages, whilst our targeted sequence data (Figs. 2 and 3) recovers resolved and moderately to strongly supported relationships among them. These six clades have previously been associated with five names at the rank of genus: *Obetia* (E), *Poikilospermum* (D), *Scepocarpus* (F), *Touchardia* (A), and *Urera* (B&C).

We thus propose to reinstate the genus name *Scepocarpus* for all Afrotropic taxa previously included in *Urera s.l.* (clade F), and to transfer the Hawaiian endemic *U. glabra* to *Touchardia*, which requires the creation of a new name *Touchardia oahuensis* T.Wells & A.K.Monro for the former. The Sanger sequencing confirms *U. glabra* and *T. latifolia* are monophyletic, and their morphological similarity was previously noted by Friis (1993), who suggested they most likely shared a common ancestor. As such their separation until now remains somewhat of a mystery.

Urera s.s. is therefore redefined to include only Neotropical taxa (clades B and C). It can now be diagnosed by a combination of the possession of fused stipules, bulbous stinging hairs, and free female perianths that remain shorter than the achene and become fleshy in fruit, as well as the lack of adventitious roots. Due to the strong morphological differences noted between clades B and C and their reciprocal monophyly, we also propose two subgenera within *Urera s.s.*. The first, subgenus *Urera*, conforms to clade B and can be diagnosed by possession of large stinging spines, pronounced leaf margin dentation, and irregularly branching panicles. The second subgenus *Capitata nom. nov.*, matches clade C, and is defined by the absence of stinging spines, and possession of only subtle leaf margin dentation, plus dichotomously branching cymose inflorescences.

Combining clades A (*Touchardia*) and D (*Poikilospermum*) was also considered, based on their sister relationship in the targeted sequencing tree, and their morphological similarity. We believe however, that given the lack of congruence between the Sanger and targeted sequencing results regarding the position of clade A, and the gene-tree discordance observed within clade I, further analysis is required, using an expanded taxonomic sampling for both genera alongside more in-depth analysis of shared morphological traits.

Our redefined generic delimitations thus retain three of the four currently recognized genera with species composition either unchanged, or with small additions (Hawaiian endemic taxa currently treated as species of *Urera* to be placed in *Touchardia*), and ensures that all genera in the 'Urera clade' are monophyletic and readily diagnosed. In resurrecting the name *Scepocarpus*, we also respond to the most comprehensive work of past specialists in the group. Our revised delimitation of the 'Urera clade' therefore reflects both evolutionary relationships and the distribution of morphological character states, and we hope that it will serve as a framework for future biogeographical and comparative biology research.

5. Conclusion:

Our results confirm the previously noted polyphyly of *Urera* (Wu et al. 2013; Kim et al. 2015), and are the first to recover the strongly supported hypotheses of relationships necessary to revise the classification and delimitation of the genera within the 'Urera clade'. This was achieved through greater taxon and genome sampling, and a two-stage approach to genome sampling in the phylogenetic analyses; in combination with comprehensive morphological and biogeographic analysis.

We use our results to propose a new classification for the '*Urera* clade', which provides a framework for interpreting the development of morphological traits and geographic distribution within it. Our new classification recognises five genera, *Obetia*, *Poikilospermum*, *Scepocarpus*, *Touchardia* and *Urera*. We resurrect *Scepocarpus* for African taxa previously assigned to *Urera*, redelimit *Touchardia* to include the Hawaiian species of *Urera*, and leave *Urera s.s.* to include only Neotropical taxa, which we divide into two subgenera based on consistent morphological differences. In the process we identify several trends with

respect to biogeography, lifeform and secondary woodiness, and the production of fleshy fruits. These have tended to be obscured by taxonomic and phylogenetic confusion in the past, and they suggest multiple biome shifts in the course of the group's evolution. We thus demonstrate that a combination of Sanger and targeted sequencing is an economical and effective approach for resolving problematic complexes of genera and providing a solid framework for the study of evolutionary developments.

6. Taxonomic treatment

6.1. Key to the genera (See Fig. 5 for example illustrations of characters)

6.2. Synopsis of the genera

Obetia Gaudich., Voy. Bonite, Bot., Atlas: t. 82. 1844. Type species: *O. ficifolia* Gaudich., Voy. Bonite, Bot., Atlas: t. 82 (1844). Fig. 4, Ei–v.

Upright or scandent shrubs to small trees; lacking adventitious roots; bearing bulbous stinging hairs; lacking bulbous spines or dark fleshy outgrowths. Stipules interpetiolar, completely free, with a single central vein. Leaf margin dentate. Inflorescences irregularly branching panicles, internal branches visible. Male flowers 5-merous. Female flowers 4-merous, perianth parts free, unequal, lateral pair largest. Stigma stalked, covered in small stigmatic hairs on all sides. Perianth parts and infructescence branches remaining dry in fruit. Perianth length exceeding achene in fruit. Achene < 2 mm in length, ovate-elliptic. Africa, Madagascar, Aldabra and the Mascarene Islands, 6 spp. (Friis, 1983), *Obetia aldabrensis* Friis, *Obetia carruthersiana* (Hiern) Rendle, *Obetia ficifolia* (Savigny) Gaudich., *Obetia madagascariensis* (Juss. ex Poir.) Wedd., *Obetia radula* (Baker) Baker ex B.D.Jacks., *Obetia tenax* (N.E.Br.)

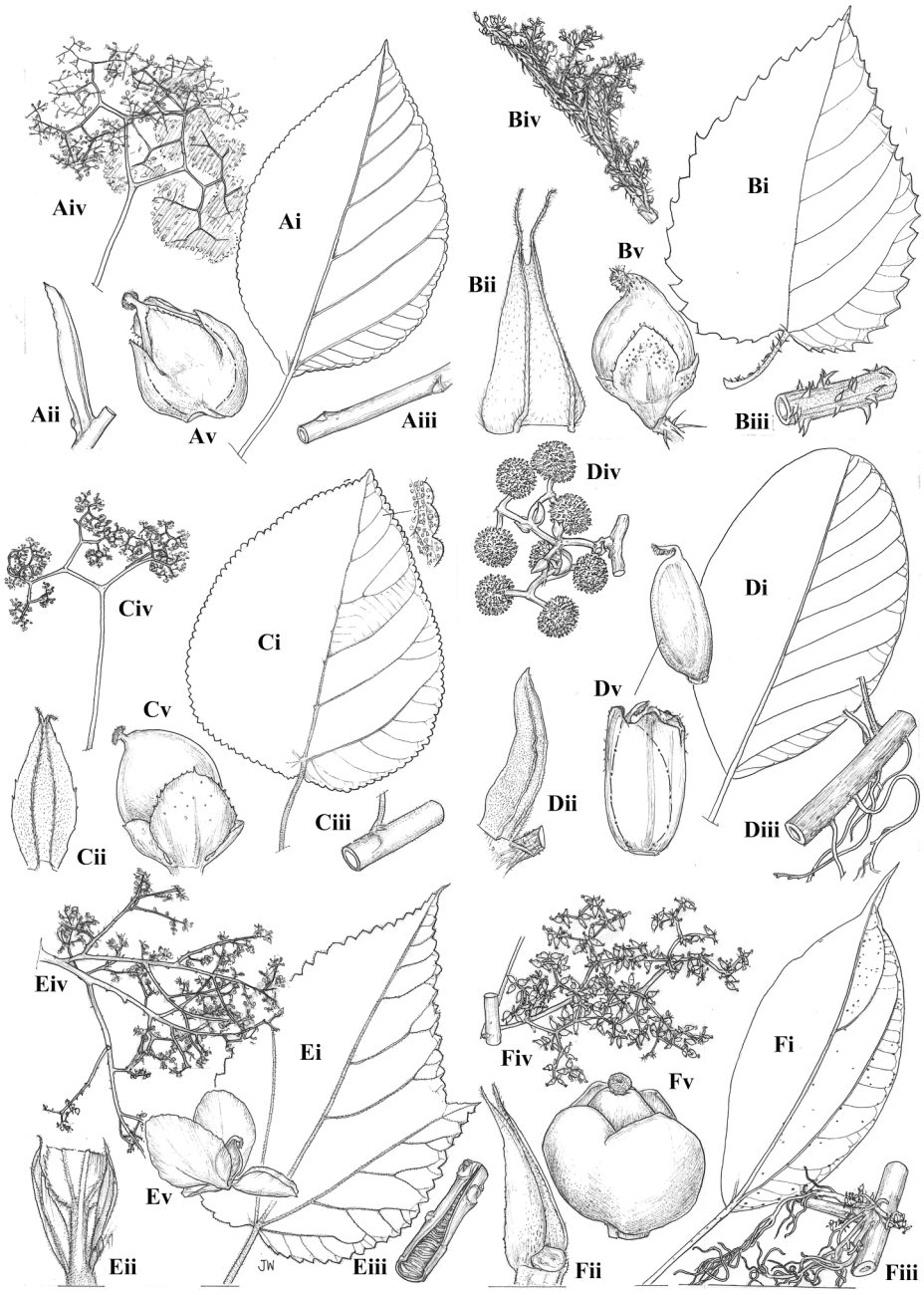

Fig. 5. Illustration of Genera. **A** *Touchardia* based on *Degener 8703*, *Heller 2065*, *Hilldebrandt 171*; **B** *Urera* subgenus *Urera* based on *Beck & Bach 23206*, *Woolston 1094*; **C** *Urera* subgenus *Capitata* based on *Mexia 8327*; **D** *Poikilospermum* based on *Dransfield 4408*, *Forest Department Sand 54775*, *Leong 433*; **E** *Obetia* based on *Fryer 17*, *Perrier 11731*; **F** *Scepocarpus* based on *Oloruinjemi 30523*, *Leeuwenberg 7187*, *Lewalle 4498*. **i** Leaf outline and venation, **ii** stipule, **iii** section of leaf-bearing stem, **iv** outline of female inflorescence, **v** female flower/fruit. Illustration by Juliet Beentje.

1. Translucent, bulbous stinging hairs absent anywhere on plant..…..……...……2

2. Hemi-epiphyte or liana, possessing adventitious roots at nodes.

Australasia/Indomalaya…….……..…….*Poikilospermum*

2. Shrub or small tree, lacking adventitious roots from nodes. Hawaii……...…*Touchardia*

1. Translucent, bulbous stinging hairs present on leaves, and/or stems, petioles, inflorescences...…….…3

3. Stipules entirely free; female perianth always dry and exceeding length of achene in fruit.

Afrotropics……...…….……...…*Obetia*

3. Stipules fused for most or all of their length; female perianth usually fleshy in fruit, but if dry then not exceeding length of achene in fruit.…….……..4

4. Shrubby liana or trailing vine, possessing adventitious roots at nodes. Female perianth generally fused for most of its length. Afrotropics…..…….....*Scepocarpus*

4. Shrub or small tree, lacking adventitious roots at nodes. Female perianth entirely free and four lobed. Neotropics…..……*Urera* (5)

5. Stinging spines at least 3mm long present on leaves and/or stems. Leaf margins with teeth at least 1cm apart. Inflorescence an irregularly branching panicle. Achenes elliptic-ovate, at least 2mm in length.……....subgenus Urera

5. Stinging spines at least 3mm long absent anywhere on plant. Leaf margins with teeth at least 1cm apart. Inflorescence a dichotomously branching cyme. Achenes lenticular, less than 2mm in diameter.…….….subgenus *Capitata*

Friis.

Poikilospermum Zipp. ex Miq., Ann. Mus. Bot. Lugduno-Batavi 1: 203. 1864. Type species: P. amboinense Zipp. ex Miq.

Conocephalus Blume, ilegitimate, non Conocephalus Neck. ex Dumort. ilegit., invalid orthographic variant; non Conocephalum J. Hill 1773 (nom. et orth. cons.)

Hemi-epiphytic woody scramblers; possessing adventitious roots; entirely lacking bulbous stinging hairs, spines or dark fleshy outgrowths. Stipules intrapetiolar, completely fused, with a pair central veins. Leaf margin entire or subtly crenulate. Inflorescences dichotomously branching cymes, internal branches sometimes visible, but often compressed, forming capitate heads of spherical glomerules. Male flowers 2- or 4-merous. Female flowers 4-merous, perianth parts partially to completely fused. Stigma stalked or subsessile, covered in small stigmatic hairs on one or all sides. Perianth parts becoming fleshy and remaining shorter than achene in fruit; achene > 2 mm in length, ovate-elliptic. 20 species (Chew 1964), Indo-Malaya & Australasia.

Poikilospermum comprises two geographically distinct groupings which differ in female inflorescence and flower morphology. Subgenus Ligulistigma from Continental Asia comprises species whose flowers are borne in tight capitate heads borne on accretions of swollen peduncle branches, whose stigma is stalked, asymmetrical and tongue-like. Subgenus Poikilospermum from Malesia comprises species whose flowers are borne in loose capitate heads and are free, and whose stigma is subsessile, symmetrical and capitate. As noted by Chew (1964) there is some similarity between *Touchardia* and *Poikilospermum* subgenus Ligulistigma with respect to inflorescence morphology. We also observed similarities in leaf secondary venation, both having parallel or weakly spreading secondary veins (Fig. 4, A & B). The two genera, however, may be distinguished on the absence bracts and presence of sessile compact cymes in the axils of the peduncle branches in *Touchardia* (Chew, 1964).

Scepocarpus Wedd., Prodr. 16: 98 (1869). Type species: S. mannii Wedd. A.P.de Candolle, Prodr. 16(1): 98 (1869).

Scepocarpus acuminatus (Poir.) T.Wells & A.K.Monro, *comb. nov. Urera acuminata* (Poir.) Gaudich. ex Decne., Nouv. Ann. Mus. Hist. Nat. 3: 490 (1834).

Scepocarpus batesii (Rendle) T.Wells & A.K.Monro, *comb. nov. Urera batesii* Rendle, J. Bot. 54: 368 (1916).

Scepocarpus cordifolius (Engl.) T.Wells & A.K.Monro, *comb. nov.* Urera cordifolia Engl., Bot. Jahrb. Syst. 33: 121 (1902).

Scepocarpus flamignianus (Lambinon) T.Wells & A.K.Monro, *comb. nov. Urera flamigniana* Lambinon, Bull. Soc. Roy. Bot. Belgique 91: 199 (1959).

Scepocarpus gabonensis (Pierre ex Friis) T.Wells & A.K.Monro, *comb. nov.*, *Urera gabonensis* Pierre ex Friis, Fl. Gabon 51: 77 (2018).

Scepocarpus hypselodendron (Hochst. ex A. Rich.) T.Wells & A.K. Monro, *comb. nov. Urtica hypselodendron* Hochst. ex A.Rich., Tent. Fl. Abyss. 2: 260 (1850). *Urera hypselodendron* (Hochst. ex A.Rich.) Wedd., Ann. Sci. Nat., Bot., sér. 3, 18: 203 (1852).

Scepocarpus oblongifolius (Benth.) T.Wells & A.K.Monro, *comb. nov. Urera oblongifolia* Benth., W.J.Hooker, Niger Fl.: 515 (1849).

Scepocarpus obovatus (Benth.) T.Wells & A.K.Monro, *comb. nov.*, *Urera obovata* Benth., Niger Fl.: 516 (1849).

1. Translucent, bulbous stinging hairs absent anywhere on plant..…..……...……2

2. Hemi-epiphyte or liana, possessing adventitious roots at nodes.

Australasia/Indomalaya…….……..…….*Poikilospermum*

2. Shrub or small tree, lacking adventitious roots from nodes. Hawaii……...…*Touchardia*

1. Translucent, bulbous stinging hairs present on leaves, and/or stems, petioles, inflorescences...…….…3

3. Stipules entirely free; female perianth always dry and exceeding length of achene in fruit.

Afrotropics……...…….……...…*Obetia*

3. Stipules fused for most or all of their length; female perianth usually fleshy in fruit, but if dry then not exceeding length of achene in fruit.…….…….……..4

4. Shrubby liana or trailing vine, possessing adventitious roots at nodes. Female perianth generally fused for most of its length. Afrotropics…..…….....*Scepocarpus*

4. Shrub or small tree, lacking adventitious roots at nodes. Female perianth entirely free and four lobed. Neotropics…..……*Urera* (5)

5. Stinging spines at least 3mm long present on leaves and/or stems. Leaf margins with teeth at least 1cm apart. Inflorescence an irregularly branching panicle. Achenes elliptic-ovate, at least 2mm in length.……....subgenus Urera

5. Stinging spines at least 3mm long absent anywhere on plant. Leaf margins with teeth at least 1cm apart. Inflorescence a dichotomously branching cyme. Achenes lenticular, less than 2mm in diameter.…….….subgenus *Capitata*

Scepocarpus repens (Wedd.) T.Wells & A.K.Monro, *comb. nov. Laportea repens* Wedd., A.P.de Candolle, Prodr. 16(1): 81 (1869). *Urera repens* (Wedd.) Rendle, Fl. Trop. Afr. 6(2): 264 (1917).

Scepocarpus rigidus (Benth.) T.Wells & A.K.Monro, *comb. nov. Boehmeria rigida* Benth., W.J.Hooker, Niger Fl.: 519 (1849). *Urera rigida* (Benth.) Keay, Kew Bull. 10: 141 (1955).

Scepocarpus robustus (A. Chev.) T.Wells & A.K.Monro, *comb. nov. Urera robusta* A.Chev., Mém. Soc. Bot. France 8: 301 (1917).

Scepocarpus sansibaricus (Engl.) T.Wells & A.K.Monro, *comb. nov. Urera sansibarica* Engl., Pflanzenw. Ost-Afrikas, C: 162 (1895).

Scepocarpus thonneri (De Wild. & T.Durand) T.Wells & A.K.Monro, *comb. nov. Urera thonneri* De Wild. & T.Durand, Bull. Soc. Roy. Bot. Belgique, Compt. Rend. 38: 48 (1899).

Scepocarpus trinervis (Hochst.) T.Wells & A.K.Monro, *comb. nov. Elatostema trinerve* Hochst., Flora 28: 88 (1845). *Urera trinervis* (Hochst.) Friis & Immelman, Nordic J. Bot. 7: 126 (1987).

Lianas (often shrubby) or trailing vines; possessing adventitious roots, and bulbous stinging hairs; lacking spines; usually with dark fleshy outgrowths 2–3 mm in length and covered in stinging hairs. Stipules intrapetiolar, partially to completely fused, with a pair central veins. Leaf margin subtly dentate, or rarely entire. Inflorescences irregularly branching panicles; internal branches visible. Male flowers 4- or 5-merous. Female flowers 4-merous, perianth partially to completely fused, or rarely free. Stigma capitate, sessile on apex of ovary, covered in small stigmatic hairs. Perianth parts and/or infructescence branches becoming fleshy in fruit. Perianth parts remaining shorter than achene in fruit. Achene $<$ 2 mm in length, ovate-elliptic. c.14 species (Wells et al. *in prep*), Afrotropics.

The genus *Scepocarpus* was described by Weddell (1869) to account for fruiting material of a plant collected in Equatorial Guinea by Gustav Mann. Weddell's description is identical to that for *Urera*, with the exception of the female perianth being fused and tubular as opposed to free for most of its length, and four-lobed. Weddell overlooked the fact that he had provided descriptions of three African *Urera* species which also had a tubular female perianth, *Urera obovata* Benth., *U. acuminata* (Poir.) Gaudich. ex Decne and *U. cameroonensis* Wedd. Bentham (1880) later placed *Scepocarpus* in synonymy with *Urera* for reasons unspecified, and it is as such that it has been treated ever since.

Touchardia Gaudich., Bot. Voy. Bonite t. 94 (1844). Type species: T. latifolia Gaudich. Type: Iles Sandwich [Hawaii], *C. Gaudichard 204* (lectotype (designated here) P [P00646355]).

Touchardia oahuensis T.Wells & A.K. Monro, *nom. nov. Urera glabra* Wedd., Arch. Mus. Hist. Nat. 9: 149 (1856).

Shrubs or small trees; lacking adventitious roots, bulbous hairs, spines, or fleshy outgrowths; stipules intrapetiolar, completely fused, with a pair central veins; Leaf margins toothed. Inflorescences dichotomously branching cymes or irregularly branching panicles, internal branches sometimes visible, otherwise compressed, forming capitate heads of spherical glomerules. Male flowers 4- or 5-merous; female flowers 4-merous, perianth parts partially or completely fused. Stigma stalked, covered in small stigmatic hairs on one side. Perianth parts and infructescence branches becoming fleshy and remaining shorter than achene in fruit. Achene $<$ 2 mm in length, ovate-elliptic. Oceania (endemic to Hawaii).

Touchardia oahuensis T.Wells & A.K.Monro is a replacement name for *Touchardia glabra* non H.St.John. Within *Touchardia*, *T. kaalae* (Wawra) T.Wells & A.K. Monro differs from the remaining species in its indeterminate asymmetrical paniculate inflorescences and converging secondary leaf veination.

Weddell (1856: 442) discusses the difficulty of assigning *Touchardia* to a tribe within the Urticaceae and suggests that it could be assigned to either the Boehmerieae, as proposed by Gaudichaud (1844), or the Elatostemeae. Weddell opted for the Elatostemeae based on the imbricate nature of the male flowers and their affinity, in this respect, to *Sarchochlamys*. It could be because of the imbricate arrangement of the male perianth parts that it did not occur to him that the Hawaiian species of *Urera* which he described could be congeneric with *Touchardia*.

Urera Gaudich., Voy. Uranie, Bot. 496. 1830. (nom. cons.). Type species (lectotype selected by N. l. Britton et P. Wilson, Scient. Surv. Porto Rico 1: 243. 10 Jan 1924) *U. baccifera* (L.) Gaudich. ex Wedd.

Shrubs, trees or scandent lianas; lacking adventitious roots; generally bearing bulbous stinging hairs; with or without stinging spines > 3 mm long; lacking dark fleshy outgrowths. Stipules intrapetiolar, partially to completely fused, with a pair of central veins. Leaf margin pronouncedly or subtly toothed, rarely deeply lobed or entire. Inflorescences dichotomously branching cymes or irregularly branching panicles, internal branches usually visible, occasionally compressed, forming capitate heads of spherical glomerules. Male flowers 4- or 5-merous. Female flowers 4-merous; perianth parts completely free, unequal, the lateral pair largest. Stigma capitate, sessile on apex of ovary, covered in small stigmatic hairs. Perianth parts and/or infructescence branches becoming fleshy in fruit, rarely remaining dry. Perianth parts remaining shorter than achene in fruit. Achene generally < 2 mm or sometimes > 2 mm in length, lenticular or ovate-ellitpic. 22 species. Neotropics.

Subgenus *Urera*

Bearing stinging spines > 3 mm in length. Leaf margin pronouncedly dentate to deeply lobed. Inflorescences irregularly branching panicles. Achene > 2 mm in length.

Urera baccifera (L.) Gaudich. ex Wedd.

Urera laciniata Wedd.

Urera nitida (Vell.) P.Brack

Subgenus Capitata T.Wells & A.K. Monro **nom. nov.**

Lacking stinging spines > 3 mm in length. Leaf margin subtly toothed to subentire or entire. Inflorescences dichotomously branching cymes. Achene < 2 mm in length.

Urera capitata Wedd., Ann. Sci. Nat., Bot., sér. 3, 18: 201 (1852). Fig. 4, Di–v.

Urera altissima Lillo, Prim. Reun. Nac. Soc. Argent. Ci. Nat.: 222 (1919).

Urera aurantiaca Wedd., Ann. Sci. Nat., Bot., sér. 3, 18: 201 (1852).

Urera caracasana (Jacq.) Gaudich. ex Griseb., Fl. Brit. W. I.: 154 (1859).

Urera chlorocarpa Urb., Symb. Antill. 1: 293 (1899).

Urera domingensis Urb., Repert. Spec. Nov. Regni Veg. 15: 159 (1918).

Urera elata (Sw.) Griseb., Fl. Brit. W. I.: 154 (1859).

Urera expansa (Sw.) Griseb., Fl. Brit. W. I.: 155 (1859).

Urera fenestrata A.K.Monro & Al.Rodr., Ann. Missouri Bot. Gard. 96: 273 (2009).

Urera glabriuscula V.W.Steinm., Acta Bot. Mex. 71: 22 (2005).

Urera guanacastensis A.K.Monro & Al.Rodr., Ann. Missouri Bot. Gard. 96: 276 (2009)

Urera killipiana Standl. & Steyerm., Fieldiana, Bot. 24(3): 427 (1952).

Urera lianoides A.K.Monro & Al.Rodr., Ann. Missouri Bot. Gard. 96: 278 (2009).

Urera lobulata Urb. & Ekman, Ark. Bot. 23A(5): 2 (1930).

Urera martiniana V.W.Steinm., Acta Bot. Mex. 71: 26 (2005).

Urera pacifica V.W.Steinm., Acta Bot. Mex. 71: 28 (2005).

Urera simplex A.P.de Candolle, Prodr. 16(1): 90 (1869).

Urera sinuata Wedd., Ann. Sci. Nat., Bot., sér. 3, 18: 201 (1852).

Urera verrucosa (Liebm.) V.W.Steinm., Acta Bot. Mex. 71: 39 (2005).

Subgenus Capitata is named after one of the earliest described and most widespread members of the neotropical *Urera*, *U. capitata*.

Excluded species: *Urera kaalae* Wawra, Flora 57: 542 (1874)

Hawaiian endemic *U. kaalae* has very distinct inflorescences to *Touchardia* and on some levels (pistillate inflorescence symmetry, perianth, stem morphology, and petiole scars) resembles members of Neotropical *Urera subgenus Urera*. It differs from these species in the absence of spines, stinging hairs and achene and fruit morphology however. Further molecular phylogenetic analysis including specimens of this species will be required to clarify its position in the new classification outlined above.

CRediT authorship contribution statement

Tom Wells: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Olivier Maurin:** Methodology, Formal analysis, Validation, Investigation, Writing - review & editing. **Steven Dodsworth:** Methodology, Formal analysis, Validation, Investigation, Writing - review & editing. **Ib Friis:** Writing - review & editing. **Robyn Cowan:** Resources, Investigation. **Niroshini Epitawalage:** Resources, Investigation. **Grace Brewer:** Resources, Investigation. **Felix Forest:** Resources, Investigation. **William J. Baker:** Project administration, Funding acquisition, Supervision, Writing - review & editing. **Alexandre K. Monro:** Conceptualization, Methodology, Writing - original draft, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Andrew Hudson (University of Edinburgh) for help with SANGER sequencing; Robyn Cowan in the Jodrell laboratory for the targeted sequencing; David Harris for his observations on *Urera* in Central Africa; Nicholas Hind for his advice on nomenclature; Juliet Beentje for Fig. 5 and the Curators of Herbaria at BM, E, and K for access to and sampling of collections. Funding for the Targeted Sequencing was provided as part of the PAFTOL project by The Sackler Trust and Calleva Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.107008.

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing., in. J. Comput. Biol.: J. Comput. Mol. Cell Biol. 455–477. https://doi.org/10.1089/cmb.2012.0021.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L., 2005. GenBank. Nucleic Acids Res. D34–D38. https://doi.org/10.1093/nar/gky989.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bolmgren, K., Eriksson, O., 2005. Fleshy fruits. Oikos 2.

Bonsen, K.J., ter Welle, B.J.H., 1984. Systematic wood anatomy and affinities of the Urticaceae. Bot. Jahrbucher 105, 49–71.

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

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. In: BMC Bioinformatics. p. 421. https://doi.org/10.1186/1471-2105-10-421.

Chew, W.-L., 1964. Florae Malesianae Precursores - XXXIV A Revision of the Genus Poikilospermum (Urticaceae). Gard. Bull. Singapore 20, 1–103.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2015. jModelTest 2: more models, new heuristics and high-performance computing Europe PMC Funders Group. In: Nature Methods. p. 772. https://doi.org/10.1038/nmeth.2109.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 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. Funct. Ecol. 252–260. https://doi.org/10.1111/j.1365-2435.2006.01096.x.
Eiserhardt, W.L., Antonelli, A., Bennett, D.J., Botigué, L.R., Burleigh, J.G., Dodsworth, S., Enquist, B.J., Forest, F., Kim, J.T., Kozlov, A.M., Leitch, I.J., Maitner, B.S., Mirarab, S., Piel, W.H., Pérez-Escobar, O.A., Pokorny, L., Rahbek, C., Sandel, B., Smith, S.A., Stamatakis, A., Vos, R.A., Warnow, T., Baker, W.J., 2018. A roadmap for global synthesis of the plant tree of life. Am. J. Bot. 614–622. https://doi.org/10.1002/ajb2.1041.
Fosberg, F.R., 1948. Derivation of the flora of the Hawaiian Islands. In: Zimmerman, E.C. (Ed.), Insects of Hawaii. I. University of Hawaii Press, Honolulu, pp. 107–119.
Friis, 1993. The Urticaceae. In: The Families & Genera of Vascular Plants, Vol. 2. Springer-Verlag, Berlin, pp. 612–639.
Friis, I., 2018. Urticaceae. In: Sosef, M.S.M. (Ed.), Flore Du Gabon Vol. 51: Oleaceae, Podostemaceae, Urticaceae. Magraf.
Friis, I., 1989a. The Urticaceae: a systematic review. In: Crane, P. (Ed.), Evolution, Systematics, and Fossil History of the Hamamelidae, Vol. 2. Clarendon Press, Oxford, pp. 285–308.
Friis, I., 1989b. Urticaceae. In: Flora of Tropical East Africa.
Friis, I., 1985. The genus Urera (urticaceae) in eastern tropical Africa. Nord. J. Bot. 5, 547–553.
Friis, I., 1983. A Synopsis of Obetia (Urticaceae). Kew Bull. 38, 221–229.
Friis, I., 1982. The identity of Urera longifolia and U. oligoloba - a supplement to Chew's monograph of Laportea (Urticaceae). Nord. J. Bot. 2, 231–233.
Gaudichaud-Beaupré, C., 1830. Urera, in: Freycinet, L.C.D. de (Ed.), Voyage Autour Du Monde, Entrepris Par Ordre Du Roi, Execute Sur Les Corvettes de S.M. l'Uranie et La Physicienne. Pillet-aine, Paris, pp. 496–497.
GBIF.org, 2019. GBIF Occurrence Download [WWW Document]. https://doi.org/10.15468/dl.jvvwkc.
Global Plants [WWW Document], n.d. URL https://plants.jstor.org/ (accessed 5.15.17).
Hadiah, J.T., Conn, B.J., Quinn, C.J., 2008. Infra-familial phylogeny of Urticaceae, using chloroplast sequence data. Aust. Syst. Bot. 21, 375. https://doi.org/10.1071/sb08041.
Harbaugh, D.T., Wagner, W.L., Allan, G.J., Zimmer, E.A., 2009. The Hawaiian Archipelago is a stepping stone for dispersal in the Pacific: An example from the plant genus Melicope (Rutaceae). J. Biogeogr. 36, 230–241. https://doi.org/10.1111/j.1365-2699.2008.02008.x.
Hawkins, J.A., Hughes, C.E., Scotland, R.W., 1997. Primary Homology Assessment, Characters and Character States. Cladistics 13, 275–283. https://doi.org/10.1111/j.1096-0031.1997.tb00320.x.
Huang, X., Deng, T., Moore, M.J., Wang, H., Li, Z., Lin, N., Yusupov, Z., Tojibaev, K.S., Wang, Y., Sun, H., 2019. Tropical Asian Origin, boreotropical migration and long-distance dispersal in Nettles (Urticeae, Urticaceae). Mol. Phylogenet. Evol. 137, 190–199. https://doi.org/10.1016/j.ympev.2019.05.007.
Johnson, M.G., Gardner, E.M., Liu, Y., Medina, R., Goffinet, B., Shaw, A.J., Zerega, N.J. C., Wickett, N.J., 2016. HybPiper: Extracting Coding Sequence and Introns for Phylogenetics from High-Throughput Sequencing Reads Using Target Enrichment. In: Applications in Plant Sciences. p. 1600016. https://doi.org/10.3732/apps.1600016.
Johnson, M.G., Pokorny, L., Dodsworth, S., Botigué, L.R., Cowan, R.S., Devault, A., Eiserhardt, W.L., Epitawalage, N., Forest, F., Kim, J.T., Leebens-Mack, J.H., Leitch, I. J., Maurin, O., Soltis, D.E., Soltis, P.S., Wong, G.K., Baker, W.J., Wickett, N.J., 2018. A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering. Syst. Biol. 1–13. https://doi.org/10.1093/sysbio/syy086.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. In: Molecular Biology and Evolution. pp. 772–80. https://doi.org/10.1093/molbev/mst010.
Kidner, C., Groover, A., Thomas, D.C., Emelianova, K., Soliz-Gamboa, C., Lens, F., 2016. First steps in studying the origins of secondary woodiness in Begonia (Begoniaceae): Combining anatomy, phylogenetics, and stem transcriptomics. Biol. J. Linn. Soc. 117, 121–138. https://doi.org/10.1111/bij.12492.
Kim, C., Deng, T., Chase, M., Zhang, D.G., Nie, Z.L., Sun, H., 2015. Generic phylogeny and character evolution in Urticeae (Urticaceae) inferred from nuclear and plastid DNA regions. Taxon 64, 65–78. https://doi.org/10.12705/641.20.
Lens, F., Davin, N., Smets, E., del Arco, M., 2013. Insular Woodiness on the Canary Islands: A Remarkable Case of Convergent Evolution. Int. J. Plant Sci. 992–1013. https://doi.org/10.1086/670259.
Lens, F., Groeninckx, I., Smets, E., Dessein, S., 2009. Woodiness within the Spermacoceae-Knoxieae alliance (Rubiaceae): Retention of the basal woody condition in Rubiaceae or recent innovation? Ann. Bot. 103, 1049–1064. https://doi.org/10.1093/aob/mcp048.
Letouzey, R., 1968. Urticaceae. In: Flore Du Cameroun 8. Museum National d'Histoire Naturelle, Paris, pp. 67–216.
Letouzey, R., 1967. Notes Sur Diverses Especes d'Afrique et de Madagascar du Genre Urera Gaudich. (Urticacees). Adansonia Ser. 2, 295–300.
Li, R., Wen, J., 2014. Phylogeny and biogeography of Asian Schefflera (Araliaceae) based on nuclear and plastid DNA sequence data. J. Systemat. Evol. 431–449. https://doi.org/10.1111/jse.12052.
Mirarab, S., Warnow, T., 2015. ASTRAL-II: Coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinformatics i44–i52. https://doi.org/10.1093/bioinformatics/btv234.
Monro, A.K., 2015. Urera. In: Davidse, G., Sousa Sanchez, M., Knapp, S., Chiang Cabrera, F. (Eds.), Flora Mesoamericana: Saururaceae a Zygophyllaceae. Missouri Botanical Garden, Saint Louis, pp. 165–171.
Monro, A.K., 2006. Revision of Species - Rich Genera : a Phylogenetic Framework for the Strategic Revision of Pilea (Urticaceae) based on cpDNA, nrDNA, and Morphology. Am. J. Bot. 93, 426–441.
Monro, A.K., Rodríguez, A., 2009. Three New Species and a Nomenclatural Synopsis of Urera (Urticaceae) from Mesoamerica 1. Ann. Missouri Bot. Gard. 96, 268–285. https://doi.org/10.3417/2006121.
Moyers, B.T., Rieseberg, L.H., 2013. Divergence in Gene Expression Is Uncoupled from Divergence in Coding Sequence in a Secondarily Woody Sunflower. Int. J. Plant Sci. 1079–1089. https://doi.org/10.1086/671197.
Olson, D.M., Dinerstein, E., 2002. The Global 200: Priority Ecoregions for Global Conservation. Ann. Mo. Bot. Gard. 199–224. https://doi.org/10.1109/VPPC.2008.4677498.
Pokorny, L., Riina, R., Mairal, M., Meseguer, A.S., Culshaw, V., Cendoya, J., Sanmartín, I., 2015. Living on the edge: timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. Front. Genet. 6, 154. https://doi.org/10.3389/fgene.2015.00154.
Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, 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. In: Systematic Biology. pp. 539–542. https://doi.org/10.1093/sysbio/sys029.
Rowe, N., Paul-Victor, C., 2012. Herbs and secondary woodiness - keeping up the cambial habit. New Phytol. 193, 3–5. https://doi.org/10.1111/j.1469-8137.2011.03980.x.
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, 1–9. https://doi.org/10.1371/journal.pone.0043808.
Sayyari, E., Mirarab, S., 2016. Fast Coalescent-Based Computation of Local Branch Support from Quartet Frequencies. Mol. Biol. Evol. 33, 1654–1668. https://doi.org/10.1093/molbev/msw079.
Slater, G.S.C., Birney, E., 2005. Automated generation of heuristics for biological sequence comparison. BMC Bioinf. 1–11. https://doi.org/10.1186/1471-2105-6-31.
Smith, S.A., Dunn, C.W., 2008. Phyutility: A phyloinformatics tool for trees, alignments and molecular data. Bioinformatics 24, 715–716. https://doi.org/10.1093/bioinformatics/btm619.
Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
Steinmann, V.W., 2017. Four new neotropical species and a new combination of Urera (Urticaceae). In: Acta Botanica Mexicana. p. 19. https://doi.org/10.21829/abm71.2005.992.
Steinmann, V.W., 2005. FOUR NEW NEOTROPICAL SPECIES AND A NEW COMBINATION OF URERA (URTICACEAE). Acta Bot. Mex. 71, 19–43.
Swofford, D.L., 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA., pp. 1–140.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of choroplast DNA. Plant Mol. Biol. 17, 1105–1109.
The Plant List [WWW Document], 2010. URL http://www.theplantlist.org/ (accessed 5.20.19).
Tseng, Y.H., Monro, A.K., Wei, Y.G., Hu, J.M., 2019. Molecular phylogeny and morphology of Elatostema s.l. (Urticaceae): Implications for inter- and infrageneric classifications. In: Molecular Phylogenetics and Evolution. Elsevier, pp. 251–264. https://doi.org/10.1016/j.ympev.2018.11.016.
Voigt, F.A., Bleher, B., Fietz, J., Ganzhorn, J.U., Schwab, D., Böhning-Gaese, K., 2004. A comparison of morphological and chemical fruit traits between two sites with different frugivore assemblages. Oecologia 141, 94–104. https://doi.org/10.1007/s00442-004-1654-8.
Wagner, W.L., Herbst, D.R., Sohmer, S.H., 1999. Manual of the flowering plants of Hawai'i, revised edition. University of Hawaii Press & Bishop Museum Press, Honolulu.
Weddell, H.A., 1869. Urticaceae, in: Candolle, D. (Ed.), Prodromus Systematis Naturalis Regni Begetabilis, Vol. 16,1. Masson, Paris, pp. 32–235.
Weddell, H.A., 1856. Monogarphie de la Famille des Urticées. Tome IX, Masson, Paris.
Wen, J., Nie, Z.L., Ickert-Bond, S.M., 2016. Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. J. Systemat. Evol. 469–490. https://doi.org/10.1111/jse.12222.
White, T.J., Birns, T., Lee, S.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322 http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1.
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. Mol. Phylogenet. Evol. 69, 814–827. https://doi.org/10.1016/j.ympev.2013.06.022.