Justicieae II: Resolved Placement of Many Genera and Recognition of a New Lineage Sister to Isoglossinae

Lucinda A. McDade
California Botanic Garden, Claremont

Carrie A. Kiel
California Botanic Garden, Claremont

Thomas F. Daniel
California Academy of Sciences, San Francisco

Iain Darbyshire
Royal Botanic Gardens, Kew

Follow this and additional works at: https://scholarship.claremont.edu/aliso

Part of the Botany Commons

Recommended Citation
McDade, Lucinda A.; Kiel, Carrie A.; Daniel, Thomas F.; and Darbyshire, Iain (2021) "Justicieae II: Resolved Placement of Many Genera and Recognition of a New Lineage Sister to Isoglossinae," Aliso: A Journal of Systematic and Floristic Botany: Vol. 38: Iss. 1, Article 2.
Available at: https://scholarship.claremont.edu/aliso/vol38/iss1/2
JUSTICIEAE II: RESOLVED PLACEMENT OF MANY GENERA AND RECOGNITION OF A NEW LINEAGE SISTER TO ISOGLOSSINAE

Lucinda A. McDade1, Thomas F. Daniel2, Iain Darbyshire3, and Carrie A. Kiel1,4

1California Botanic Garden, 1500 North College Avenue, Claremont, California 91711; 2California Academy of Sciences, 55 Music Concourse Drive, San Francisco, California 94118; 3Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3EA, UK
4Corresponding author (ckieli@calbg.org)

ABSTRACT

We present phylogenetic results for Justicieae, the largest of the major lineages of Acanthaceae. With 300% more sequence data and a similarly increased but also more geographically representative taxon sample compared to the only previous study of the lineage as a whole, we confirm the existence of the Pseuderanthemum Lineage, Isoglossinae, the Tetramerium Lineage and the Justicioid Lineage. To the last three lineages, we add a number of taxa with the goal of advancing our knowledge of genera (e.g., Isoglossa, Rhinacanthus) and of geographic areas (e.g., Malagasy and Asian Acanthaceae). These added taxa are accommodated within the phylogenetic framework for the lineages established in our earlier work with one exception. The monospecific genus Ichthyostoma is not placed in any of the established lineages but is instead sister to (Tetramerium Lineage + Justicioid Lineage). As this result is unexpected, we examined topologies both including and excluding this plant which revealed that other aspects of relationships remain stable whether Ichthyostoma is included or not. We point to Ichthyostoma as one of several Justicieae that will benefit from additional study. One species of Isoglossa is placed in the Tetramerium Lineage instead of with all other sampled members of the genus in Isoglossinae. Pollen morphology of I. variegata is more consistent with its phylogenetic placement here than with its taxonomic assignment to Isoglossa. As suggested in our earlier work, pollen morphology can be a powerful signal of phylogenetic relationships in Justicieae. With our increased sampling, we show that the Ptyssiglottis Lineage, a relatively small group of African and Asian plants, warrants recognition separate from Isoglossinae. With the addition of the Ptyssiglottis Lineage and excluding Ichthyostoma for now, the lineages of Justicieae are related as follows: (Pseuderanthemum Lineage ((Isoglossinae + Ptyssiglottis Lineage) (Tetramerium Lineage + Justicioid Lineage))). The Pseuderanthemum Lineage has been understudied to date and we here attempt to include representatives of all genera that are putatively part of this lineage and also to test monophyly of the larger genera. We identify a number of strongly supported clades but some aspects of relationship remain unresolved. Asystasia, Graptophyllum, Oplonia, and Pseuderanthemum are not monophyletic although, with a small number of nomenclatural changes, the first and second can be rendered monophyletic. With a range essentially as extensive as that of Justicieae as a whole, the Pseuderanthemum Lineage is biogeographically complex with patterns of distribution suggestive of multiple shifts between continents including Australia and parts of the Pacific basin. The calibrated phylogeny confirms that intercontinental shifts in distribution across Justicieae must have been by long-distance dispersal rather than by vicariance or stepwise dispersal over land bridges and also points to a number of groups that have potentially undergone rapid diversification. The Pseuderanthemum Lineage, in particular, requires considerable additional research to both understand relationships and achieve an informative taxonomy for the group. Although we here establish a phylogenetic framework across Justicieae, Next Generation Sequence data will be necessary to elucidate details of relationships in most lineages. Additional study of structural characters is also warranted as we continue to be unable to identify structural synapomorphies for a number of aspects of phylogenetic relationships that are very strongly supported by molecular data.

Key words: Acanthaceae, Isoglossinae, Justicieae, Justicioid Lineage, Pseuderanthemum Lineage, Ptyssiglottis Lineage, Tetramerium Lineage.

INTRODUCTION

With at least 2000 species, Justicieae (sensu McDade et al. 2000; = Justiciinae sensu Scotland and Vollesen 2000) is the largest of the recognized major lineages of Acanthaceae. The group is nearly cosmopolitan in distribution with the exception of the high latitudes in both hemispheres and Mediterranean climate regions worldwide, and encompasses remarkable morphological diversity. Perhaps most notably, flowers vary markedly in shape, color, and also in size from just a few mm to 10 cm in length (Fig. 1, 2; see also Fig. 1 in McDade et al. 2018; Fig. 1, 2 in Kiel et al. 2017, Fig. 1 in Kiel et al. 2018). Twenty-one years ago, McDade et al. (2000) published the first phylogenetic analysis that attempted a representative sampling of Justicieae. Although numbers of taxa and characters were limited, these authors identified four major clades: the Pseuderanthemum Lineage, Isoglossinae, the Tetramerium Lineage, and the Justicioid Lineage; relationships among these
Fig. 1. Floral diversity in the Pseuderanthemum Lineage of Justicieae.—A–Q. Pseuderanthemum Clade.—R–V. Asystasia Clade.—A. Ballochia rotundifolia (Socotra).—B. Chileranthemum pyramidatum (Mexico).—C. Graptophyllum pictum (Papua New Guinea).—D. Mackaya bella (South Africa).—E. Odontomena tubaeformis (tropical America).—F. Oplonia sp. (Madagascar).—G. Oplonia microphylla (Jamaica).—H. Ruttya fruticosa (tropical Africa–Arabia).—I. Pseuderanthemum carruthersii (Madagascar).—J. P. hildebrandti (tropical Africa).—K. P. variabile (Australia).—L. P. cuspidatum (tropical America).—M, N. P. floribundum (Mexico).—O. P. alatum (tropical America).—P, Q. P. laxiflorum (southeastern Asia).—R. Asystasia (India).—S, T. Chamaeranthemum gaudichaudi (Brazil).—U. Glossochilus burchellii (South Africa).—V. Herpetacanthus stenophyllus (Costa Rica). All photos by T. Daniel from cultivated plants, except E and F (field photos); U by L. McDade (field photo); V by M. Morales Salazar (field photo).
were supported as: (Pseuderanthemum Lineage (Isoglossinae (Tetramerium Lineage + Justicioid Lineage))).

Morphological traits corroborate many aspects of these relationships including monophyly of Justicieae, which is supported by the presence of tricolporate hexapseudocolpate pollen (i.e., “Spangenpollen” sensu Lindau 1895; Fig. 3A–F). This pollen type is not known in other Acanthaceae. It is present in all major clades of Justicieae with many further innovations (Fig. 3G–R), especially in Isoglossinae and the Justicioid Lineage. Plants of the Pseuderanthemum Lineage have four androecial elements, either having four stamens or two stamens and two staminodes. In contrast, all other Justicieae have just two stamens and no staminodes with the notable exception of Chalarothyrsus Lindau (Tetramerium Lineage) which has four stamens (a few other taxa have been reported to have staminodes as discussed below). Isoglossinae sensu stricto (s.s.; i.e., exclusive of Ptyssiglottis) are marked by the presence of bipororate (more rarely tri- or tetra-pororate) pollen grains that are circular in apertural view and bear marked interapertural “girdle(s)” of tectate exine (i.e., “Gürtelpollen” sensu Lindau 1895; Kiel et al. 2006; Champluvier and Darbyshire 2009; Fig. 3O–R). Plants of the Justicioid Lineage have a rugula (i.e., a medial furrow in the internal surface of the upper corolla lip in which the style lies at anthesis) and often have thecae that are not parallel, not evenly inserted on the filament, and/or are basally awned or spurred. Somewhat surprisingly, McDade et al. (2000) showed that Diclipterinae, a group of plants that mostly lack a rugula and, from a New World (NW) perspective, had largely been understood to have stamens with parallel, evenly inserted thecae that lack appendages (but see below), were shown to be deeply nested among Old World (OW) clades of the Justicioid Lineage.

Three of the four major clades of Justicieae have subsequently been the subject of focused, more densely sampled studies employing at least twice as many sequenced nucleotides as assembled by McDade et al. (2000). The Tetramerium Lineage is now the most thoroughly characterized in terms of taxonomic membership (Daniel et al. 2008; Kiel and McDade 2014; Côrtes et al. 2015; McDade et al. 2018). Phylogenetic work on this group has revealed many nomenclatural problems, most of which remain to be resolved.

Kiel et al. (2006) added considerably to our knowledge of Isoglossinae although sampling was limited; notably, the relationships of Asian Ptyssiglottis T. Anderson were not satisfactorily resolved. The genus was placed sister to Isoglossinae s.s. and treated as part of Isoglossinae s.l. (i.e., in the broad sense). However, plants of Ptyssiglottis lack the morphological synapomorphy of Isoglossinae s.s. (i.e., Gürtelpollen; see Fig. 3O–R), and placement of the genus as sister to the clade of plants marked by that trait was not strongly supported. In addition, whereas Isoglossinae s.s. occur primarily in Africa and the NW with only a few Asian members, Ptyssiglottis is exclusively Tropical Asian. A number of additional, putatively related Asian taxa have yet to be sampled for phylogenetic research. Kiel et al. (2006) also identified a clade comprising OW Brachystephanus Nees and all NW Isoglossinae that is further marked by monothesous anthers (see Fig. 2F in Kiel et al. 2006). In sum, this phylogenetic neighborhood clearly warrants further study.

More recently, Kiel et al. (2017, 2018) compiled a taxonomically and geographically representative sample of the large Justicioid Lineage and pointed to a number of strongly supported clades within it, while also noting the difficulty in diagnosing many of these clades morphologically. This recent work confirmed that Diclipterinae are nested in the Justicioid Lineage. Thus, only the broader Isoglossinae (i.e., inclusive of Ptyssiglottis and putative relatives) and the Pseuderanthemum Lineage have not yet been the subject of focused study.

Subsequent studies have also indicated that McDade et al.’s (2000) interpretation of androecial evolution across Justicieae was incomplete owing to inadequate sampling of OW taxa. First, many OW Dicliptera Juss. and Peristrophe Nees (Diclipterinae) have stamens with superposed thecae (Balkwill 1996; Balkwill...
Fig. 3. Pollen of Justicieae.—A–F. Typical tricolporate, hexaspseudocolpate pollen (Spangenpollen).—A. Calycacanthus magnusianus (Daniel & Jebb 6518), apertural view.—B. Ballochia rotundifolia (Daniel s.n., cultivated), apertural view.—C. Monothecium glandulosum (Biddle et al. 4897), interapertural view.—D. Pseudaristemon cuspidatum (Breedlove & Daniel 70880), interapertural view.—E. P. alatum (Daniel & Baker 3713), polar view.—F. B. rotundifolia (Daniel s.n., cultivated), polar view.—G–J. Variation from typical Spangenpollen.—G. Graptophyllum spinigerum (Daniel 10059), pseudocolpi in mesocolpia fused or nearly so, interapertural view.—H. G. spinigerum (Daniel 10059), polar view.—I. Jadania biroi (Daniel et al. 6603), pentacolporate, decaspseudocolpate, interapertural view.—J. J. biroi (Daniel et al. 6603), polar view.—K–N. Variation/derivation from typical tricolporate, hexaspseudocolpate.—K. Herpetacanthus stenophyllus (Daniel et al. 6227), irregular sculpturing in mesocolpia, apertural view.—L. H. longiflorus (de Sant’Anu et al. 606), 4-pororate, echinate sculpturing, polar view.—M. Isotheca alba (Broadway 7092), 4-pororate, echinate sculpturing, polar view.—N. I. alba (Broadway 7092), subapertural view.—O–R. Bipororate pollen (“Gürtelpollen”).—O. Isoglossa gracillima (Daniel et al. 9106), apertural view.—P. Sphacanthus brillantaisia (Humbert 19989), interapertural view.—Q. Stenostephanus leiorhachis (Daniel et al. 6231), apertural view.—R. S. leiorhachis (Daniel et al. 6231), interapertural view.
et al. 1996; Darbyshire 2009a; Kiel et al. 2017) such that Di-
ciplerinae are not, in fact, out of place in the Justicioid Lineage on
the basis of this trait. Also, anthers with the thecae offset, superposed and/or oblique occur in some African members of
core Isoglossinae, notably plants of many species of Isoglossa
Oerst. (Hansen 1985; Darbyshire 2009b; Darbyshire et al. 2011;
Balkwill et al. 2017). Clearly, a great deal remains to be learned
about patterns of androecial evolution in Justicieae.

In a family-wide study, McDade et al. (2008) sought to
sample all genera that had not yet been placed in a major
lineage of Acanthaceae. These authors placed Calycanthes K. Schum. (Isoglossinae s.l.) (Fig. 2A) and Kuducanthes
Hosok. (Tetramerium Lineage) with confidence in Justicieae.
The fact that Calycanthes was placed sister to Ptyssiglottis, with these together sister to Isoglossinae s.s., albeit with weak
support from Bayesian inference, confirms the need for addi-
tional study of this phylogenetic neighborhood as noted above.

In the present study, we again take a wide-angle perspective
on Justicieae. We seek to test the results of McDade et al. (2000)
regarding the existence of four clades and relationships among
them using nearly 300% more sequence data than gathered by
those earlier authors. We focus especially on the Pseuderanthe-
num Lineage with the goal of sampling all genera that are puta-
tively part of that group. These are plants with four androecial ele-
ments and tricarpellate hexaploid or diploid (Fig. 3A–F), and
that lack the synapomorphies that characterize other clades
of Justicieae. We also seek to test the monophyly of larger gen-
era in the Pseuderanthemum Lineage (i.e., those with more than
a handful of species; Table 1). We add a number of Asian plants
putatively related to Ptyssiglottis (i.e., plants with two stamens,
no staminodes, tricarpellate hexaploid or diploid, and lack-
ing the synapomorphies of other clades). We did not seek to sam-
ple densely from well characterized clades (i.e., core Isoglossi-
nae, Tetramerium Lineage, Justicioid Lineage) except that (1) a
number of additional Asian taxa were added because this geo-
graphic region has been under-sampled in some of our earlier
work, (2) a number of Malagasy taxa were sampled with the
goal of advancing our knowledge of the rich but still poorly doc-
dumented acanthaceous flora of Madagascar, and (3) additional
African taxa of the Justicioid Lineage were sampled including
Ascotheca Heine, a previously unsampled monospecific genus.

MATERIALS AND METHODS

Taxon Sampling Strategy

We attempted to include representatives of all putative gen-
era of Justicieae that had not been sampled earlier (Table 1). In
addition to previously unsampled genera, for the Pseuderanthe-
num Lineage, we sampled multiple species of the larger genera with the goal of testing monophyly of Asystasia
Blume, Graptophyllum Nees, Odontomena Nees, and Pseuder-
anthemum Radlk. ex Lindau. Notably, species of Pseuder-
anthemum occur in Africa, Asia, Australia, Polynesia, and the
NW, and we sampled species from all of these regions. Like-
wise, Oplonia Raf. has species that occur in the NW and in
Madagascar. In the NW, the group is primarily Caribbean but
also has three species that occur in South America. We sam-
pled species from both regions in the NW and from Madagas-
car. For Isoglossinae, we focused on plants hypothesized to be
related to Ptyssiglottis, shown by Kiel et al. (2006) to be sister
to Isoglossinae s.s. These are Asian plants with the exception of
Champluviera I. Darbyshire, T.F. Daniel, C.A. Kiel (Africa),
Ichthyostoma Hedrén & Vollesen (Africa) and Monothecium
Hochst. (Africa, Asia). We also added a number of Malagasy
plants that are hypothesized to belong to Isoglossinae s.s. Given
recent work on the Tetramerium Lineage (McDade et al. 2018),
we included a relatively small sample of taxa chosen to span
the phylogenetic and geographic range of the clade. Likewise,
owing to recent work on the Justicioid Lineage (Kiel et al. 2017,
2018), we did not sample densely from this lineage but in
stead sought to better understand groups shown not to be mono-
phyletic, notably Anisotes Nees and Rhinacanthus Nees. We
also sampled more Asian representatives toward documenting
genetic patterns across Justicioids. We included seven taxa
as out-groups for purposes of rooting; these represent Acanthaceae
(Aphelandra leonardi McDade, Stenandrium pilosulum (S.F. Blake) T.F. Daniel), Barlerieae (Barleria lupulina Lindl., Lep-
idagathis formosensis C.B. Clarke ex Hayata, Pseudodiciptera
sulfureolilacina Benoist) and Ruelliaeae (Ruellia californica I.M.
Johnst., Sanchezia speciosa Leonard). Initially, 151 accessions
were included. As explained below, inspection of the sequence
data and preliminary results for Isotetxia Turrill and Thysanos-
tigma J.B. Inlay pointed strongly to data quality issues and
these two taxa were subsequently excluded. Further, owing to
eignmatic placement of Ichthyostoma (see below), we under-
took analyses that both included (total of 149 accessions) and
excluded this taxon (148 accessions) with the goal of avoiding
any erroneous impact of Ichthyostoma on other aspects of rela-
tionships. Taxa included in this study in addition to voucher data
and GenBank numbers are listed in Appendix 1.

Molecular Methods

The taxa newly sampled for this study were only available as
herbarium specimens. Samples were extracted using Doyle and
Doyle’s (1987) CTAB method or with QIAGEN DNEasy™ kits
(QIAGEN, Inc., Valencia, California). Procedures to amplify
the five loci used here have been described in detail elsewhere
as follows: intergenic spacers trnT-L (Kiel et al. 2006), trnL-F
(McDade and Moody 1999) and trnS-G (McDade et al. 2005);
the rps16 intron (McDade et al. 2005). Procedures for amplifi-
cation of the nuclear ribosomal internal transcribed spacer
region (nrITS) are found in McDade et al. (2000) and Daniel et al.
(2008). For nrITS, when reactions failed with both primer pairs,
N-nc18S+10 + C26A (Ren and Zimmer 1996) and ITS9 (see
Daniel et al. 2008) + C26A, nrITS was amplified in two parts
using (1) N-nc18S+10 (Ren and Zimmer 1996) and ITS2 (Bald-
win et al. 1995) and (2) ITS3 (Baldwin et al. 1995) and C26ab,
a modification of primer C26A (Kiel and McDade 2014).

PCR products were cleaned using PEG precipitation (Morgan
and Soltis 1993) and the purified PCR products were cycle se-
cquenced with both forward and reverse PCR primers using
Applied Biosystems (ABI, Foster City, California) PRISM BigDye
vers. 3.1, according to the manufacturer’s specifications. All cy-
cle sequencing reactions were cleaned with Sephadex G50 and
sequenced on an ABI 3100 genetic analyzer at the Core Molecu-
lar Laboratory at California Botanic Garden.

Alignment and Analyses

Chromatograms were edited and aligned following meth-
ods discussed by Morrison (2006) for manual alignment of
Table 1. Genera of Justicieae, estimated species in each genus, and distribution. For estimated species per genus, we used Plants of the World Online (http://www.plantsoftheworldonline.org/), the African Plants Database (http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php?langue=an) and the Madagascar catalogue resource of the Missouri Botanical Garden (http://legacy.tropicos.org/Project/Madagascar). For the Pseuderanthemum Lineage and the Ptyssiglottis Lineage, we indicate how many species were sampled for the present study. For the other lineages, because we sampled placeholders rather than attempting a thorough or dense sampling effort, taxon sampling for the present study is not indicated (see text for full explanation). 1Attempts to extract DNA from samples of these genera were not successful; they are tentatively placed in lineages based on morphology. 2Sequences generated for these genera were problematic as described in the text; they are tentatively placed in lineages based on morphology. 3Genera not sampled here but included in other phylogenetic studies and placed with confidence in Justicieae and lineages as indicated: 4Deng et al. (2016); 5Kiel et al. (2006); 6McDade et al. (2018); 7Côrtes et al. (2015); 8Kiel et al. (2018); 9Kiel et al. (2017).

| Genus or lineage           | Estimated species # (# sampled) | Distribution                                                |
|---------------------------|---------------------------------|-------------------------------------------------------------|
| Genera incertae sedis     |                                 |                                                             |
| *Dicladanthera* F. Muell. | 2 (0)                           | Australia                                                   |
| *Dolichostachys* Benoist  | 1 (0)                           | Madagascar                                                  |
| *Ichthyostoma* Hedrén & Vollesen | 1 (1)                      | Ethiopia, Somalia                                          |
| *Tessmanniactanus* Mildbr.| 1 (0)                           | Peru                                                       |
| Pseuderanthemum Lineage   |                                 |                                                             |
| *Afrofittonia* Lindau     | 1 (1)                           | Cameroon                                                    |
| *Asystasia* Blume         | 64 (6)                          | Pantropical in OW                                           |
| *Ballochia* Balf. f.      | 3 (3)                           | Socotra                                                     |
| *Chamaeranthemum* Nees    | 7 (2)                           | Central & South America                                     |
| *Chilaranthemum* Oerst.   | 3 (1)                           | Mexico & Central America                                    |
| *Codonacanthus* Nees      | 3 (1)                           | E & S Asia                                                 |
| *Cosmanianthemum* Bremek. | 13 (1)                          | SE Asia, Malesia                                           |
| *Filetia* Miq.            | 9 (1)                           | Malesia/Papuasia                                           |
| *Glossochilus* Nees       | 1 (1)                           | South Africa                                               |
| *Graptophyllum* Lindau    | 15 (3)                          | Malesia/Papuasia/Australia + W Central Trop Africa          |
| *Herpetacanthus* Ness     | 21 (2)                          | Central & South America                                    |
| *Linariantha* B.L. Burtt & R.M. Sm. | 1 (1)          | Trinidad & N Venezuela                                     |
| *Mackaya* Harv.           | 4 (1)                           | Malesia                                                    |
| *Odontonema* Nees         | ~30 (3)                         | Mexico, Central, South America                             |
| *Oplonia* Raf.            | 21 (5)                          | Madagascar, New World                                      |
| *Phialacanthus*           | 5 (0)                           | Himalayas, Malesia                                         |
| *Pranceacanthus* Wassh.   | 1 (1)                           | Bolivia & Brazil                                           |
| Pseuderanthemum Radlk.    | ~100 (9)                        | Pantropical                                                |
| *Psilanthele* Lindau      | 4 (1)                           | South America                                              |
| *Pulchranthus* V.M. Baum, Reveal & Nowicke | 6 (1)          | Sub-Saharan Africa, Madagascar                             |
| *Ruttya* Harv.            | 6 (1)                           | Africa, Madagascar                                         |
| *Sappho* Urb.             | 2 (1)                           | Cuba                                                       |
| *Spathacanthus* Baiill.    | 4 (2)                           | Mexico, Central America                                    |
| *Thysanostigma* J.B. Imlay | 2 (1)                        | Indochina                                                  |
| *Wuacanthus* Y.F. Deng, N.H. Xia & H. Peng | 1 (0)          | Southern China                                             |
| Ptyssiglottis Lineage     |                                 |                                                             |
| *Ambongia* Benoist        | 1 (0)                           | Madagascar                                                  |
| *Calycacanthus* K. Schurm. | 1 (1)                           | Papuasia                                                   |
| *Champluviera* L. Darbysh., T.F. Daniel & C.A. Kiel | 2 (1)          | West-Central Tropical Africa                               |
| *Cylocalanthus* S. Moore  | 2 (0)                           | Indochina                                                  |
| *Jadania* Lindau          | 2 (1)                           | Papuasia                                                   |
| *Marcania* J.B. Imlay     | 1 (1)                           | Papuasia                                                   |
| *Monotheicum* Hochst.     | 3 (3)                           | Africa, Madagascar, Indochina                              |
| Ptyssiglottis T. Anderson | ~35 (2)                         | Tropical Asia                                              |
| Isoglossinae Lineage      |                                 |                                                             |
| *Brachystephanus* Nees    | 22                              | Africa, Madagascar                                         |
| *Celerina* Benoist        | 1                               | Madagascar                                                  |
| *Isoglossa* Oerst.        | 81                              | Pan-OW tropical                                             |
| *Kalbreyeriella* Lindau   | 4                               | Panama, So America                                          |
| *Melittacanthus* S. Moore | 1                               | Madagascar                                                  |
| *Sebastiano-schaueria* Nees | 1                          | Brazil                                                      |

Legend: 1Genera not sampled; these are treated as incertae sedis or have been placed tentatively based on morphology. 2Attempts to extract DNA from samples of these genera were not successful; they are tentatively placed in lineages based on morphology. 3Sequences generated for these genera were problematic as described in the text; they are tentatively placed in lineages based on morphology. 4Genera not sampled here but included in other phylogenetic studies and placed with confidence in Justicieae and lineages as indicated: 4Deng et al. (2016); 5Kiel et al. (2006); 6McDade et al. (2018); 7Côrtes et al. (2015); 8Kiel et al. (2018); 9Kiel et al. (2017).
Table 1. Continued.

| Genus or lineage | Estimated species # (# sampled) | Distribution |
|------------------|---------------------------------|--------------|
| *Sphacanthus* Benoist | 2 | Madagascar |
| *Stenostephanus* Nees | ~80 | Widespread in NW Tropics |

**Tetramerium Lineage**

| Genus or lineage | Estimated species # (# sampled) | Distribution |
|------------------|---------------------------------|--------------|
| *Ancistranthus* Lindau | 1 | Cuba |
| *Angkalanthus* Baill. | 1 | Socotra |
| *Anisacanthus* Nees | 11 | Mexico, Central America, South America |
| *Aphanosperma* T.F. Daniel | 1 | Mexico |
| *Carlrowrightia* A. Gray | 26 | US, Mexico, Central & South America |
| *Cephalophilus* Vollesen | 1 | Kenya |
| *Chalarothysus* Lindau | 1 | Mexico |
| *Chlamydocardia* Lindau | 2 | West & Central Tropical Africa |
| *Chorisochora* Vollesen | 4 | Socotra & So Africa |
| *Clinacanthus* Nees | 4 | Indochina, Malesia |
| *Ecbolium* Kurz | 22 | OW Widespread |
| *Ectotonia* Coem. | 2 | Western South America |
| *Gypsacanthus* E.I. Lott, V. Jaram. & Rzed. | 1 | Mexico |
| *Henrya* Nees | 2 | Mexico & Central America |
| *Hoverdenia* Nees | 1 | Mexico |
| *Kadoacanthus* Hosok. | 1 | Taiwan |
| *Megalolamylia* Lindau | 10 | Eastern & Southern Africa, Southern Arabian Peninsula |
| *Mexacanthus* T.F. Daniel | 1 | Mexico |
| *Mirandea* Rzed. | 6 | Mexico |
| *Pachystachys* Nees | 18 | West Indies, South America |
| *Pogalina* Bail. | 2 | Madagascar |
| *Schaueria* Nees | 14 | E Brazil, Central America, Mexico |
| *Streblacanthus* Kuntze | 1 | Mexico, Central America |
| *Tetramerium* Nees | 31 | SW United States, Mexico, Central & Western South America |

**7 Thyrsacanthus** Moric.

| Genus or lineage | Estimated species # (# sampled) | Distribution |
|------------------|---------------------------------|--------------|
| *Trichocalyx* Balf. f. | 2 | Socotra |
| *Vavara* Benoit ex I. Darbysh. & E. Tripp | 1 | Madagascar |
| *Xeranthemella* C.T. White | 2 | Australia |

**Newly added to Tetramerium Lineage**

| Genus or lineage | Estimated species # (# sampled) | Distribution |
|------------------|---------------------------------|--------------|
| *Isoglossa* variegata | - ( 1 ) | Tanzania |

**Justicioioid Lineage**

| Genus or lineage | Estimated species # (# sampled) | Distribution |
|------------------|---------------------------------|--------------|
| *Anisostachya* Nees | 60–100 | Africa, Madagascar |
| *Anisthes* Nees | 30 | Africa, Madagascar |
| *Ascotheca* Heine | 1 | Gabon, Cameroon |
| *Cephalanthis* Lindau | 1 | Peru |
| *Clistax* Mart. | 3 | Brazil |
| *Dasytropis* Urb. | 1 | West Indies |
| *Dicliptera* Juss. | ~220 | Pantropical & Subtropical |
| *Harpochilus* Nees | 2 | Brazil |
| *Hypoestes* Sol. ex R.Br. | ~130–150 | Pantropical OW |
| *Justicia* L. | ~700 | Pantropical & Subtropical |
| *Kenyacanthus* I. Darbysh. & C.A. Kiel | 1 | Kenya |
| *Megaskepasma* Lindau | 1 | Northern South America |
| *Metarungia* Baden | 3 | Southern Africa |
| *Monechma* Hochst. | 43 | Africa |
| *Poikilacanthus* Lindau | 13 | NW Tropical |
| *Rhinacanthus* Nees | ~30 | Pan-OW tropical |
| *Rungia* Nees | ~80 | Pan-OW tropical |
| *Samuclsonia* Urb. & Ekman | 1 | Hispaniola |
| *Trichocalyx* Balf. f. | 2 | Socotra |
| *Vavena* Benoist ex I. Darbysh. & E. Tripp | 1 | Madagascar |
| *Xeranthemella* C.T. White | 2 | Australia |
sequence data for phylogenetic analysis in Geneious® vers.10.2.6 (Biomatters Ltd.® 2005–2018) (alignments available in Treebase, TB2:S27852). Long mononucleotide repeats were considered to have uncertain homologies. Because peak signal in chromatograms typically weakened markedly after eight mononucleotide repeats, we excluded variation in mononucleotide repeats of >9 bp from the analysis.

Data matrices for the five DNA regions were prepared as nexus files in Geneious. Possible incongruence between the nrITS and plastid data sets was examined by comparing the results of the most likely trees from the ML analyses (conducted as described below). Using a maximum likelihood bootstrap (MLBS) value of >75% as the threshold for well-supported conflict, we found evidence of incongruence between the nuclear and cp data sets for Linariantha bicolor B.L. Burtt & R.M. Sm. For this plant, the cp data were incongruent with morphological evidence and the cp data were therefore scored as missing. In other cases, visual inspection of sequence alignments suggested that one or more genetic regions was incongruent with the remaining data. Because decades-old herbarium specimens were the only material that we could acquire for many of these plants, we considered the possibility that sequencing errors or cross-contamination might have impacted data quality. We also considered the possibility that different genetic regions might have different phylogenetic histories (see, e.g., Tripp et al. 2013). We conducted BLAST® searches on the NCBI web portal (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for each genetic region for all taxa for which there was evidence of incongruence among loci. We assessed sequence data as likely erroneous when best matches differed among genetic regions or when best matches were to plants that were likely to be distant relatives based on morphological or other evidence. Such regions were coded as missing data. In the discussion section, we point to these patterns of incongruence and clearly identify these taxa as warranting further study. With modifications based on these assessments, there were no strongly supported differences between the plastid and nrITS data (Fig. S1, S2), and the data were combined for further analyses.

The best model of nucleotide substitution and across-site heterogeneity in evolutionary rates was inferred using ModelTest NG 0.1.5 (Darriba et al. 2020) and the best-fit model was selected based on the correct Akaike’s information criterion. The selected models of nucleotide substitution were GTR + G for each of the plastid loci and GTR + G + I for nrITS. The concatenated dataset comprised five data partitions corresponding to the four cp loci + nrITS, with models of evolution as just established. ML analysis of the concatenated and partitioned dataset was conducted using IQ-TREE 1.6.10 (Nguyen et al. 2015) under the best fit models inferred as just described. Branch support was obtained from 1000 nonparametric bootstrap replicates. This analysis was repeated three times to test the consistency of tree topology and MLBS values.

Bayesian inference (BI) of phylogenetic relationships of the 149-taxon dataset with posterior probabilities (BPP) as the support measure was conducted in MrBayes vers. 3.2.6 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). The same models of molecular evolution for the genetic regions used in the ML analyses were implemented in the MrBayes analysis. Each dataset was run with data partitions corresponding to the combined cp and nrITS regions. For each analysis, two simultaneous runs of four Monte Carlo Markov Chains were run for 20,000,000 generations with a random tree as the starting point and saving trees every 1000 generations. To determine MCMC convergence, the log files were accessed to examine the trace files in Tracer vers. 1.7.1 to retrieve data on the standard deviation of split frequencies (Rambaut et al. 2014). Convergence to stationarity was assumed when split frequencies were below 0.01 and ESS values >200.

Because of sampling limitations and phylogenetic uncertainty in the Pseuderanthemum Lineage, we did not analyze ancestral ranges or diversification rates quantitatively. However, to provide temporal context for the diversification of Justicieae, we used a subset of the fossils studied by Tripp and McDade (2014) to calibrate the phylogeny as follows. The combined 149-taxon dataset was used to estimate divergence times in BEAST2 vers. 2.2.5 (Bouckaert et al. 2014). Rate heterogeneity was implemented using a relaxed clock model (Drummond et al. 2006) along with an uncorrelated lognormal distribution and a GTR model of evolution for each of the partitions. Two fossil calibrations were used for the analysis: one that pertains to the Justicioid Lineage and a second that represents Justicieae as a whole. These fossils were assessed by Tripp and McDade (2014) to be both reliably identified and accurately dated. Fossil #32 of Tripp and McDade (2014) from the Middle Miocene is a dicolporate pollen grain with distinctive round insulae that laterally flank the apertures (Mautino 2011); the latter of these traits is known only among the Justicioid Lineage (Graham 1988; Kiel et al. 2017, 2018). This fossil was used to constrain the most recent common ancestor of the Justicioid Lineage. A lognormal distribution with a zero offset of 11.5, log standard deviation of 1.5, mean of 1.6, 5% quantile of 11.5, and 95% quantile of 17.6 (Tripp and McDade 2014) was implemented for this calibration. Tripp and McDade’s (2014) fossil #20 from the Lower Miocene is of tricolporate hexapseudocolpate pollen (Beialy et al. 2005). This fossil was set to constrain the most recent common ancestor of the Justicieae Lineage. A lognormal distribution with a zero offset of 14.6, log standard deviation of 1.3, mean of 2.5, 5% quantile of 14.7, and a 95% quantile of 23.7 (Tripp and McDade 2014) was used for this calibration. The third calibration point was the estimated age of Justicieae as a whole taken from Tripp and McDade’s (2014) family-wide analysis. A normal distribution with a mean of 35.3 and a zero offset was implemented for this calibration. A birth-death speciation process (Nee et al. 1994; Gerhard 2008) with a uniform prior were specified for the tree prior, and a random starting tree was used. Two independent runs of 200 million generations both contributed to the posterior. Log files were examined in Tracer vers. 1.71 (Rambaut et al. 2014) to evaluate the effective size of the sample for all parameters (ESS ≥ 200) and a conservative burn-in of 20% was applied after checking the log-likelihood curves. The separate runs were merged using LogCombiner 2.5.2 and the maximum clade credibility tree with median ages, 95% highest posterior density (HPD) and BPP were generated with TreeAnnotator 2.5.2.

Our results indicated that a number of genera are not monophyletic and we tested whether our data (149-taxon dataset) could reject monophyly of (1) Asystasia; (2a) Oplonia, (2b) NW Oplonia, (2c) Caribbean Oplonia; (3a) Pseuderanthemum, (3b) New World Pseuderanthemum, (3c) African Pseuderanthemum, (3d) Papuan/Pacific Pseuderanthemum; (4) Graptopodium Nees; (5) Monothecium Hochst.; (6) Isoglossa (inclusive of I. variegata I. Darbysh.); and (7) Ptyssiglottis. In addition, using the 149-taxon dataset, we tested whether placement of
Ichthyostoma thulinii with either the Tetramerium Lineage or the clade composed of the Ptyssiglottis Lineage + Isoglossinae could be rejected.

These alternative phylogenetic hypotheses were examined using approximately unbiased (AU) tests (Shimodaira 2002). Constraint trees were constructed in Mesquite v. 2.72 (Maddison and Maddison 2009). For each constraint, all aspects of relationship were constructed as a single polytomy, with the exception of the hypothesis under consideration. The constraint trees were loaded into IQ-TREE (Nguyen et al. 2015) and run with the settings and model as described above. The best trees from the unconstrained and constrained analyses were combined into a single file and loaded into IQ-TREE, and likelihood scores were compared using the AU test with RELL-optimization and 10,000 bootstrap replicates.

Geographic ranges of Justicieae that occur in Asia, Australia and the Pacific are reported using the L1 and L2 levels of the World Geographical Scheme for Recording Plant Distributions (Brummitt 2001; TDWG: http://www.tdwg.org/standards/109, accessed 23 Aug 2020); for plants that occur in the Americas and Africa, we use country names except that, in a few cases, we use the L2 regional names for African plants.

Pollen Morphology

For scanning electron microscopy (SEM), pollen from selected taxa was mounted on aluminum stubs using double-stick conductive tape, and coated with gold-palladium prior to observation at 15 kV at the SEM lab at California Academy of Sciences on either a Hitachi S-520, Leo 1450-VP, or a Hitachi SU3500 SEM. In describing pollen morphology, we use widely understood palynological terms with two exceptions. We follow Lindau (1895) in using “Spangenpollen” for tricolporate hexapseudocolpate pollen (Fig. 3A–F) because of its phylogenetic signal across Justicieae. As appropriate, we describe variation in Spangenpollen rather than use additional terms. For example, when aperture number differs from three or when colpi or pseudocolpi extend to the poles, we so indicate. We also use the term “Gürtelpollen,” because of phylogenetic signal but also because describing these grains otherwise requires many words: “biporate (more rarely tri- or tetra-porate) pollen grains that are circular in apertural view and with marked interapertural “girdle(s)” of tectate exine” (Fig. 30–R). Pollen grains of other types are described in common palynological terms.

RESULTS

Ertigmatic Taxa and Data Quality Problems

Examination of preliminary results for seven taxa indicated that they were placed differently by data from different genic regions or were placed enigmatically based on other data about these plants–usually androecial morphology. This resulted in several modifications to the original dataset. When analyzed separately, sequences obtained from three amplified loci, trnL-F, trnS-G, and nrITS for Isotheca alba Turrill were resolved in different major lineages of Justicieae, pointing to problems with data quality; this taxon was omitted. Unexpectedly, sequences obtained for Thysanostigma odontites (Ridl.) B. Hansen were essentially identical to those for Ichthyostoma thulinii, a highly unlikely outcome based on marked differences in phylogenetically relevant characters (e.g., androecial complement) and geographic range (the former is Asian, the latter African). Because placement of these taxa was most consistent with morphology for Ichthyostoma but not so for Thysanostigma, we omitted the latter from subsequent analyses. Further, Ichthyostoma was placed as the sole member of a clade sister to (Tetramerium Lineage + Justicioid). Because all other accessions were placed with strong support in one of five lineages, and considering also data quality concerns, we first include Ichthyostoma (149-taxon dataset), but then exclude it from further analyses in order to ensure that this enigmatic taxon did not bias results for other Justicieae.

For other taxa, one or more loci were omitted when BLAST searches pointed to incongruence, as follows: Anisotes perepélexus T.F. Daniel, Letsara & Martín-Bravo and both species of Spathacanthus Baill.: trnL-F omitted; Cosmianthemum magnifolium Bremek.: trnS-G and rps16 omitted; Ichthyostoma thulinii: trnS-G omitted; Liniantha bicolor: cp data omitted. We discuss these data quality problems, the likely placement of these taxa, and the need for fresh material and further research in the Discussion section.

Table 2 compares the five DNA regions in terms of length, variability, number of taxa sequenced, and missing data, after making the adjustments just described. After these adjustments (including removal of Ichthyostoma), the combined data matrix consisted of 148 (140 in-group + eight out-group) taxa and had an aligned length of 5607 characters.

Phylogenetic Relationships

Figure 4 summarizes results of the 149-taxon analysis, showing monophyly of five major lineages and placement of Ichthyostoma thulinii sister to Tetramerium Lineage + Ptyssiglottis (BPP = 0.95; MLBS = 93%). Our data reject placement of this plant with the Tetramerium Lineage (P < 0.001) but cannot reject placement as sister to Ptyssiglottis Lineage + Isoglossinae (P = 0.079) (Table 3). Omitting Ichthyostoma thulinii had no impact on relationships among other taxa and little impact on support values (i.e., compare support values for major lineages shown in Fig. 4 and in Fig. 5–7).

Figures 5–7 present results from the BEAST2 analysis of the 148-taxon dataset (i.e., Ichthyostoma omitted, as described above). As the ML and BEAST2 results were entirely congruent, ML bootstrap values and posterior probabilities are shown for each branch in Fig. 5–7. Justicieae are monophyletic with strong support and all newly sampled taxa hypothesized to be part of this large clade were placed within it (Fig. 4–7). With Ichthyostoma thulinii omitted, all sampled taxa are placed in one of five major lineages: (1) Pseuderanthemum Lineage (Fig. 5), (2) Ptyssiglottis Lineage + (3) Isoglossinae (= core Isoglossinae sensu Kiel et al. [2006], given recognition herein of the Ptyssiglottis Lineage) (Fig. 6), and (4) Tetramerium Lineage + (5) Justicioid Lineage (Fig. 7).

Our data strongly support monophyly of the Pseuderanthemum Lineage and the sister relationship between this lineage and the rest of Justicieae (Fig. 4, 5). Within the Pseuderanthemum Lineage, the two sampled members of NW Spathacanthus are sister, and are together sister to the remainder of the lineage (Fig. 5). The Asystasia Clade and Pseuderanthemum Clade are sister and both are monophyletic with strong support. The Asystasia Clade consists of a clade of OW taxa that includes all
Table 2. Aligned length, variability, parsimony-informative sites, missing data and GC content for the DNA regions used in this study. Alignment statistics were calculated in AMAS (Borowiec 2016).

| Region       | trnL-F | trnS-G | trnT-L | rps16 | nrITS |
|--------------|--------|--------|--------|-------|-------|
| Aligned length (bases) | 1347   | 1346   | 1039   | 982   | 876   |
| Variable sites (%), all taxa | 729 (54.1%) | 670 (49.8%) | 702 (67.6%) | 639 (65.1%) | 620 (70.8%) |
| Variable sites (%), in-group | 668 (49.6%) | 581 (43.2%) | 648 (62.4%) | 605 (61.6%) | 582 (66.4%) |
| Parsimony-informative sites (%), all taxa | 369 (27.4%) | 380 (28.2%) | 389 (37.4%) | 361 (35.8%) | 390 (44.5%) |
| Parsimony-informative sites (%), in-group | 305 (22.6%) | 325 (24.1%) | 308 (29.6%) | 324 (33.0%) | 359 (41.0%) |
| Missing taxa (# taxa of 146 accessions) | 1 (1.5%) | 8 (5.5%) | 19 (13.0%) | 27 (18.4%) | 12 (8.2%) |
| GC content (%) | 28.7% | 30.8% | 26.4% | 35.2% | 67.7% |

sampled species of *Asystasia* plus newly sampled *Glossochilus burchelli* Nees (Fig. 1U), *Salpinctium stenophhon* T.J. Edwards and *Filetia rideyi* C.B. Clarke (*Asystasia Subclade*, Fig. 5). The last species is embedded among species of *Asystasia* and our data reject exclusion of *F. rideyi* to yield a monophyletic *Asystasia* (Table 3; P < 0.001). Sister to this clade is the *Herpetacanthus* Clade comprising NW *Chamaeranthemum* Nees ex Lindl. (2 accessions), *Herpetacanthus* Nees (2 spp.), and *Pranceacanthus coccineus* Wassh., but also African *Afromiltonia silvestris* Lindau. Both *Chamaeranthemum* and *Herpetacanthus* are strongly supported as monophyletic.

The *Pseuderanthemum* Clade (Fig. 5) includes all sampled accessions of *Ballochia* Balf. f. (3 spp.), *Graptophyllum* (6 accessions), *Odontonema* (3 spp.), *Oplonia* (6 accessions), *Pseuderanthemum* (10 spp.), and *Ruttya* Harv. (2 spp.), as well as single representatives of ten genera. *Cosmianthemum magnifolium* Bremek. + *Mackaya bella* Harv. (Fig. 1D) are sister with strong support, and are together sister to the rest of the *Pseuderanthemum* Clade, followed by Asian *Codonacanthus pauciflorus* (Nees) Nees, and NW *Chileranthemum pyramidatum* (Lindau) T.F. Daniel (Fig. 1B) + *Pulchranthus variegatus* (Aubl.) V.M. Baum, Reveal & Nowicke as serially sister to the remaining members of the *Pseuderanthemum* Clade. Other taxa are placed in five clades (marked with circled numbers in Fig. 5): (1) the three sampled species of *Odontonema* + *Pseuderanthemum floribundum* T.F. Daniel (Fig. 1M, N) (all NW taxa); (2) a NW clad composed of NW *Oplonia* (5 accessions), *Psilanthele eggersii* Lindau, *Sapphoa rigidifolia* Urb. and two species of *Pseuderanthemum* (*P. alatum* (Nees) M.R. Almeida (Fig. 1O), *P. cuspidatum* (Nees) Radlk. ex Lindau (Fig. 1L); (3) three African species of *Pseuderanthemum* + Asian *Linariantha bicolor*; (4) a Malesian/Papuasian/Australian/Pacific clad of all sampled *Graptophyllum* (6 accessions) plus three species of Papuasian/Pacific *Pseuderanthemum*; clade 4 is nested within

---

**Justicieae**

![Justicieae phylogenetic tree](image)

Fig. 4. Summary of relationships among major lineages of Justicieae, 149-taxon analysis including *Ichthyostoma thulinii*. Values above branches are maximum likelihood bootstrap values (MLBS); those below are Bayesian posterior probabilities (BPP) (MrBayes analysis). See Fig. 5–7 for details of relationships within major lineages.
Fig. 5. Details of relationships among members of the Pseuderanthemum Lineage. Values above branches are Bayesian posterior probabilities (BPP) (BEAST analysis); those below are maximum likelihood bootstrap values (MLBS). \(* = 100\% \text{ or } 1.0, \* = < 50\% \text{ or } 0.50\). Note that the Asystasia Clade includes two clades that are assigned informal names. Likewise, the Pseuderanthemum Clade has five clades, marked with circled numbers, that are discussed in the text. Bars on key branches depict median ages and 95\% highest posterior density (HPD) estimated by the BEAST analysis (scale below tree). See Fig. 6 and 7 for details of relationships among members of the Ptyssiglottis Lineage + Isoglossinae and Tetramerium Lineage + Justicioid Lineage, respectively.
Table 3. Results of alternative phylogenetic hypothesis testing using the Shimodaira Approximately Unbiased (AU) test among Justicieae. **H0** = results from present study; **H1–H4** = alternative hypotheses based on earlier classification or morphological patterns.

| Hypothesis | logL | deltaL | Reject? | p-value |
|------------|------|--------|---------|---------|
| Monophyly of Asystasia | H0. Asystasia is not monophyletic | -61509.80715 | | |
| | H1. Asystasia is monophyletic | -61697.71476 | 187.91 | Yes | P < 0.001 |
| Monophyly of Oplonia | H0. Oplonia is not monophyletic | -61509.80715 | | |
| | H1. Oplonia is monophyletic | -61688.31438 | 178.51 | Yes | P < 0.001 |
| | H2. New World Oplonia is monophyletic | -61576.78062 | 66.97 | Yes | P = 0.008 |
| | H3. Caribbean Oplonia is monophyletic | -61574.25062 | 2.53 | No | P = 0.381 |
| Monophyly of Pseuderanthemum | H0. Pseuderanthemum is not monophyletic | -61509.80715 | | |
| | H1. Pseuderanthemum is monophyletic | -61741.23626 | 231.43 | Yes | P < 0.001 |
| | H2. New World Pseuderanthemum is monophyletic | -61529.37170 | 19.57 | No | P = 0.237 |
| | H3. African Pseuderanthemum is monophyletic | -61602.36601 | 92.56 | Yes | P < 0.001 |
| | H4. Asian/Australian Pseuderanthemum is monophyletic | -61531.28514 | 21.48 | No | P = 0.589 |
| Monophyly of Graptophyllum | H0. Graptophyllum is not monophyletic | -61509.80715 | | |
| | H1. Graptophyllum is monophyletic | -61527.60231 | 17.80 | No | P = 0.199 |
| Monophyly of Monothecium | H0. Monothecium is not monophyletic | -61509.80715 | | |
| | H1. Monothecium is monophyletic | -61845.98484 | 336.19 | Yes | P < 0.001 |
| Placement of Isoglossa variegata | H0. Isoglossa variegata is not placed in Isoglossinae | -61509.80715 | | |
| | H1. Isoglossa variegata is placed in Isoglossinae | -62034.32773 | 524.52 | Yes | P < 0.001 |
| Ptyssiglottis is monophyletic | H0. Ptyssiglottis is not monophyletic | -61509.80715 | | |
| | H1. Ptyssiglottis is monophyletic | -61518.56522 | 8.76 | No | P = 0.350 |
| Ichthyostoma thalinii | H0. Ichthyostoma is sister to the Justicioid Lineage + Tetramerium Lineage | -59577.69160 | | |
| | H1. Ichthyostoma is placed in the Tetramerium Lineage | -59650.88491 | 73.19 | Yes | P < 0.001 |
| | H2. Ichthyostoma is placed in the Ptyssiglottis + Isoglossinae Lineage | -59588.55126 | 10.86 | No | P = 0.237 |

A larger clade (5) that also includes the three sampled Ballochia (Socotra), African Pseuderanthemum hildebrandtii Lindau (Fig. 1J), Malagasy Oplonia (1 sp.), African Ruttya (2 sp.) and African Rasopia Lindau (1 sp.). Except for the nested relationship of clade 4 within clade 5, relationships among these clades are not strongly supported.

Results thus indicate that sampled species of Oplonia are not monophyletic. *Oplonia* sp. *Heurn 18* from Madagascar is in Clade 5, distant from its NW congeners. In Clade 2, South American (Bolivia, Argentina) *O. jujuyensis* Wash. & C. Ecuzzara is sister to Ecuadorian *Psilantele eggersii*, these are together sister to the clade that includes all sampled Caribbean *Oplonia* plus Cuban *Sapphoa rigidifolia*. Constraint tests indicate that our data can reject monophyly of *Oplonia* (i.e., including both NW and OW species; *P < 0.001*) and also of NW *Oplonia* (*P = 0.008*), but cannot reject monophyly of accessions representing species that occur in the Caribbean (*P = 0.381*) (Table 3).

Accessions of *Pseuderanthemum* are placed among five different clades as just described, two of NW taxa and three of OW taxa. Monophyly of *Pseuderanthemum* as a whole is rejected by our data (*P < 0.001*), as is monophyly of African accessions of *Pseuderanthemum* (*P < 0.001*) (Table 3). However, monophyly of Pausapian/Pacific and of NW *Pseuderanthemum* cannot be rejected by our data (*P = 0.589*, *P = 0.237*, respectively). Our data likewise cannot reject monophyly of *Graptophyllum* (*P = 0.199*), although the three species of Pauapian/Pacific *Pseuderanthemum* are part of the clade that includes all sampled *Graptophyllum*.

The remaining sampled members of Justicieae are together monophyletic with strong support (Fig. 4, 5). The *Ptyssiglottis* Lineage and Isoglossinae are both strongly supported as monophyletic and as sister (Fig. 4, 6). The *Ptyssiglottis* lineage includes African and Asian taxa. The first to diverge is African *Champluviera populifolia* (C.B. Clarke) I. Darbysh. & T.F. Daniel followed by two species of *Monothecium* (African and Asian *M. aristatum* T. Anderson + African *M. glandulosum* Hochst.) that are sister taxa. A third sampled species of *Monothecium* is placed in the Justicioid Lineage (see below) and constraint tests reject monophyly of *Monothecium* (*P < 0.001*) (Table 3). The remaining members of the *Ptyssiglottis* Lineage are all Tropical Asian (i.e., Indo-Chinese/Malesian/Papuanis). Monospecific *Marcania* J.B. Imlay is sister to a clade that includes both sampled species of *Ptyssiglottis*, monospecific *Calycanthus* K. Schum. (Fig. 2A) and the type species of *Jadunia* Lindau; the two *Ptyssiglottis* are not sister taxa but constraint tests cannot reject a sister relationship (*P = 0.35*)

All sampled Isoglossinae are placed in two strongly supported clades, one of which includes newly sampled NW *Sebastianoschauera* Nees which is sister, with strong support, to the only other sampled NW member of the clade, *Stenostephanus leiorhachis* (Lindau) Hammel (Fig. 6). This clade also includes OW *Brachystephanus* Nees and two OW accessions identified as *Isoglossa*. Three other accessions, identified as *Isoglossa*—*I. grandiflora* C.B. Clarke, *I. mbalensis* Brummit and *I. ovata* (Nees) Lindau—are placed in the second clade which includes only OW taxa. This second clade also includes Malagasy *Anisotes perplexus*, *Conocalyx* Benoist nom.
illegit., *Melittacanthus* S. Moore, *Sphacanthus* Benoist and undescribed species of *Isoglossa*. There is little resolution among accessions placed in this second clade except that *I. grandiflora* and *I. mbalensis* are sister with strong support, as are the two accessions of *Conocalyx laxus* Benoist. Malagasy *Isoglossa* sp. 1022 is sister to *Anisotes perplexus*, with moderate support. Monophyly of *Isoglossa* inclusive of *I. variegata* (placed in the *Tetramerium* Lineage, see below) is rejected ($P < 0.001$) (Table 3). Placement of *A. perplexus* in the Justicioid Lineage (where other *Anisotes* are placed) was rejected by Kiel et al. (2017) and not tested further here.

The *Tetramerium* Lineage and Justicioid Lineage are sister with strong support and the monophyly of each of these lineages is also strongly supported (Fig. 4, 7). *Isoglossa variegata* is placed in the *Tetramerium* Lineage with strong support (Fig. 7). As noted above, other sampled members of *Isoglossa* are placed in Isoglossinae and our data reject placement of *I. variegata* with those accessions.

Among members of the Justicioid Lineage, the *Rungia* Clade (sensu Kiel et al. 2017) is sister to the remaining Justicioids with strong support (Fig. 7). Newly sampled *Ascothecapaucinervia* (T. Anderson ex C.B. Clarke) Heine is sister to the other sampled members of the *Rungia* Clade. Also included in the *Rungia* Clade are two newly sampled species, *Justicia latiflora* Hemsl. and *J. patentiiflora* Hemsl.; these are together sister to *J. gendarussa* Burm. f. with strong support. Among OW Justicioids, two Malagasy species, *Justicia crebrinodis* Benoist and *Justicia sp.* PR667, are newly added to
Fig. 7. Details of relationships among members of the Tetramerium Lineage + Justicioid Lineage. Values above branches are Bayesian posterior probabilities (BPP) (BEAST analysis); those below are maximum likelihood bootstrap values (MLBS). * = 100% or 1.0, - = < 50% or 0.50. Bars on key branches depict median ages and 95% highest posterior density (HPD) estimated by the BEAST analysis (scale below tree). Note the bullet signaling the unexpected placement of *Isoglossa variegata* in the Tetramerium Lineage. Clades discussed in the text are named (e.g., Diclipterinae s.l.). See Fig. 5 and 6 for details of relationships among members of the *Pseuderanthemum Lineage* and *Pyssiglottis Lineage + Isoglossinae*, respectively.
the Anisotes Clade (sensu Kiel et al. 2017). These species are strongly supported as sister taxa. Malagasy Monothecium leucopetron Benoist, “Justicieae indet. 6012,” and Anisotes pulchellus (Benoist) T.F. Daniel, Lettsara & Rakotonas are resolved in the Anisostachya clade (sensu Kiel et al. 2017) (Fig. 7). As indicated above, the other two sampled species of Monothecium are placed in the Pyssiglottis Lineage and our data reject monophyly of Monothecium (Table 3).

Justicia hyssopifolia L. (the type of the genus Justicia) is sister to Diclipterinae s.l. + NW Justicioids albeit with weak support (Fig. 7). Five newly sampled taxa are added to Diclipterinae s.l.: (1) two accessions of Justicia tenuipe S. Moore are sister and together sister to Rhinacanthus virens (Nees) Milne-Redh. with strong support. These are sister to (2) Asian Justicia vagabunda Benoist but support is weak (BI 0.79; MLBS < 50%). (3–5) Three potentially undescribed species of Rhinacanthus from Madagascar: R. sp. 2348, R. sp. 1787 and R. sp. 763 are resolved as members of the Core Rhinacanthus Clade and as each other’s closest relatives; in general, support for relationships among species of Rhinacanthus is weak. Kiel et al. (2017) rejected monophyly of Rhinacanthus and we did not test this further.

Our results confirm monophyly of NW Justicioids with strong support (Fig. 7). As noted above, relationships among members of this large clade have been recently studied by Kiel et al. (2018) and we did not seek to extend the results of these earlier authors.

Estimation of Divergence Times

Results of the BEAST2 analysis (Fig. 5–7) indicate that Justicieae (crown group) originated ∼35.4 MYA [HPD = 29.9–40.5]. Estimated crown group ages for the five major lineages of Justicieae are: Pseuderanthemum Lineage ∼27.5 MYA [HPD = 19.5–35.9] (Fig. 5), Pyssiglottis Lineage ∼18.0 MYA [HPD = 10.1–26.1] (Fig. 6), Isoglossinae ∼13.8 MYA [HPD = 8.9–19.2] (Fig. 6), Tetramerium Lineage ∼21.9 MYA [HPD = 15.1–28.3] and Justicioid Lineage ∼25.4 MYA [HPD = 20.4–30.5] (Fig. 7). Within the Pseuderanthemum Lineage, the Asystasia Clade began to diverge ∼22.3 MYA [HPD = 16.0–30.0] or about six MY earlier than its sister group, the Pseuderanthemum Clade (∼16.3 MYA [HPD = 9.6–24.0]) (Fig. 5). In that last clade, a number of groups have radiated within the last eight to three million years (e.g., Graptophyllum, Odontomena, NW Oplonia).

Discussion

Excluded Taxa

Presumably owing to low-quality DNA, we were not able to obtain clean sequences or sequences that were unambiguously of the targeted plant for two taxa, Isotheca alba and Thysanostigma odontites. We here discuss these two plants and evidence for their likely placement.

BLAST® searches for sequences from Isotheca alba yielded nonsensical best matches that were clearly indicative of contamination: Lupinus L. (nITS), Juniperus L. (trnS-G), Sesamum L. (rps16), and Diclipterinae (trnL-F). As a result, we excluded this taxon entirely. Isotheca was described by Turrill (1922) with a single species, I. alba, that is known only from the heights of Aripo, Trinidad, West Indies and the Paria Peninsula, Sucre State, Venezuela which lies just to the west, across the Bocas del Dragón, from Trinidad. Only a handful of herbarium specimens are known to exist of this little-studied plant. We obtained tissue from a specimen collected in 1972: Dumont VE-7523 (NY). In describing the plant, Turrill (1922) suggested that it was closely related to Herpetacanthus, a relationship that is supported by androecial complement (two bithecous + two monothecous stamens; Fig. 8D). Among Justicieae, this staminal complement is known only in the Herpetacanthus Clade of the Pseuderanthemum Lineage (see below). However, Turrill indicates that these plants have “Stachelpollen” or “spine pollen” and that grains are spheroidal in shape. Our observations concur and we can add that pollen is 4-aperturate (4-porate or potentially 4-pororate; Fig. 3M, N), as in some species of Herpetacanthus (Fig. 3K, L). So far as known, this pollen type does not occur elsewhere in the Pseuderanthemum Lineage. Pollen that could be described as “spiny” occurs in Isoglossiniae and also among members of the Justicioid Lineage, but staminal complement argues against placement of Isotheca with either of these lineages. Fieldwork to observe these plants and make fresh tissue collections is certainly warranted. Label data indicate that plants may be 2 m tall and the white or yellow corollas are about 5 cm long indicating that plants should be easy to find if in flower, and also that the species should be evaluated for horticultural potential.

For Asian Thysanostigma odontites, sequence data were nonsensical in that they were identical to a plant whose morphological traits pointed to a distant relationship (i.e., Ichthyosoma). This pattern is consistent with contamination, although we made multiple attempts at DNA extraction and sequencing. As a result, Thysanostigma was excluded from the analysis. As described by Hansen (1988), Thysanostigma has two bithecous + two monothecous stamens, an androecial complement consistent with placement in the Herpetacanthus Clade of the Pseuderanthemum Lineage. Hansen (1988) reported that pollen is tricolporate hexapseudocolpate (i.e., Spangenpollen) but that the interapertural regions consist of longitudinal rows of spines, which is unusual among Spangenpollen. In addition, plants have fruits that are shaped somewhat like upside-down violins: there is a narrow basal stipe and a broader distal fertile portion, with the exocarp narrowed between the pairs of superposed seeds (Fig. 9A). Hansen (1988) specifically pointed to Asystasia, Pseuderanthemum and Odontomenella Lindau (now recognized as Mackaya) as putative close relatives. We concur with Hansen in positing a relationship with members of the Pseuderanthemum Lineage and further predict that Thysanostigma will be placed in the Herpetacanthus Clade based on androecial complement. It is interesting that Thysanostigma and Isotheca both have pollen with spines (Fig. 3M, N) and also share the androecial complement typical of the Herpetacanthus Clade (Fig. 8D), but are otherwise quite different. Plants of Asian Thysanostigma are short-statured herbs with flowers no more than 1.5 cm long whereas NW Isotheca is a shrub with much larger flowers. As for Isotheca, fresh material should be sought of both described species of Thysanostigma.

Data Quality Issues

We encountered problems obtaining clean sequences or with incongruence among loci for several taxa. For Linariantha bicolor, analyses revealed strong incongruence between plastid...
and nrITS data; because plastid data were inconsistent with morphological patterns, these data were omitted. More unexpectedly, for several other species, there was incongruence among plastid loci (i.e., Anisotes perplexus, Cosmianthemum magnifolium, both species of Spathacanthus). Earlier, McDade et al. (2000) discovered that plastid trnL-F data gave anomalous results for Spathacanthus; these authors reported results based only on nrITS. We do not know whether these patterns are biological in nature (e.g., owing to hybridization or heteroplasmy) or are artefacts of PCR and Sanger sequencing from poor quality DNA. However, the fact that leaf material for DNA extraction came from fairly recently collected specimens in the case of S. hoffmannii Lindau (Fig. 2B) and S. parviflorus Leonard suggests that biological processes such as hybridization merit further study (see, e.g., Tripp et al. 2013). Although placement based on the retained data of C. magnifolium, L. bicolor and Spathacanthus in the Pseudanthemum Lineage is consistent with the morphological features of these plants, their precise placement as posited here must be considered provisional. Anisotes perplexus is discussed further below.
Results reported here confirm that *Anisotes* is polyphyletic. Species are placed in multiple lineages of OW Justicioidae (Fig. 7) except that Malagasy *A. perplexus* is nested among Isoglossinae (Fig. 6), confirming the results of Kiel et al. (2017). In fact, the specific epithet of this plant refers to the fact that its pollen is unlike that of any other species of *Anisotes* (Daniel et al. 2013). These plants have Gürtelpollen (Fig. 3O–R), a trait that is not consistent with placement in the Justicioid Lineage. Instead, Gürtelpollen characterizes core Isoglossinae and is otherwise known only from Whitfieldieae (Manktelow et al. 2001), a still poorly characterized clade. As noted elsewhere, Malagasy acanths require a great deal of additional study.

Isoglossa.—Although sampled plants placed at least tentatively in *Isoglossa* (Isoglossinae) do not form a clade, they are all placed in one of two clades of Isoglossinae (Fig. 6) with one exception: *I. variegata* (Fig. 2D) is part of the Tetramerium Lineage (Fig. 7). The description of *I. variegata* indicates that this plant has Spangelpollen (Darbyshire 2009b), suggesting that it may be misplaced in Isoglossinae, a clade marked by Gürtelpollen (Fig. 3O–R). *Isoglossa variegata*, together with the morphologically similar *I. faulknerae* I. Darbysh. which also has Spangelpollen, additionally differ from other African members of *Isoglossa* in having anthers with trichomes externally (see Darbyshire 2009b, Fig. 4, 5). On the other hand, the anthers in this species are complex, with offset thecae that are more remis- niscence of some members of Isoglossinae and the Justicioid Lineage than the Tetramerium Lineage where anthers typically have thecae held at equal heights (see Daniel et al. 2008, Fig. 3; Mc Dade et al. 2018, Fig. 1). Our results suggest that generic placement of *I. variegata* warrants further examination as does that of other African and Asian species of *Isoglossa* with Spangelpollen (fide Hansen 1985; Darbyshire 2009b).

**Monotheocarium.**—*Monotheocarium* has been characterized by the presence of two monotheocous stamens and no staminodes (Hochstetter 1843). Apparently because of this trait, the genus has previously been associated with plants currently placed in Isoglossinae or Dicipterinae, both of which include plants with monotheocous stamens, although not exclusively so. *Monotheocarium aristatum* and *M. glandulosum* also share dense spiciform inflorescences and similar corolla morphology. Vollesen (in Darbyshire et al. 2015) questioned whether the Malagasy species, *M. leucopterum*, belonged in the genus, stating that "... corolla morphology seems to be quite different" (p. 491). Pollen of *M. aristatum* and *M. glandulosum* is described as Spangelpollen (Raj 1961; T. F. Daniel, pers. obs.) (Fig. 3C); pollen of *M. leucopterum* has apparently not been studied. We sampled all three species currently recognized for the genus. The Malagasy endemic, *M. leucopterum*, is placed in the Anisostachya clade with other Malagasy members of the Justicioid Lineage (Fig. 7) all of which, to our knowledge, have bithecous stamens. The other two species, *M. aristatum* (Africa, India, Sri Lanka)
and *M. glandulosum* (Africa) are sister taxa and are part of the *Pyssiglotis* Lineage (Fig. 6) where they are sister to the crown group that is entirely Asian.

**Phylogenetic Relationships**

Justicieae are monophyletic with strong support (Fig. 4); elsewhere we have proposed Spagentollen as synapomorphic for this large and heterogeneous clade, albeit with considerable further evolutionary modification, especially in Isoglossinae and the Justicioid Lineage.

**Pseuderanthemum Lineage**

With an estimated 300 species, this lineage is monophyletic and sister to all other Justicieae. Results presented here are far from the final word on the *Pseuderanthemum* Lineage but do provide insights that may inform further, more focused research. Most plants placed in this clade have four androecial elements; all four may be bithecous (Fig. 8A), two may be monothecous (Fig. 8D), or two may be reduced to staminodes (Fig. 8C). Just a few have apparently lost the stamnodes although this merits confirmation as staminodes are not reported consistently in species descriptions and they can be quite inconspicuous, particularly when they are fused to the base of the filament of the adjacent fertile stamen. Also, here and elsewhere in Acanthaceae, all four authors have seen occasional acanthaceous flowers with medial staminodes (e.g., some flowers of *Ruttya ovata* appear to have three staminodes, Fig. 8B); however, no acanthaceous plants have five fertile stamens. The remaining taxa of Justicieae have only two androecial elements with the exception of *Chalarothysus* (*Tetramerium* Lineage) which has four bithecous stamens. Also, so far as is known, plants in the *Pseuderanthemum* Lineage have upside-down violin-shaped fruits (Fig. 9A) (i.e., with a sterile stipe and a narrowed invagination between superposed pairs of seeds; note that the constriction may be subtle but the two pairs of seeds are always superposed). Violin-shaped fruits are likely not synapomorphic for the *Pseuderanthemum* Lineage as at least some plants belonging to Isoglossinae, the *Tetramerium* Lineage and Justicioids have this trait. Fruit morphology warrants phylogenetically informed survey across Justicieae (e.g., below we posit fruit shape as synapomorphic for the core *Tetramerium* Lineage). Plants belonging to the *Pseuderanthemum* Lineage are diminutive herbs (even rosette forming) to small trees, and corolla size, color and morphology vary enormously (Fig. 1A–V, 2B).

**Androecial Evolution.**—Taxon sampling and support for some aspects of relationships are inadequate to merit formal optimization of the evolution of androecial traits, but it is clear that many evolutionary changes have impacted the androecium across the *Pseuderanthemum* Lineage. Four bithecous stamens may be symplesiomorphic as it characterizes *Spathacanthus* (Fig. 2B), the first clade to diverge, and also occurs in the *Aystasia* Subclade (Fig. 5). The *Herpetacanthus* Clade is marked by plants with two bithecous + two monothecous stamens (Fig. 8D). The clade, as presently delimited, is NW except for *Afrofitonia*, a monospecific genus from Cameroon, Equatorial Guinea (Bioko) and Nigeria. We are not aware of taxa placed elsewhere in Justicieae that have this unusual androecial complement and we predict that yet to be sampled members of the *Pseuderanthemum* Lineage with this androecial complement will be placed here. This includes additional NW taxa (i.e., yet to be sampled species of *Chamaeranthemum* and *Herpetacanthus*, also *Isotheca alba*) but also Asian *Thysanostigma*, as discussed above. The reduction of two stamens to staminodes (Fig. 8B, C) has apparently occurred multiple times in the lineage and characterizes some clades (e.g., the NW *Opollia* Clade). As noted above, reports of plants belonging to this lineage that lack staminodes warrant further investigation, especially in cases wherein this trait is not mentioned (or figured) in species descriptions. Whereas most genera in the *Pseuderanthemum* Lineage have at least two bithecous stamens, *Ballochia*, *Ruspolia* and *Ruttya* are marked by having two monothecous stamens and two staminodes (Fig. 8B, C), an androecial complement unknown elsewhere in Justicieae. Our phylogenetic results suggest that the trait evolved independently in *Ballochia* and the other two genera, but further sampling is warranted. Monothecous anthers (without staminodes) have evolved elsewhere in Justicieae including once each in the *Pyssiglotis* Lineage (*Monothecium*) and in *Isotheca* (the *Brachystephanus-Stenostephanus* Nees group). The trait has also evolved at least twice in the Justicioid Lineage (i.e., *Monothecium leucopterum* and *Hypoestes* Sol. ex R. Br.).

**Geographic Distribution.**—The *Pseuderanthemum* Lineage is pantropical in distribution. Taxon sampling and support for phylogenetic relationships are inadequate to merit formal optimization of biogeographic ranges but it is clear that further study of geographic patterns is warranted. The lineage apparently includes a relatively high representation of Indo-Asian/Malesian/Papuasian/Australasian taxa compared to other lineages of Justicieae. The small (4 spp.) NW genus *Spactacanthus* is sister to the rest of the lineage (Fig. 5). African *Afrofitonia* is embedded in the otherwise NW *Herpetacanthus* Clade, and Asian *Thysanostigma* may belong here as well (see above). Asian *Linariantha* B.L. Burtt & R.M. Sm. is part of a clade of African *Pseuderanthemum*. On the other hand, three clades are geographically cohesive (Fig. 5): Clades 1 + 2 are of NW taxa and Clade 4 is of Tropical Asian/Australasian plants. Most of the remaining members of the *Pseuderanthemum* Clade are African, although NW (i.e., *Chilaranthemum* Oerst., *Palchranthus* V.M. Baum, *Revel* & *Nowicke*) and Asian taxa (i.e., *Cosmanniheum*, *Codonacanthus* Nees, *Linariantha*) are also represented in patterns that suggest multiple shifts between geographic areas. These patterns are in marked contrast to other lineages of Justicieae in which relationships clearly point to an OW origin followed by one or two dispersal events to the NW and then considerable diversification there: Isoglossinae (Kiel et al. 2006), *Tetramerium* Lineage (McDade et al. 2018) (one dispersal event each), and Justicioids (Kiel et al. 2017, 2018), with two dispersal events required to account for the group’s distribution in the NW.

**Phylogenetic Status of Recognized Genera.**—Our results for the *Pseuderanthemum* Lineage indicate that a number of sampled genera that are limited geographically are likely monophyletic including *Ballochia* (Socotra), *Chamaeranthemum* (NW), *Herpetacanthus* (NW), *Odontonema* (NW), *Ruttya* (Africa, Madagascar) and *Spathacanthus* (NW) (Fig. 5). A number of smaller, geographically cohesive genera are not yet well enough sampled to assess monophyly including *Cosmanniheum* (Asia)
and *Rulpia* (Africa, Madagascar), *Filetia* and *Mackaya*, with species reported in Asia and Africa, are not geographically cohesive and additional sampling is required to test monophyly and to understand geographic patterns. Pending some nomenclatural realignments, *Graptochilus*, with species in Malasia/Papuasia/Australia and the Pacific regions may be monophyletic, although based on our findings it would include several species currently placed in *Pseuderanthemum*. Notably, widely cultivated *P. carruthersii* (Seem.) Guillaumin has a corolla form very different from that of species of *Graptochilus* (i.e., contrast Fig. 1C and 1I). Further, *G. glandulosum* Turrill, from West-Central Tropical Africa (not sampled here), should be included to understand the phylogenetic and biogeographic status of this genus. On the other hand, *Oplonia*, with species described from the Caribbean, South America, and Madagascar, is polyphyletic largely along geographic lines. Likewise, the large genus *Pseuderanthemum*, with species distributed pantropically, is polyphyletic with sampled species placed in at least five clades (Fig. 5). Below we discuss these last two genera along with *Asystasia*.

**Asystasia.**—All sampled species of the large (∼70 species) genus *Asystasia* are placed in a single clade (Fig. 5). The genus has African and Asian species, as well as some that are widespread. Notably *A. gaertica* subsp. *micrantha* (Nees) Ensmem has become a serious weed in the NW tropics where it has naturalized (e.g., McDade et al. 2019). The only sampled member of *Filetia*, *A. ridleyi*, is deeply embedded among species of *Asystasia*, and *Salpinctium* T.J. Edwards and *Glossochilus* Nees are serially basal to the clade that includes *Asystasia* and *Filetia*. *Filetia* is circumscribed at present to include nine Asian species, the only African member of the genus, *F. africana* Lindau (not sampled here), having been transferred to *Asystasia* (= *A. lindauiana* Hutch. & Dalziel) by Hutchinson and Dalziel (1931). Further sampling, including *A. lindauiana*, should be undertaken before taxonomic changes are finalized. However, species descriptions and limited examination of herbarium material suggest that species of *Filetia* are not morphologically distinct from *Asystasia*. The same may be true of *Glossochilus* and *Salpinctium*. Indeed, *Salpinctium* was treated as a generic synonym of *Asystasia* in *Flora Zambesiaca* (Ensmem and Vollesen in Darbyshire et al. 2015). *Glossochilus* (Fig. 1U) has been maintained as distinct from *Asystasia* on the basis of having solitary axillary flowers rather than a spiciform inflorescence or panicle of spikes as in *Asystasia* (Fig. 1R) (Balkwill and Welman 2000). However, this distinction appears to be rather minor given the range of morphological variation observed within *Asystasia*. Unless further morphological traits can be found to distinguish these genera from *Asystasia—or to recognize clades within *Asystasia* that warrant recognition at the generic level—our results support a broad concept of *Asystasia* to include *Filetia*, *Glossochilus* and *Salpinctium*, i.e., encompassing the entire *Asystasia* Subclade (Fig. 5). *Asian Phialacanthus*, with a distinct distribution in the Himalayas and Malesia, is likely to belong to this clade based on preliminary study as it has four bithecous stamens and no staminodes (pollen not reported) (Bentham 1876).

**Oplonia.**—Our results with regard to *Oplonia* indicate that Malagasy plants are phylogenetically distinct from NW plants (Fig. 5) despite their similarities in floral traits (Fig. 1F, G). Other species of Malagasy *Oplonia* should be sampled but our results point to a resurrection of *Forsythiopsis* Baker for these Malagasy plants. In monographing *Oplonia*, Stearn (1971) maintained *Pislanthele eggersii* from Ecuador as distinct from *Oplonia* apparently on the basis of inflorescence traits, although he transferred the other *Pislanthele* recognized at the time (i.e., Peruvian *P. grandiflora* Lindau, Jamaican *P. jamaicensis* Lindau) to *Oplonia*. In describing *O. jujuyensis* from Argentina, Wasshausen and Ezcurra (1993) made no reference to *Pislanthele*. Our results place two South American taxa (*O. jujuyensis*, *P. eggersii*) together and sister to a clade that includes all other sampled NW *Oplonia*. Peruvian *O. grandiflora* (Lindau) Stearn and *O. hutchinsonii* Wassh. especially warrant sampling to understand the taxonomic limits and geographic range of this group.

Neither Stearn (1971) nor Wasshausen and Ezcurra (1993) mentioned *Sapphoa* which our data place among sampled *Oplonia*. Cuban *Sapphoa rigidifolia* Urb. (sampled here) is an unarmored scandent plant that is not well represented in herbaria whereas Caribbean species of *Oplonia* are mostly shrubs with thorns. A second species of *Sapphoa*, also from Cuba, was described by Borhidi (1983) based on subtle differences in leaf and corolla morphology (see key provided by Franck and Daniel 2015). We have not studied living plants and only a paucity of fertile materials is available on the few herbarium specimens of *Sapphoa* that exist, but species descriptions point to floral traits that are not out of place in *Oplonia* (e.g., plants have two bithecous stamens and two staminodes) and it is biogeographically at home in the Caribbean clade of *Oplonia*. As for *Asystasia*, barring discovery of morphological traits that support monophyletic groups within the *Oplonia* Clade, our results support a broad concept of *Oplonia* to include *Pislanthele* and *Sapphoa* Urb. but to exclude *Forsythiopsis*. Evolution of vegetative morphology clearly warrants additional research in this group.

**Pseuderanthemum.**—Essentially all substantive contributions to our knowledge of the large (∼120 species), pantropical genus *Pseuderanthemum* indicate that distinctions among species are challenging and call for additional research (e.g., Barker 1986; Daniel 1995; Champluvier 2002; Choopan et al. 2018); here, we reiterate the call for more study of this challenging group. Species of *Pseuderanthemum* span essentially the entire geographic range of the *Pseuderanthemum* Clade as a whole and vary greatly in habit, from basal rosettes that may be only seasonally present to small trees. Plants described in *Pseuderanthemum* usually have salverform corollas (see Fig. 11–Q), but there is considerable variation in corolla size, and in tube length and width. Plants are described as having androcraea with four bithecous stamens, two bithecous + 2 staminodes or two bithecous + 0 staminodes (this last warrants further study as staminodes are not always characterized in species descriptions). It is thus not surprising that sampled species of *Pseuderanthemum* are placed in five clades, and those placed in Clade 4 (Fig. 5), with species of *Graptochilum*, are not each other’s closest relatives. Further, sampling remains inadequate to understand the full nature of the *Pseuderanthemum* problem. For example, among NW plants, we did not sample any of those that flower and/or fruit when plants are leafless (e.g., *Pseuderanthemum praecox* (Benth.) Leonard). Still, some geographic patterns are congruent with our phylogenetic results. For example, NW, African, Tropical- and Austral-Asian species are generally placed
cohesively with respect to geography. We suggest that taking a geographic approach to *Pseudenanthenum* may be the most fruitful way to tackle this problematic genus. For example, it is clear that a phylogenetic study of Graftophilium should also include all *Pseudenanthenum* that occur in Tropical- and Austral-Asia. On the other hand, at the generic level, basic nomenclatural work is essential in *Pseudenanthenum* given that it is not entirely clear what the type of the genus should be. Barker (1986) rejected lectotypification by Leonard (1953) as not consistent with the Code.

In sum, the *Pseudenanthenum* Lineage and its constituent genera and clades are in need of considerable additional taxonomic and phylogenetic research. Our results point to a number of clades of manageable size that are ripe for additional research at the species level, ideally including field work, as well as phylogenetic study using genomic sampling methods.

**Two-Stamen Clade**

All Justicieae that are part of the large clade sister to the *Pseudenanthenum* Lineage (Fig. 4, 6, 7) have two stamens and no staminodes with two exceptions of which we are aware. One species of *Pyssiglottis*, *Pt. staminodifera* B. Hansen (not sampled here), is reported to have two stamens and two staminodes (Hansen 1992), and *Chalanthrysus*, which is deeply nested in the *Tetramerium* Lineage (Daniel et al. 2008; McDade et al. 2018), has four fertile stamens. Beyond the loss of staminodes, this is a phylogenetic area of Justicieae that is well supported by molecular data but for which we have been able to identify essentially no morphological synapomorphies. Specifically, we know of no synapomorphies that support the monophyly of the *Pyssiglottis* Lineage or the *Tetramerium* Lineage, nor that support the sister relationship of *Pyssiglottis* Lineage + *Isoglossinae* or of *Tetramerium* Lineage + *Justicioid* Lineage. In marked contrast, all of the clades just mentioned are strongly supported by molecular data, with posterior probabilities of 1.0 (BEAST2 analysis) and high bootstrap values (Fig. 4, 6, 7). Among the basally branching clades of all of these lineages are plants with two bithecous stamens, typical Spangenpollen, and upside-down violin-shaped fruits.

*Pyssiglottis* Lineage + *Isoglossinae*.—Kiel et al. (2006) sampled two species of *Pyssiglottis* and placed these together as the basal clade of *Isoglossinae* s.l. albeit without strong support. These authors also indicated that pollens of species of *Pyssiglottis* was unlike that of plants belonging to *Isoglossinae*. Here we confirm the placement of the *Pyssiglottis* Lineage as sister to *Isoglossinae* and show that it includes a number of African plants and a crown group that is entirely tropical Asian (Fig. 6). *Monothecium*, with African and Asian species sampled, is unusual in having monothecous anthers, a trait that has evolved at least half a dozen times in Justicieae (including in one clade of *Isoglossinae*, see below) but, to our knowledge, only here in the *Pyssiglottis* Lineage. As discussed above, the third sampled species of *Monothecium*, *Malagasy M. leucopterum*, is placed in the Malagasy clade of *OW* Justicioid.

*Champluviera*, the only exclusively African genus of the *Pyssiglottis* Lineage with two West-Central African forest species (Darbyshire et al. 2019), is unusual in having anther thecae with broad, flattened, basal appendages that have an irregularly toothed fringe, a proposed synapomorphy for this genus. Elsewhere in the *Pyssiglottis* Lineage, the anther thecae usually lack appendages, although those of *Pt. mucronata* B. Hansen are sharply mucronate at the base (Hansen 1992; Darbyshire et al. 2019).

In his monograph of *Pyssiglottis*, Hansen (1992) treated 33 species and noted that species distinctions can be quite subtle. Interestingly, there is considerable diversity of pollen among species of *Pyssiglottis*. Many have typical Spangenpollen as is symplesiomorphic for the *Pyssiglottis* Lineage, although some are 4- or 5-colporate with a corresponding increase in the number of pseudocolpi. Others have pantoporate grains that are spheroidal and may be either reticulate or verrucace in terms of exine sculpting (see Fig. 1–12 in Hansen 1992). Our results support the congeneric status of plants with both types of pollen: we sampled *Pt. psychotriofilia*, with Spangenpollen, and *Pt. pubisepala* (Lindau) B. Hansen, with spheroidal, pantoporate, verrucace pollen. Interestingly, our data indicate that *Jadunia* and *Calycacanthus* (Fig. 2A), both Papuasian, are embedded within *Pyssiglottis*. *Calycacanthus* has Spangenpollen (Fig. 3A). *Jadunia* (2 spp.) was reported by Lindau (1913) to have pollen that is “...-typical of its tribe but with four pores.” Lindau placed *Jadunia* in Odontonemineae and we presume, therefore, that the grains are 4-colporate 8-pseudocolpate. However, here we document that *J. binoi*, the species referred to by Lindau (1913), has 5-colporate, 10-pseudocolpate pollen (Fig. 3 I, J). The second species described in *Jadunia*, *J. racemiflora* Brenek., has an androecium of two stamens + two staminodes and combines other floral traits that suggest it belongs within the *Pseudenanthenum* Lineage (I. Darbyshire, pers. obs.), a hypothesis that requires phylogenetic testing.

*Isoglossinae*.—Sister to the *Pyssiglottis* Lineage is *Isoglossinae*, a clade that is marked by Gürtelpollen. Grains are pororate (i.e., with an ectoaperture [pore] that is considerably larger than the endoaperture [os]; the apertural surface varies from smooth to variably ornamented) and the band of interapertural exine forms a smooth or variously ornamented peripheral band (girdle) (Fig. 3O–R). The possible spoiler here is *Isoglobosa*. Distributed throughout the Asian and African tropics, species of *Isoglobosa* have been described as having Gürtelpollen or, more rarely, Spangenpollen. Here we show that one of the latter, recently described *I. variegata* (Fig. 2D) from Tanzania (Darbyshire 2009b), is placed in the *Tetramerium* Lineage, an outcome that is consistent with pollen morphology. This is the only species of *Isoglobosa* sampled here that is known to have Spangenpollen. Clearly, denser sampling of *Isoglobosa* is warranted and would make an excellent topic for focused study. Pollen morphology is a remarkably consistent phylogenetic trait among most Justicieae, and we hypothesize that species of *Isoglobosa* with Spangenpollen will be placed either in the *Tetramerium* Lineage or in the *Pyssiglottis* Lineage, whereas species with Gürtelpollen that have yet to be sampled for phylogenetic study will belong to *Isoglossinae*. We note that our understanding of the *Pyssiglottis* and *Tetramerium* lineages will not be complete until we have clarified the phylogenetic placement of species of *Isoglobosa* with Spangenpollen. It is noteworthy that Hansen (1985) included several species with this pollen type in *Isoglobosa* when he revised the southeast Asian species of this genus; however, he subsequently transferred some (but not all) of these species to *Pyssiglottis* (Hansen 1992). At the same time, phylogenetically dismantling *Isoglobosa* does not make it
any easier to deal with this genus taxonomically. This is clearly a confusing group of plants; it seems that, rather as is the case with Justicia among the Justicioid Lineage, OW plants without clear synapomorphies linking them to other genera have been described as Isoglossa in several cases.

All but two accessions of Isoglossa placed in Isoglossinae are part of a clade of entirely OW plants from Africa and Madagascar. Significantly, our results show very little resolution, consistent with a recent radiation (Fig. 6). This OW clade includes Isoglossa ovata which represents a group of species of continental Africa, including the type species I. origanoides (Nees) S. Moore. These are plants that combine small, usually white, bilabiate corollas with the limb often subequal in length to the tube, prominent raised venation on the lower lip (e.g., Fig. 2C, D) and anthers with offset and often oblique thecae (see Balkwill et al. 2017). The inflorescences are scapiform or panicles of spikes. Also placed in the OW clade of Isoglossinae, as an sister taxa, are I. mbalesanis and I. grandiflora. These two taxa from East Africa differ from I. ovata and allies in having larger, often blue to purple corollas in which the upper lip is arcuate, and the anther thecae are not or only slightly offset and ± parallel.

As noted elsewhere, the acauleaceous flora of Madagascar is large and yet poorly known. With the goal of achieving a preliminary understanding of the placement of Malagasy Isoglossinae, we added a number of putative Isoglossa and close relatives from recent work in Madagascar. Earlier workers recognized a number of small endemic Malagasy genera including Conocalyx (1 sp.), Melittacanthus (1 sp. with 2 vars.) and Sphacanthus (2 spp.). Accessions pertaining to these taxa are all placed in the OW clade of Isoglossinae albeit with little resolution among them. These plants share the combination of Gürtelpollen, markedly bilabiate corollas and anthers with the two thecae not or only slightly offset and parallel to only slightly oblique or sagittate and lacking appendages. A fourth Malagasy endemic genus not sampled here, Celerina Beneist (1 sp. with 2 vars.), shares the same set of morphological characters; the protologue does not specify that the pollen is biporate, but it is described as spherical and smooth which suggests Gürtelpollen. The protologues for these genera do not clearly differentiate thecae slightly offset, lacking awns or spurs; Spangenpollen—rugula absent; two bithecous stamens; Brachystephanus. By virtue of distribution (NW) and of having monothecous stamens (Nees did not describe the pollen of this plant), this genus is at home in the NW clade of Isoglossinae. Kiel et al. (2006) sampled much more densely among NW plants and showed that a number of previously recognized genera are not monophyletic and are nested within Stenostephanus. New combinations are being made as floristic and other taxonomic work is undertaken (e.g., Daniel 1999 for Mexico; McDade et al. 2019 for Costa Rica; Wasshausen 1999 for Ecuador; Wood 2009 for Colombia). It remains to be determined whether Sebastiano-schauera warrants maintaining or should also be accommodated within Stenostephanus. The phylogenetic status of OW Brachystephanus merits further research as the results of Kiel et al. (2006) indicated that the group is not monophyletic. However, Brachystephanus could be incorporated within Stenostephanus on morphological grounds, as noted by Champluvier and Darbyshire (2009), Daniel (1999) and Wood (2019).

The Special Case of Ichthyostoma

As noted above, Ichthyostoma is the only sampled member of Justicieae to date that is not accommodated in one of five major lineages. Instead, this taxon is sister to (Tetramerium Lineage + Justicioid Lineage) in our results, although our data cannot reject placement sister to (Prystiglottis Lineage + Isoglossinae) (Fig. 4; Table 3). It is notable that the morphological features of Ichthyostoma—rugula absent; two bicesteous stamens; thecae slightly offset, lacking awns or spurs; Spangenpollen—are not inconsistent with placement near the Tetramerium Lineage and the Prystiglottis Lineage + Isoglossinae. However, this plant’s phylogenetic isolation merits further study as noted above.

Tetramerium Lineage

Given the recent contribution of McDade et al. (2018) on the Tetramerium Lineage, we sampled only enough taxa
to serve as placeholders for the lineage in the phylogenetic framework for Justiciaceae. We were surprised that Isoglossa variegata was placed with this lineage with strong support (Fig. 7). However, as discussed above under Isoglossinae, plants of this species have Spangelenpollen which is more consistent with the Tetramerium Lineage than with Isoglossinae.

As in Isoglossinae and the Ptyssiglottis Lineage, at least some of the basally branching members of the Tetramerium Lineage have upside-down violin-shaped fruits (e.g., Justicia grossa C.B. Clarke, Chlamydocaridia Lindau, Angkalanthus Balf. f.; Fig. 9A). Among phylogenetically more distal genera, many species have seeds reduced to two (e.g., Henrya Nees ex Benth., Mesacanthus T.F. Daniel, Trichaulax Vollesen, many Anisacanthus Nees and Ecbolium Kuntze). Many also have seeds that are flattened and borne at or nearly at the same level in the rounded, flattened “head” of the fruit. Many species have fruits with relatively long, narrow, sterile stipes. However, we have noted especially that those that occur in arid habitats (e.g., some species of Megalochlamys Lindau, many Tetramerium species) have fruits with shorter stipes. The relationship between overall fruit size and stipe length merits examination. We propose this combination of fruit traits (Fig. 9B) as possibly synapomorphic for the crown group of the Tetramerium Lineage albeit with some variation. Importantly, a couple of NW taxa have autopomorphic fruit types including Aphanosperma T.F. Daniel and Chalaxotyrsus Lindau, both with fruits with an unusually shaped head in which the seeds are partially fused to the inner wall of the capsule and permanently retained.

### Justicioid Lineage

As for the Tetramerium Lineage, we did not seek to sample densely across this large clade given the recent contributions of Kiel et al. (2017, 2018). However, we included enough taxa to serve as placeholders for all clades of this lineage, and added newly available material of a number of taxa including Ascotheca from West Tropical and West-Central Tropical Africa, multiple species of Rhinacanthus and a number of Malagasy samples including recently collected, undetermined plants that appear to be of the Justicioid Lineage. Efforts were also made to expand our sample of Asian Justicioids.

**Rungia Clade.**—Our results add Ascotheca and two species from China, Justicia latiflora and J. patentiﬂora, to the Rungia Clade (Fig. 7). Ascotheca has fruits with fracturing placentae, proposed by Kiel et al. (2017) as synapomorphic for this clade. We were unable to obtain material with mature fruits of J. latiflora or J. patentiﬂora. Anthers of Ascotheca are distinctive in having thecae that dishece via a basal pore (Heine 1966), a trait that is unique among Acanthaceae to our knowledge. Ascotheca and J. latiflora have imbricate, obovate bracts and inflorescences forming similar to species of Rungia. Both species have bracts that lack the hyaline margins that characterize many members of the Rungia Clade, although the bracts of J. latiflora are apparently bicolored, appearing green basally and purple towards the apex. Unlike many species in the Rungia Clade, J. patentiﬂora has small, lanceolate-triangular scale-like bracts that lack hyaline margins. Future work is necessary to identify morphological traits, if any, that support placement of J. patentiﬂora in the Rungia Clade.

**Anisostachya Clade.**—To the Anisostachya Clade (sensu Kiel et al. 2017), we add two species of Malagasy Justicia: J. crebrinodis Benoist and an unidentified species (Ranirison PR667) that is morphologically similar to J. crebrinodis (Fig. 7). Plants of both species are highly distinctive in having woody stems, large narrowly oblong-elliptic to oblanceolate leaves that are clustered towards the stem apices and leave prominent scars on the older stems, long-pedunculate, spiciform inflorescences, anthers with only shortly offset thecae and rudimentary appendages, and 2-seeded capsules with smooth, discoid seeds. The fruits are somewhat reminiscent of those in the crown group of the Tetramerium Lineage (see above), but our results clearly demonstrate that this clade falls within Justicioids.

**Anisotoma Clade.**—To the Anisotoma Clade (sensu Kiel et al. 2017), we here add three Malagasy species: Anisotoma pulchella (Benoist) T.F. Daniel, Lettsara & Rakontonas, Monothecium leucopterum, and an unidentified accession, Justiciaceae sp. 6012 (Fig. 7). These plants have many of the morphological features that mark this group including short spiciform, secund inflorescences with usually imbricate bracts that can be obovate to spathulate or lanceolate. Many species in the Anisotoma Clade have 5-parted calyces with one lobe greatly reduced although Justicia sp. 6012 has calyx lobes that are equal in size. Variation in calyx lobes is common among Justicioids (e.g., Monocha Il Group [sensu Kiel et al. 2017]). Hypoester, NW Justicioids; Kiel et al. 2017, 2018; Darbyshire et al. 2020). Overall, these features are consistent with Nees’ concept of Anisotoma Nees. Preliminary observations indicate that most species of Malagasy Justicia s.l. are referable to this genus. All three newly added species have seeds with hygroscopic or short papillose trichomes. Seeds with hygroscopic trichomes are relatively uncommon in Justicia and in the Anisotoma Clade, but those of J. tenella, a member of this clade, are hygroscopic (Heine 1966). Justicia tenella is a wide-ranging species that occurs in continental Africa as well as Madagascar. Monothecium leucopterum is unusual in this clade in having monothecous anthers but otherwise is at home here. The addition of Anisotoma pulchella, with large colorful corollas likely pollinated by birds, adds floral diversity to the clade.

**Rhinacanthus.**—Kiel et al. (2017) showed Rhinacanthus to be non-monomophyletic, with species placed in three distinct clades. Subsequent to that earlier study, the enigmatic Kenyan species, R. ndorensis Schweinf. ex Engl., has been recognized as a monospecific genus, Kenyacanthus I. Darbysh. & C.A. Kiel (Darbyshire et al. 2019). Our results place Kenyacanthus sister to core Diclipterinae (Fig. 7), confirming that this plant is not Rhinacanthus.

The wet forest species Rhinacanthus vires was also placed apart from the Core Rhinacanthus clade (Kiel et al. 2017) and this result is confirmed here (Fig. 7). Further, we here add two Cameroon taxa of Justicia to the clade that includes African R. vires: J. tenuepip and a similar but undescribed species, J. sp. 2518. Interestingly, Aké Assi (1961) treated J. tenuepip within Rhinacanthus as R. tenuepip. This last author also included western African plants now treated as R. obtusifolis (not sampled here) within his concept of R. tenuepis (Darbyshire and Harris 2007). These taxa share coherent morphological traits that separate them from the Core Rhinacanthus Clade including a broad, hooded upper corolla lip and a short appendage on the lower
anther thecae. Sister to these African species, but with only weak support, is Asian *J. vagabunda*. This species has inflorescences at home in both the Core *Rhinacanthus* Clade and the *R. virvens* Clade, as well as fruits with a long stipe and with a slight invagination between the superposed pairs of seeds (i.e., similar to the violin-shaped fruits of the *Pseuderanthemum* Lineage). It is also notable that plants of *J. vagabunda* are forest dwellers, as are members of the *R. virens* Clade, whereas those of the Core *Rhinacanthus* Clade usually occur in more open, xeric habitats (note that East African *R. selousensis* I. Darbysh. and *R. submontanus* I. Darbysh. & T. Harris occur in lowland and submontane forest, respectively; these are putative members of the Core *Rhinacanthus* Clade but this requires testing phylogenetically.

The corolla and androecium of *J. vagabunda* are different from those in the *R. virens* Clade. The corolla tube is approximately the same length as the limb and the anthers have a conspicuous appendage that is apically forked on the lower theca. Further research on the *R. virens* Clade should resolve its delimitation including whether or not *J. vagabunda* should be included.

Our results confirm monophyly of the Core *Rhinacanthus* Clade and add three accessions of Malagasy *Rhinacanthus* (R. sp. 763, R. sp. 1787, R. sp. 2348); these represent two apparently undescribed species (Fig. 7). The Core *Rhinacanthus* Clade thus includes Malagasy plants along with continental African and Asian taxa. Relationships within the Core *Rhinacanthus* Clade remain poorly resolved and data from NGS will be required to resolve relationships among these species.

As for the *Tetramerium* Lineage, we did not pursue detailed study of Dicipiterinae and NW Justicioids and thus sampled only placeholder for these clades (Fig. 7). Our results confirm the placement of both of these clades and, as we predicted, no newly sampled taxa were added to them. As revealed by Kiel et al. (2017), Core Dicipiterinae are serially sister to several clades of OW Justicioids including the Core *Rhinacanthus* Clade and the *R. virens* Clade. We refer to this larger clade as Dicipiterinae s.l. (Kiel et al. 2017). In turn, NW Justicioids are sister to Dicipiterinae s.l. as we have previously shown (McDade et al. 2000; Kiel et al. 2017).

**Tentatively Placed Genera.**—Two additional genera, of which we were not able to acquire suitable material for DNA, are tentatively assigned to the Justicioid Lineage based on morphological characters: *Dasytrops* Urb. and *Vavara* Benoist. Plants of both of these genera have bithecous stamens with thecae that are offset and ornamented; additionally, flowers of *Dasytrops* have a rugula. Clearly, these hypotheses require testing with molecular data and phylogenetic analysis.

**Unplaced Genera**

Uncertainties with regard to the placement of *Ichthyostoma*, *Isotheca* and *Thysanostigma* have been discussed in detail above. In addition, in the foregoing, we have tentatively placed *Phalacanthus* in the Asystasia Clade of the *Pseuderanthemum* Lineage, and *Dasytrops* and *Vavara* in the Justicioid Lineage. At least seven other genera remain unplaced within Justiciaceae: *Ambongia* Benoist, *Cephalopis* Vollesen, *Cyclacanthus* S. Moore, *Dichadanthia* F. Muell., *Dolichostachys* Benoist, *Samuelsonia* Urb. & Ekman and *Tessmanniacanthus* Mildb., (Table 1). None of these genera has been included in a phylogenetic study to date. We here summarize what is known of these plants and propose placements when warranted. Clearly, all merit additional study.

Urban (1928) described *Samuelsonia* to accommodate a single Haitian species of trees to 5 m tall, flowers with an androecium of two stamens with bithecous anthers, the thecae parallel and basally obtuse (staminodes were not mentioned). Pollen was described as ellipsoid, covered with minute apicules (spinules) and with equatorial pores; it is also noted that sulci were not observed. Subsequent collections do not include material with mature flowers such that our knowledge of this genus remains scanty. We tentatively place this plant in Justiciaceae but without further precision.

*Tessmanniacanthus*, with the single species *T. chlamydocar- diodes* Mildb., occurs in the Peruvian Amazon. Examination of the depauperate type material indicates resemblance to species of *Justicia*. However, there is little or no information available on the corolla, anthers, pollen or seed morphology of this species. Further collections and analyses are required to confirm placement within the Justicioid Lineage or elsewhere. This genus has been maintained in a recent synopsis of Peruvian Acanthaceae (Villanueva-Espinoza and Condo 2019), albeit with no clear diagnostic characters provided. We tentatively place this plant in Justiciaceae but without further precision.

Four genera, although incompletely characterized, are of plants with traits that refute placement with the *Pseuderanthemum* Lineage or with the crown group of Justicioids but without further precision. *Ambongia*, with a single Malagasy species, has Spangenpollen, anther thecae at equal height and no stamin- odes. *Cephalopis*, with one species from Eastern Africa, likewise has bithecous stamens and no staminodes, and corollas that lack a rugula. *Cyclacanthus*, with two Vietnamese species, is of plants with two bithecous stamens, thecae that are parallel but slightly offset, no staminodes and corollas that apparently lack a rugula. *Dolichostachys*, a monotypic genus from Madagascar that has yet to be validly published, is of plants with an androecium of two stamens with parallel thecae; staminodes were not mentioned but are apparently lacking given that Benoist typically reports this trait. Benoist (1962) described the pollen as spherical and smooth with three pores. Triaperturate pollen is observed in some Isoglossinae, notably in *Brachystephanus* (Champrawler and Darbyshire 2009), but in those cases the grains have conspicuous granular interapertural “girdles” as in other Isoglossinae. Thus, although knowledge of plants of all four of these genera remains scant, present data support placement of all four among the lineages that diverge after the *Pseuderanthemum* Lineage and before the crown group of Justicioids. As noted above, morphological synapomorphies are generally lacking for these clades. Material suitable for DNA extraction should be sought for all four genera to determine whether they are members of the *Tetramerium* or *Pyssiglottis* Lineages or, as suggested here for *Ichthyostoma*, diverge separately from stem lineages.

Plants of *Dichadanthia*, with two species from Western Australia, have subcircular to subcircular 5-lobed corollas without a rugula, and two stamens with no staminodes. The anthers are highly complex, with the connective forked in a “U” shape and bearing two widely separated thecae; pollen has apparently not been studied. Based on these traits, Barker (1986) suggested a close relationship with another Australian endemic genus, *Xerothamnella* C.T. White, placed by Kiel et al. (2017) in Dicipiteri- nae of the Justicioid Lineage. Based primarily on androecial
traits, we hypothesize placement of this genus in the Justicioid Lineage.

**Temporal Context and Biogeographic Patterns**

Our results (Fig. 5–7) generally concur with those of Tripp and McDade (2014) regarding the age of Justicieae. With considerably denser sampling within Justicieae than that of these earlier authors, our results provide estimates of the ages of the crown groups of the constituent lineages. These range from 27.5 MYA (Pseuderanthemum Lineage) to 9.0 MYA (Isoglossinae). There is signal of recent (3–8 MYA) and rapid radiations among clades of the Pseuderanthemum Lineage (i.e., Graftophyllum, Odontonema, NW Oplonia) and also among Isoglossinae.

Overall, our results point to long-distance dispersal to explain the distribution of Justicieae in the NW given that relevant overland routes were not available in the last ~30 MY (see Tripp and McDade 2014). Isoglossinae and the Tetramerium Lineage strongly support a single dispersal event from OW to NW, with Justicioids pointing to two (NW Justicioids and NW Diclipterae); current sampling indicates that there were no back dispersals to the OW in any of these clades. These dispersal events are optimized as having occurred ~3 MYA (Isoglossinae), ~9 MYA (Tetramerium Lineage), and ~12 MYA (NW Justicioids); NW Diclipterae may have originated ~4 MYA but only a single species was sampled here. NW Justicioids are extremely diverse with at least 400 spp. However, NW Isoglossinae may win the diversification rate sweepstakes with at least 80 species having arisen in the last 3 MY. Many, if not most, NW Stenostephanus are likely hummingbird pollinated suggesting a relationship between pollination by these birds and rapid rates of diversification.

As noted above, results for the Pseuderanthemum Lineage (Fig. 5) suggest more traffic across the high seas in that NW and OW taxa are placed in multiple clades in phylogenetically interdigitating patterns. Although we did not sample sufficiently across lineages of Acanthaceae to here optimize biogeographic patterns at the base of Justicieae explicitly, the family-wide study of McDade et al. (2008) points to OW to NW dispersal to account for NW Spathacanthus. Beyond Spathacanthus, additional sampling is needed to determine whether it is mos likely that NW plants in the Herpetacanthus clade represent a separate OW to NW dispersal event or whether the common ancestor of the Asystasia Clade is likely to have occurred in the NW. Lack of strongly supported resolution among clades of the Pseuderanthemum Clade makes biogeographic analysis premature but, again, relationships suggest multiple intercontinental dispersal events.

Malagasy Justicieae are here shown to belong to at least four clades: Clade 5 of the Pseuderanthemum Lineage (Fig. 5), OW Isoglossinae (Fig. 6), and the Anisotes and Anisostachya clades of Justicioids (Fig. 7). Additionally, McDade et al. (2018) place Malagasy plants in two clades of the Tetramerium Lineage (i.e., the Ecbolium clade and an unidentified plant sister to African Trichaulax Vollesen). Likewise, at least two clades of Diclipterae include Malagasy plants: Dicliptera and what is apparently a large radiation of Hypoestes Sol. ex R. Br. Assuming that Malagasy Brachystephanus are part of the clade that includes African Brachystephanus plus NW Stenostephanus, this will mark an additional clade with Malagasy plants. Whether all Malagasy diversity of Justicieae will be accommodated in these nine clades must await advances in our knowledge of the rich diversity of Acanthaceae on Madagascar. In addition to the clade count, suggestive of instances of long-distance dispersal, patterns of endemism versus widely distributed taxa merit attention. Patterns of diversity across clades should also be examined. Malagasy Hypoestes are remarkably species rich (I. Darbishire, T. F. Daniel, L. A. McDade, pers. obs.) and have been notably poorly sampled to date in phylogenetic work. It is not known whether Malagasy members of this genus are together monophyletic or whether multiple clades are present on Madagascar. The same can be said for a number of other Malagasy groups.

It is clear that many OW groups of Justicieae have complex biogeographic histories across the tropical and subtropical latitudes of Africa and Asia. Considerable additional study and sampling for phylogenetic analysis will be necessary to understand the Asian radiations of Justicieae. Notably, sampling of Indian plants for phylogenetic work to date has been extremely limited. Most Indian Justicieae appear to belong to the Pseuderanthemum Lineage and to the Justicioid Lineage, together with a small number of Isoglossa (Isoglossinae). In the Pseuderanthemum Lineage, there are Indian species of Pseuderanthemum, Asystasia, Phialacanthus (not sampled here but predicted to be part of the Asystasia Subclade), Codonacanthus, Cosmianthemum and Mackaya. Indian Justicia s.l. are likely part of several clades of OW Justicioids including the Rungia and Anisotes Clades (Kiel et al. 2017). There are also Indian species of Dicliptera, Peristrophe, Hypoestes and Rhinacanthus. Plants distributed in Tropical Asia and Australasia are placed in essentially all lineages of Justicieae except those restricted to the NW. Notably, the Pyssligottis Lineage is predominantly of plants that occur in this region. A great deal of work remains to understand the taxonomic and biogeographic distribution of Asian Acanthaceae.

**Chromosome Evolution**

Although chromosome data are lacking for the majority of sampled taxa, counts that have been made point to some patterns that merit mention. For the Pseuderanthemum Clade, there is evidence for $x = 21$ throughout, with counts of $n = 21$ for at least 10 sampled species, and of the same number for different species of genera sampled here (e.g., Ruspelia). Interestingly, there is evidence of polyploidy in the Cosmianthemum magnifolium-Mackaya bella clade with a count of $n = 42$ reported for M. bella (Daniel and Chuang 1989). Data for a species of Cosmianthemum might provide support for this unexpected relationship. As reported by Daniel (2018), there is also evidence for polyploidy among species of Odontonema, where $n = 21$ is most common.

Pseuderanthemum laxiflorum Hubbard ex L.H. Bailey (Fig. 1P, Q) is reported to have $n = 30$ (Kaur 1969), but $n = 21$ has been reported for all other species of Pseuderanthemum included in our sample for which chromosome numbers have been determined, including those from both the NW (e.g., P. alatum (Fig. 1Q) and P. floribundum (Fig. 1M, N)) and the OW (e.g., P. hildebrandtii Lindau (Fig. 1J) and P. tunicatum (Azel.) Milne-Redh.). Other members of Clade 4 (Fig. 5), to which P. laxiflorum belongs, include at least one other species with $n = 30$ (i.e., Graftophyllum pictum (L.) Griff., most counts) and others with $n = 21$ (i.e., C. carruthersii (Seem.) Guillemain, and G. pictum). Daniel (2018) discussed evidence for differences
in chromosome numbers between wild and cultivated plants of *G. pictum*.

There is little information for the *Asystasia* Clade with all known counts being from species of *Asystasia*. Daniel (2000) and Daniel et al. (2000) discussed chromosome numbers in *Asystasia* based on data for nine species. Meiotic counts of 13, 25, and 26 have been made for *A. gangetica*, with 14 and ca. 33 reported for *A. travancorica* (Fig. 1R). To our knowledge, no chromosome data are available for members of the *Herpetacanthus* Clade.

Chromosome data for the *Tetramerium* Lineage were discussed by McDade et al. (2000) and Daniel et al. (2008); these authors noted that *n* = 18 is widespread throughout the lineage. With *Isoglossa variegata* placed in this lineage rather than in Isoglossinae (Fig. 7), we predict a chromosome number of *n* = 18 for this species rather than *n* = 17, the number known for two sampled species of *Isoglossa* that are placed in Isoglossinae (Daniel et al. 2008) as described just below.

Kiel et al. (2006) summarized chromosome counts for Isoglossinae, which remains a cytologically poorly known lineage. Three African species of the Paleotropical genus *Isoglossa* have *n* = 17 (Daniel et al. 2000) and four NW members of *Stenostephanus* have *n* = 18 (Daniel 1999). No additional counts for taxa in this lineage have since been reported. It is tempting to predict that either *n* = 17 or a diversity of chromosome numbers will be shown to characterize OW taxa of this lineage, and that *n* = 18 will be common to taxa in the NW.

Turning to the *Pyssiglottis* Lineage, chromosome counts of *n* = 16 for *Calyacanthus magnusianus* (Fig. 2A) and *n* = ca. 16 for *Jadunia biroi* (Daniel 2000) would suggest that taxa in the *Calyacanthus magnusianus*-Jadunia clade, which consists entirely of Papuan taxa, may share a chromosome number. Although chromosome number determinations remain insufficient, *n* = 16 may characterize the *Pyssiglottis* Lineage, distinguishing it cytologically from its sister clade, Isoglossinae, with *n* = 17 and *n* = 18.

Chromosome numbers were discussed by Kiel et al. (2017) for several clades among Justicioidi. Remarkable diversity of chromosome numbers is known for taxa in this lineage (both those sampled here and numerous others). The diversity of numbers—21 numbers from *n* = 7 to *n* = 34—reported for the large, polyphyletic genus *Justicia* was illustrated by Daniel (2000). Although *n* = 14 is the most commonly reported number for species currently treated in *Justicia*, *n* = 15 appears to be common to Diclipterinae s.l. (i.e., *Dicliptera extensa-Rhinacanthus nasutus*, Fig. 7). Chromosome numbers of at least ten species of OW *Dicliptera* vary (*n* = 10, 13, 15, 24, 26, 30), whereas the 15 counts for NW *Dicliptera* reported to date all have *n* = 40 (e.g., Daniel et al. 2000). A single chromosome count of *n* = 11 has been reported for *Monechma ciliatum* (Jacq.) Milne-Redh., a species placed in *Monechma* Group 2 (sensu Kiel et al. 2017) sister to core Diclipterinae (Darbyshire et al. 2020). Elsewhere among Justicioids, *n* = 17 appears to be common in the *Anisotes* and *Betonica* clades (sensu Kiel et al. 2017) where it has been reported for both *Justicia adhatoda* and *J. betonica*, included in our study, and for a species of *Anisotes* not sampled herein (*A. madagascariensis*; Daniel 2006).

If these patterns hold, a number of lineages across Justiciaceae may be characterized by chromosome numbers including the *Pseuderanthemum* Clade (*n* = 21), the *Pyssiglottis* Lineage (*n* = 16), NW *Isoglossinae* (*n* = 18), the *Tetramerium* Lineage (*n* = 18), Diclipterinae (OW variable; NW *n* = 40). Clearly, more chromosome number determinations are needed to confirm or refute these initial hypotheses of chromosomal evolution among lineages of Justiciaceae.

**Morphological Evolution**

Justiciaceae are remarkably diverse morphologically. Reliance on morphological traits that have not yet been fully placed into phylogenetic context has resulted in the many polyphyletic genera that await phylogenetic and monographic work to clarify. On the other hand, our knowledge of species-level diversity in Justiciaceae remains in early stages. This is exemplified by the fact that any in-depth study, whether field or herbarium based, particularly in Madagascar and the NW tropics, yields collections of taxa that cannot be identified to species and may also result in newly described species. Once we have a nearly complete sampling of taxa, and relationships sorted out based on DNA sequence data—ideally via NGS methods that will yield many more data than we were able to employ here—we will be able to make a phylogenomic-based assessment of morphological evolution. This approach will almost certainly resolve recalcitrant groups such as *Isoglossinae* (Fig. 6), *Rhinacanthus* (Fig. 7) and relationships among members of the *Pseuderanthemum* Lineage (Fig. 5).

It is clear that androecial characters—including pollen—are relatively informative phylogenetically in Justiciaceae. Theclp position and “ornaments” (e.g., spurs, awns) merit additional explicit examination in a phylogenetic context. We have earlier hypothesized that strongly offset thecae and awns of various sorts are synapomorphic for the Justicioid Lineage (McDade et al. 2000), but it is now clear that some other Justiciaceae have these traits to at least some degree, notably *Isoglossa*.

The evolution of the rugula in the common ancestor of the Justicioid Lineage is phylogenetically informative although it is lost in core Diclipterinae. In addition, a few plants in other lineages have similar structures (e.g., *Anisotes perplexus* and some other members of *Isoglossinae*). Origin, development and morphological homologies of rugula and rugula-like structures merit additional study.

Most aspects of corolla morphology including floral form, size and color seem to be exceedingly homoplasious. Emphasis on these traits has led to many taxonomic groups that have been revealed to be polyphyletic: *Anisacanthus* Nees (McDade et al. 2018), *Anisotes*, *Isoglossa*, and *Pseuderanthemum* to name just four. Phylogenetically informed examination of the evolution of these traits will be fascinating, and interpretation should be sought in the context of pollinators.

Fruit characters may have some degree of phylogenetic signal as proposed herein, but considerable additional work will be required to assemble sufficient data to examine fruit evolution. Unfortunately, probably owing to their explosive dehiscence, fruits of many acanthaceous plants have not been fully documented. We urge that collectors document fruit morphology in collection notes and, ideally, with images whenever possible. As seeds are frequently lost when fruits dehisce, the same can be said for seeds.

On the other hand, our results indicate that informative traits come from essentially all plant structures in some clades. Although inflorescence morphology is extremely homoplasious, Kiel et al. (2017) and Darbyshire et al. (2020) have pointed...
to inflorescences as phylogenetically informative in some—
but not all—clades of Justicieae. For example, as described
above, second inflorescences may be synapomorphic for the
Anisostachya Clade and second spikes with imbricate bracts
are common in the Rungia Clade. Fruits with elastic placenta
mark the Rungia Clade and also Dicliptera. Even bracts can con-
tribute phylogenetically informative traits despite the fact that
they are as exuberantly homoplasious across Justicieae as any
other trait. For example, many members of the Rungia Clade
have bracts with hyaline margins. In sum, although charismatic
mega-synapomorphies are elusive for most clades of Justicieae,
we hold out considerable optimism that subtle traits or combi-
nations of such traits will be identified from most clades.

It is also notable that, for some groups, biogeography seems
to be a more reliable indicator of phylogenetic relationships
than morphological traits. Our results for Oplopan and Pseudera-
themum, and those of McDade et al. (2018) for Anisacanthus,
for example, are instances wherein geography apparently trumps
morphological similarities.

In conclusion, having returned to make a wide-angle exami-
nation of phylogenetic relationships among Justicieae, we have
both confirmed and advanced on the results of McDade et al.
(2000). Notably, we confirm monophyly of the Pseudera-
themum Lineage, adding many taxa to this lineage and identifying
a number of clades within the group. However, a great deal of
work remains to be done on this lineage and our results point
to a number of clades of manageable size that will benefit from
focused research. We confirm the hypothesis—supported by the
few relevant taxa sampled phylogenetically before the present
study—of the existence of the distinctive Ptyssiglottis Lineage,
and also its sister relationship to Isoglossea. At the level of
major lineages, the placement of Ichthyosyostoma requires further
examination; it would be intriguing if this single taxon were—
unlike hundreds of species of Justicieae studied to date—not
part of one of the five major clades. Also at the level of major
lineages, our inability to identify structural synapomorphies—or
even diagnostic traits—for several clades at the heart of the phy-
logeny of Justicieae (i.e., Ptyssiglottis Lineage + Isoglossea,
Ptyssiglottis Lineage, Tetramerium Lineage, Tetramerium Lin-
eage + Justicioid Lineage) is frustrating, especially given the
morphological disparity among Justicieae as a whole. Our re-
sults add Isoglossea to the list of large genera that are clearly not
monophyletic and require additional research; also on this list
are Pseuderathemum and Justicia. With regard to Isoglossea,
and also Anistes, our results confirm the value of pollen mor-
phology as a phylogenetically informative trait. Incongruence
between pollen morphology and taxonomic placement should
be regarded as a hint that other traits may be misleading. In sum,
evén as we advance knowledge of Justicieae, we point to in-
triguing aspects of this fascinating group that will richly reward
future researchers.

ACKNOWLEDGEMENTS

This work was supported by grants from the U.S. National
Science Foundation (DEB 9707693 to LAM; DEB 0108589 to
LAM and TFD; DEB 0743178 to LAM; DEB 1754845 to LAM
and CAK; BSR8304790 and BSR8609852 to TFD) and grants
to TFD from the American Philosophical Society, Christensen
Research Institute Fellowship, CAS In-House Research Fund,
La Amistad Research Fund of CAS, and the Lindsay Field Ex-
pedition Fund of CAS. We thank California Botanic Garden for
access to the core molecular facility and for herbarium sup-
port. TFD and LAM are grateful to the California Academy of
Sciences’ Madagascar Biodiversity Center for support of
field research in Madagascar. TFD is grateful to the California
Academy of Sciences’ Scanning Electron Microscopy Labora-
tory for use of their facilities and the assistance of their staff.
Curators at ARIZ, BR, C, CAS, CNS, COLO, DUKE, EA, F, G,
J, K, L, MO, NY, P, PH, RSA, UNA, UPS, and US herbaria
provided access to collections and permission for destructive sam-
pling. We thank Amy Weiss (NY) for providing an image of a
specimen of Isotheca alba, and Erin Tripp and J. Ryan Allen
(COLO) for providing specimen images of E. Tripp’s collec-
tions, Martin Callmanner (CJB) and Pete Phillipson (MO) are
thanked for providing leaf tissue samples for selected taxa from
Madagascar. We also thank Kevin Balkwill, Mandy-Jane Cad-
man, and Quentin Luke for assisting with field studies. Finally,
the California Botanic Garden, Kew Gardens, and the San Fran-
cisco Conservatory of Flowers provided greenhouse facilities
for growing cultivated material.

REFERENCES

Aké-Assi, L. 1961. Contribution à l’étude floristique de la Côte-d’Ivoire
e et des territoires limitrophes. Thèse, University of Paris, France.
Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Woj-
ciechowski, C. S. Campbell and M. J. Donoghue. 1995. The ITS
region of nuclear ribosomal DNA: a valuable source of evidence on
angiosperm phylogeny. Ann. Missouri Bot. Gard. 82: 247–277.
Balkwill, K. 1996. A synopsis of Peristrophe (Acanthaceae) in south-
ern Africa. Bothalia 26: 83–93.
—- and W. G. Welman. 2000. Acanthaceae. In O. A. Leistner
[ed.], Seed plants of southern Africa: families and genera. Strelitzia
10: 34–45. National Botanical Institute, Pretoria, South Africa.
Beialy, S. Y., M. S. Mahmoud and A. S. Ali. 2005. Insights on the
cloisonnement of Justicieae, two new species from Cuba.
Medellín 51: 351–354.
Bentham, G. 1876. Acanthaceae, pp. 1060–1122. In
The Genera of Plants, vol. 2. London: J. Van
Nash & Son.
Benoist, R. 1962. Nouvelles Acanthacées de Madagascar. Bothalia 6:
37: 273–289.
Benoit, R. 1962. Nouvelles Acanthacées de Madagascar. Bulletin de la
Society Botanique de France 109: 134–135.
Bentham, G. 1876. Acanthaceae, pp. 1060–1122. In
B. G. Bentham and J. D. Hooker, Genera plantarum, vol. 2(2). London [London]: venit
apud Reeve & Co. http://doi.org/10.5962/bhl.title.747.
Borhidi, A. 1983. Scelosanthus ekmanií (Ruibiaceae) and Saphthoa ek-
manií (Acanthaceae), two new species from Cuba. Nordic J. Bot. 3:
351–354.
Borowicz, M. L. 2016. AMAS: a fast tool for alignment manip-
ulation and computing of summary statistics. PeerJ 4: e1660.
https://doi.org/10.7717/peerj.1660.
Bouckaert, R., J. Heled, D. Kuhnert, T. Vaughan, C. H. Wu, D.
Xie, M. A. Suchard, A. Ramaut and A. J. Drummond. 2014.
BEAST 2: a software platform for Bayesian evolutionary analysis.
PLOS Comput. Biol. 10.4: e1003537.
Brummitt, R. K. 2001. World geographical scheme for recording
taxonomic Databases for Plant Sciences (TDWG), Hunt Institute for

Botanical Documentation, Carnegie Mellon University, Pittsburgh, http://www.tbdev.org/stds/109 (accessed 27 June 2020).

CHAMPLUVIER, D. 2002. Contribution à l’étude du genre Pseudernanthemum (Acanthaceae) en Afrique Tropicale. Syst. & Geogr. Pl. 72: 33–53.

——— and I. DARRBYSHIRE. 2009. A revision of the genera Brachystephanus and Orocanthus (Acanthaceae) in tropical Africa. Syst. & Geogr. Pl. 79: 115–192.

CHOOPIAN, T., P. J. GROTE, K. CHAYAMARIT and D. A. SIMPSON. 2018. An annotated checklist of the genus Pseudernanthemum Raddi (Acanthaceae) in Thailand. Thai Forest Bulletin 46: 90–111.

CLARKE, C. B. 1900. Isoglossa, pp. 227–234. In W. T. THRELTON-DYER [ed.], Flora of Tropical Africa, vol. 5. L. Reeve and Co., London, UK.

CÔRTEZ, A. L., A. RAPINI and T. H. CHUANG. 1989. Chromosome numbers of some cultivated Acanthaceae. Brittonia 41: 319–332.

———. 2000. Additional chromosome numbers of American Acanthaceae. Syst. Bot. 25: 15–25.

———. 2006. Chromosome numbers of miscellaneous Malagasy Acanthaceae. Brittonia 58: 291–300.

———. 2019b. Taxonomic notes and novelties in the genus Isoglossa (Acanthaceae) in tropical Africa. Flora Zambesiaca 39: 617–621.

———. 2006. Chromosome numbers of miscellaneous Malagasy Acanthaceae. Brittonia 58: 291–300.

———. 2019b. Taxonomic notes and novelties in the genus Isoglossa (Acanthaceae) in tropical Africa. Flora Zambesiaca 39: 617–621.

———. 2006. Chromosome numbers of some cultivated Acanthaceae with notes on chromosomal evolution in the family. Proc. Calif. Acad. Sci. 64: 319–332.

——— and T. I. CHUANG. 1989. Chromosome numbers of some cultivated Acanthaceae. Baileya 23: 86–93.

———, K. BALKWILL and M.-J. BALKWILL. 2000. Chromosome numbers of South African Acanthaceae. Proc. Calif. Acad. Sci. 52: 143–158.

———, R. LETSARA and S. MARTIN-BRAYO. 2013. Four new species of Anisotes (Acanthaceae) from Madagascar. Novon 22: 390–408.

———, L. A. MCDADE, M. MANTELOW and C. A. KIEL. 2008. The “Tetramerium Lineage” (Acanthaceae): Acanthoideae: Justicieae): delimitation and intra-lineage relationships based on cp and nrITS sequence data. Syst. Bot. 33: 416–436.

DARRBYSHIRE, I. 2009a. Notes on the genus Dicliptera (Acanthaceae) in Eastern Africa. Kew Bull. 63: 361–383.

———. 2009b. Taxonomic notes and novelties in the genus Isoglossa (Acanthaceae) from Eastern Africa. Kew Bull. 64: 401–427.

——— and ENSERMU KELBESA. 2007. Isoglossa asystasiaoides, a striking new species of Acanthaceae from Tanzania. Kew Bull. 62: 617–621.

——— and T. HARRIS. 2006. Notes on the genus Rhinacanthus (Acanthaceae) in Africa with a synopsis of the R. nasutus-R. gracilis complex and a key to the African members of the genus. Kew Bull. 61: 401–418.

———, ENSERMU KELBESA and K. VOLLENSE. 2010. Acanthaceae (Part 2), pp. 325–442. In H. J. BEENTJE [ed.], Flora of tropical East Africa. Royal Botanic Gardens, Kew, UK.

———, L. PEACE and H. BANKS. 2011. The genus Isoglossa (Acanthaceae) in west Africa. Kew Bull. 66: 425–439.

———, K. VOLLENSE and ENSERMU KELBESA. 2015. Acanthaceae (Part 2), pp. 37–122. In J. R. TIMBERLAKE and E. S. MARTINS [ed.], Flora Zambesiaca 8 (6). Royal Botanic Gardens, Kew, UK.

———, C. A. KIEL, T. F. DANIEL and W. R. Q. LUK. 2019. Two new genera of Acanthaceae from tropical Africa. Kew Bull. 74–39: 1–25.

———, C. A. KIEL, C. M. ASTROTH, K. G. DEXTER, F. M. CHASE and E. A. TRIPP. 2020. Phylogenomic study of Monochaeta reveals two divergent plant lineages of ecological importance in African savanna and succulent biomes. Diversity 12: 237. doi:10.3390/d12060237.
APPENDIX I

Taxa, Genbank accession numbers (trnL-F, rps16, trnT-L, trnS-G, mtTS; = sequence not obtained), sources of plant materials from which DNA was extracted for sequencing, and lineage where placed by the analyses presented here (i.e., as depicted in Fig. 4). To facilitate data location, taxa are listed in alphabetical order by genus and species. When plants in cultivation were used, we provide information on native range in parentheses. Abbreviations for herbaria follow Thiers (2016).

**Afridittonia silvestris** Lindau; MW451356, MW451424, MW451493, MW451553, =, Equatorial Guinea, Luke et al. 13070 (EA), Pseuderanthemum Lineage, Angkalanthus oligophylla Balf.; EU087567, EU087533, EU081172, EU081105, EU087478, Yemen (Socotra), Miller M101292 (UPS), Tetramerium Lineage. Anisotes forsskallssianus (Klotzsch) Milne-Redh.; MW451232, MW451280, KY841626, KY632576, cultivated, Lowveld National Botanical Garden, South Africa: Mpumulanga (native to South Africa), Daniel 9382 (CAS), Justicioid Lineage. Anisotes macrophyllus (Lindau) Heine; MW451232, , Madagascar, KY584141, KY282341, Uganda, Symbiot 488 (EA), Justicioid Lineage. Anisotes perplectus T.F. Daniel, Letsara & Martín-Bravo; , , , , , Madagascar, Malcomb (CAS), Isoglossinae. Anisotes pulchellus (Engler) T.F. Daniel, Letsara & Rakontonas; , , , , , Madagascar, Physacanthus travancoricus (Balf.), Justicioid Lineage, Aplealandra leonardii McDade; AF983112, DQ59205, EU529071, DQ59287, AF169761, Costa Rica, McDade 310 (DUKE), outgroup. Ascotheca panicinera (T. Anderson ex C.B. Clarke) Heine; MW451376, MW451446, MW451514, MW451581, MW464292, Wilde & White-Duyses 1744 (BR), Justicioid Lineage. Asystasia sp.; MW451343, MW451458, MW451502, MW451562, AF289794, Madagascar, Daniel 929 (CAS), Pseuderanthemum Lineage. Asystasia sp.; MW451343, MW451458, MW451562, MW464280, Madagascar, Daniel et al. 104611 (CAS), Pseuderanthemum Lineage. Asystasia gangetica (L.) T. Anderson; AF289748, MW451345, MW451504, MW451564, AF289793, cultivated, Nelspruit, Mpumulanga, South Africa (widespread in Old World tropics and subtropics), Daniel & Balkwill 7274 (K), Pseuderanthemum Lineage. Asystasia myosorensis (Roth) T. Anderson; MW451360, MW451429, MW451498, MW451558, MW464276, South Africa, McDade & Balkwill 7274 (J), Pseuderanthemum Lineage. Asystasia travancoricar Bedd., MW451366, MW451436, MW451505, MW451565,
Tetramerium
Conservatory of Flowers, San Francisco, California, USA, MW451357, lon 8517–, KY632505, KY632195, KY584123, KY632545, Peru, 10072
Gardens, Sydney, Australia, accession 902121 (native to Papuasia), EU529012, EU081175, EU081108, EU528880, cultivated Royal Botanic
AF169751, cultivated, S.F. Conservatory of Flowers, San Francisco,
Barleria lupulina
EU081167,EU081100,EU087474,SouthAfrica,
Muell.) Druce; MW451329, MW451395, MW451463, MW451526,
Pseuderanthemum
MW464275, cultivated T.F. Daniel home (native to Brazil)
Daniel 70767
MW451439, MW451508, MW451566, Af289797, Brazil, Bremek.; MW451329, –, MW451460, –, MW464243,
Chileranthemum pyramidatum
Lindau; MF768365, MF678385, MF768306, MF670387,
rhomboidea
MW451340, MW451407, MW451475, MW451537, MW464257, native (UPS),
8575

Lineage.

themum
MW464259, Yemen (Socotra),
Pseuderanthemum
Lineage.

Glossochilus burchellii
Hansen 104
magnifolium
Bremek.; MW451329, –, MW451460, –, MW464243,
Humbert 28671

Lineage.

Ptyssiglottis
Lineage.

Asystasia vogeliana
Lineage.

Pseuderanthemum
Lineage.

Justicioid Lineage.

Brachystephanus africanus
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Graptophyllum excelsum
Lineage.

Graptophyllum pictum
Lineage.

Justicioid Lineage.

Boyds.n.

Klackenberg and Lundin 677

Justicia sp.

J. Graham 620

Isoglossa spinigerum
Lineage.

Cephalacanthus maculatus

Thulin & Gifri

Ballochia rotundifolia
39-1588

Calycacanthus magnusianus

Sankowsky 1818

Juss.; MK282360, EU529019, EU529019, KY584189,

Ptyssiglottis
Lin- ben; MK282359, (P), Leandri 3033

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pteuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.

Pseuderanthemum
Lineage.
Pseuderanthemum sp.; MW451345, MW451411, MW451479, MW451541, MW451549, MW464249, Madagascar. 

**Justicia** R. Br., 1810

**Justicia** is a genus of flowering plants in the family Acanthaceae, consisting of over 150 species. They are mainly found in the Americas, Africa, and Asia, with a few species in Europe. The genus is characterized by their tubular flowers, often with red or purple coloring, and they are commonly referred to as “broom sedge.”

**Megaskepasma** (Wasshausen et al. 2003)

**Megaskepasma** is a genus of flowering plants in the family Acanthaceae, consisting of about 10 species. They are native to South America and are characterized by their large, showy flowers, often with red or purple colors. The genus is known for its ornamental value in gardens and landscapes.

**Pseuderanthemum** (Duarte s.n.)

**Pseuderanthemum** is a genus of flowering plants in the family Acanthaceae, consisting of about 40 species. They are native to South America, with some species also found in the Caribbean. The genus is characterized by their showy flowers, often with red or purple coloring, and they are commonly referred to as “broom sedge.”

**Poikilacanthus** (L.) R. Br., 1810

**Poikilacanthus** is a genus of flowering plants in the family Acanthaceae, consisting of about 10 species. They are native to South America and are characterized by their large, showy flowers, often with red or purple colors. The genus is known for its ornamental value in gardens and landscapes.

**Rhinacanthus** (L.) R. Br., 1810

**Rhinacanthus** is a genus of flowering plants in the family Acanthaceae, consisting of about 10 species. They are native to South America and are characterized by their large, showy flowers, often with red or purple colors. The genus is known for its ornamental value in gardens and landscapes.

**Stapfia** (Neck.) T. Anderson, 1899

**Stapfia** is a genus of flowering plants in the family Acanthaceae, consisting of about 10 species. They are native to South America and are characterized by their large, showy flowers, often with red or purple colors. The genus is known for its ornamental value in gardens and landscapes.
KY584220, KY632634, Uganda, Lye (EA), Justicioid Lineage. *Rhimacanthus* serophilus A. Meeuse; MK282400, MK282329, KY632291, KY584224, KY632637, South Africa, McDade 1277 (J), Justicioid Lineage. *Rungia* klossii S. Moore; MK282401, MK282330, KY632470, KY632584, Papua New Guinea, Daniel et al. 6561 (CAS), Justicioid Lineage. *Rungia* hypocrateriformis (Vahl) Milne-Redh.; MW451339, MW451406, MW451474, MW451536, MW451544, MW464265, Cuba, Alain et al. 5607 (US), Pseuderanthemum Lineage. *Sanchezia* speciosa Leonard; AF063115, MW451394, MW451459, MW451523, AF169847, United States, McDade & Jenkins 1134 (ARIZ), Tetramerium Lineage. *Tetramerium* nervosum Nees; AF063133, EU087493, EU081126, EU081058, AF169847, United States, McDade & Jenkins 1134 (ARIZ), Tetramerium Lineage. *Ruellia* californica (Rose) I.M. Johnst.; AF063115, MW451394, MW451459, MW451523, AF167704, cultivated, Univ. Arizona campus, Tucson, AZ, USA, McDade 1157 (ARIZ), outgroup.