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## PHYLOGENETIC RELATIONSHIPS OF *MUEHLENBECKIA*, *FALLOPIA*, AND *REYNOUTRIA* (POLYGONACEAE) INVESTIGATED WITH CHLOROPLAST AND NUCLEAR SEQUENCE DATA

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The monophyly of *Muehlenbeckia* and evolutionary relationships with other genera in Polygonoideae have been unclear. The traditional placement of *Muehlenbeckia* in Coccolebeae (Eriogonoideae) along with *Antigonon*, *Brunnichia*, and *Coccoloba* has not been supported in previous studies, and it is more closely related to *Fallopia* in Polygonoideae. Molecular data from 22 species of *Muehlenbeckia* and from representatives of *Atraphaxis*, *Fallopia* (including *Reynoutria*), *Polygonella*, and *Polygonum* s.s. were generated from five chloroplast regions (*matK*, *ndhF*, 3'*rps16-5'trnK*, *trnL-trnF* 3'*trnV-ndhC*) and two nuclear gene regions (second intron of *LEAFY*, internal transcribed spacer). Analyses used maximum likelihood and maximum parsimony methods. Neither *Fallopia* or *Muehlenbeckia* is monophyletic, with most species of *Muehlenbeckia* related to *Fallopia*. Four clades are named as a result of this study: *Duma*, *Fallopia*, *Muehlenbeckia*, and *Reynoutria*. *Duma* contains three Australian species of *Muehlenbeckia* that form a clade sister to the clade containing *Polygonum* and *Polygonella*. Relationships within *Muehlenbeckia* correspond to geographic distribution patterns, and relationships in the *Reynoutria* and *Fallopia* clades correlate with base chromosome numbers.

**Keywords:** Australia, *Duma*, extrafloral nectaries, *Polygonum* s.l., phylogeny, Southern Hemisphere.

**Online enhancements:** appendixes.

### Introduction

Polygonaceae Juss. form a clade that includes ~49 genera and ~1,200 species (Sanchez et al. 2011). Members of the family are found in a spectrum of habitats ranging from the Arctic to the lowland tropics and from aquatic to xeric conditions. Most species occur in the temperate zone of the Northern Hemisphere. For example, 35 of the 49 genera and ~50% of the described species occur in North America (Freeman and Reveal 2005). Polygonaceae are for the most part easily recognized, and the group was known to Dioscorides (~40–90 AD; Holm 1927). Diagnostic characters for the family are the presence of an ocrea, a tissue that sheathes the stem at the nodes and is often considered to be fused stipules (Graham and Wood 1965), nodes thickened relative to the stem, and quincuncial aestivation. Although these characters are useful at the family level, there are exceptions to these traits in some of the subgroups (e.g., lack of ocreae in *Eriogonum* Michx. and no quincuncial aestivation in Rumiceae Dumort.; see discussion in Burke et al. 2010). Other groups within Polygonaceae are difficult to distinguish because of their high level of morphological variability. This is evident in the convoluted taxonomic history for Polygonaceae. The emphasis on particular morphological characters by different

authors has led to a range of often conflicting taxonomic arrangements and proliferation of names (Meisner 1826, 1856; Endlicher 1847; Bentham and Hooker 1880; Dammer 1893; Perdrigaet 1900; Gross 1913a, 1913b; Danser 1927; Jaretsky 1925, 1928; Steward 1930; Hedberg 1946; Löve and Löve 1956; Roberty and Vautier 1964; Hara 1966; Haraldson 1978; Ronse Decraene and Akeroyd 1988; Brandbyge 1993; Tutin 1993). Haraldson (1978) and Ronse Decraene and Akeroyd (1988) give good overviews of the taxonomic history of Polygonaceae based on features of morphology, anatomy, and palynology.

More recently, several authors have investigated evolutionary relationships of Polygonaceae using molecular data. These molecular studies have confirmed that Polygonaceae are monophyletic and showed that Plumbaginaceae Juss. are its sister group (Chase et al. 1993; Fay et al. 1997; Lledó et al. 1998; Chase et al. 2002; Cuénoud et al. 2002). Others undertook large-scale analyses of Polygonaceae and found some unexpected relationships within the group (Lamb-Frye and Kron 2003; Sanchez and Kron 2008; Galasso et al. 2009; Sanchez et al. 2009). For example, *Antigonon*, *Brunnichia*, and *Coccoloba* were placed in an expanded Eriogonoideae Arn. rather than in their traditional position in Polygonoideae Eaton (Sanchez and Kron 2008). In addition, *Muehlenbeckia* was shown to be a member of Polygonaceae (Lamb-Frye and Kron 2003) rather than Coccolebeae (table 1), where it had been placed by most authors who utilized

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**Table 1**  
**Placement of *Duma*, *Fallopia*, *Homalocladium*, *Muehlenbeckia*, and *Reynoutria* in Recent Classifications**

Tribe	This study	Galasso et al. (2009)	Brandbyge (1993)	Ronse Decraene and Akeroyd (1988)	Haraldson (1978)
Coccolobeae Dumort.	N/A	N/A	<i>Antigonon</i> Endl. *, <i>Brunnichia</i> Banks ex Gaertn. *, <i>Coccoloba</i> P. Browne ex L. *, <i>Muehlenbeckia</i> , <i>Podopterus</i> Humb. & Bonpl. *, <i>Neomillspaughia</i> S.F. Blake *	N/A	<i>Antigonon</i> *, <i>Brunnichia</i> *, <i>Coccoloba</i> *, <i>Fallopia</i> , <i>Harpagocarpus</i> Hutch. et Dandy, <i>Muehlenbeckia</i> , <i>Podopterus</i> *, <i>Reynoutria</i>
Polygonaceae Rchb.	<i>Duma</i> T.M.Schuster, <i>Atraphaxis</i> L., <i>Fallopia</i> (incl. <i>Knorringia</i> (Czuk.) S.P.Hong, <i>Muehlenbeckia</i> Meisn. (incl. <i>Homalocladium</i> (F.Muell.) L.H.Bailey), <i>Polygonum</i> L. s.s. (incl. <i>Polygonella</i> Michx.), <i>Reynoutria</i> Houtt.	<i>Atraphaxis</i> , <i>Calligonum</i> L. *, <i>Fallopia</i> s.l. (incl. <i>Reynoutria</i> ), <i>Homalocladium</i> , <i>Muehlenbeckia</i> Meisn., <i>Oxygonum</i> Burch. ex Campd., <i>Parapteropyrum</i> A.J.Li *, <i>Polygonum</i> (incl. <i>Polygonella</i> ), <i>Pteropyrum</i> Jaub. ex Spach. *, x <i>Reyllopia</i> Holub, <i>Reynoutria</i>	<i>Atraphaxis</i> , <i>Calligonum</i> *, <i>Fallopia</i> , <i>Oxygonum</i> , <i>Polygonum</i> s.s., <i>Pteropyrum</i> *, <i>Reynoutria</i>	<i>Atraphaxis</i> , <i>Calligonum</i> *, <i>Fallopia</i> (incl. <i>Reynoutria</i> ), <i>Oxygonum</i> , <i>Polygonella</i> (later incl. in <i>Polygonum</i> ), <i>Polygonum</i> s.s., <i>Pteropyrum</i> *	<i>Atraphaxis</i> , <i>Calligonum</i> *, <i>Fagopyrum</i> Mill. * (incl. <i>Pteroxygonum</i> ), <i>Oxygonum</i> , <i>Polygonella</i> , <i>Polygonum</i> s.s., <i>Pteropyrum</i> *

Note. Asterisk denotes placement different from the position in this study (fig. 1). N/A = not applicable.

morphological and anatomical data (Meisner 1856; Bentham and Hooker 1880; Gross 1913a, 1913b; Jaretsky 1925, 1928; Haraldson 1978).

Although significant progress has been made in understanding the complex evolutionary history of Polygonaceae on a large scale, the relationships of many smaller groups are in need of study. In particular, delimitation and relationships of genera associated with the polyphyletic *Polygonum* s.l. are still problematic. Many groups that were at one time included in *Polygonum* s.l. are now recognized as distinct genera (e.g., *Aconogonon* (Meisn.) Rchb., *Bistorta* (L.) Scop., *Coccoloba*, *Fallopia*, *Knorringia*, *Persicaria* Mill., *Reynoutria*) and neither their monophyly nor their relationships have been studied in detail except for *Persicaria* (Kim and Donoghue 2008a, 2008b; Kim et al. 2008). Meisner (1856) divided *Polygonum* s.l. into nine sections comprising 215 species. Members of his *Polygonum* section *Tiniaria* Meisn. contained species that are now recognized as *Fallopia* and *Reynoutria* (table 2), but he regarded *Muehlenbeckia* as distinct. The relationships of *Fallopia*, *Muehlenbeckia*, and *Reynoutria* to each other have sometimes been disputed but they were often considered closely related (e.g., Haraldson 1978). Although the circumscription of *Muehlenbeckia* has been consistent across treatments, the delimitation of *Reynoutria* and *Fallopia* has not. *Reynoutria* has been included within *Fallopia* in some classifications (Ronse Decraene and Akeroyd 1988; Bailey and Stace 1992; Yonekura and Ohashi 1997), but in others, *Fallopia* and *Reynoutria* have been considered separate genera (Nakai 1926; Holub 1970; Haraldson 1978; Tzvelev 1987, 1989; Brandbyge 1993). *Fallopia* and *Reynoutria* are north-temperate in distribution and often occur in weedy or disturbed areas.

Many species of *Muehlenbeckia* also respond to natural disturbance such as wildfires and cyclones. The genus is restricted to the Southern Hemisphere, with 21 species in Australia, New Guinea, and New Zealand, and nine that occur in Central and South America. They are lianas, vines or scandent to erect (sub)shrubs, and most occur in xeric environments and/or at high elevations such as the páramo or the Southern Alps of New Zealand. Several Australian species, for example *M. declina* and *M. costata*, are fast-growing colonizing species that dominate soon after wildfires (Hunter et al. 1998) or after cyclone damage that causes light breaks in the forest canopy (e.g., *M. zippelii*; K. L. Wilson, personal observation). In both cases, they gradually disappear as they are crowded out by taller but slower-growing species. In horticulture, species of *Muehlenbeckia* including *M. astonii*, *M. axillaris*, *M. complexa*, and *M. platyclada* (previously segregated as *Homalocladium platycladum* (F.Muell.) L.H.Bailey) are used as ornamentals or in the florist trade. The fruits of some species with a fleshy perianth have been used locally as food in Australia (e.g., *M. gunnii*; Cribb and Cribb 1975).

*Muehlenbeckia* was erected by Meisner (1841) and included *M. adpressa*, *M. complexa*, *M. tannifolia*, and *M. sagittifolia*. He considered these closely related, based on their having a succulent mature perianth, fimbriate stigmas, dioecy, and/or polygamy. In his treatment of the genus in 1856, Meisner included 18 species in the sections *Andinia* Wedd., *Eumuehlenbeckia* Endl., and *Sarcogonum* Endl. Currently 30 species of *Muehlenbeckia* are recognized (Meisner

1856; Danser 1940; Brandbyge 1989, 1992; Wilson 1990; Makinson and Mallinson 1997). Brandbyge (1992) monographed the Central and South American species of *Muehlenbeckia* and included a few of the Australasian species for comparison.

This molecular study addresses the evolutionary relationships among the species of *Muehlenbeckia* and other members of Polygonoideae, specifically *Fallopia* and *Reynoutria*. Seven gene regions were sequenced, including five chloroplast markers: *matK* and *ndhF* genes, 3'*rps16*-5'*trnK*, *trnL-trnF*, 3'*trnV-ndhC* intergenic spacer regions (IGS), and two nuclear markers—internal transcribed spacer (ITS) and the second intron of *LEAFY* (*lfy2*). Data were analyzed with maximum likelihood and maximum parsimony methods. The following questions were addressed. (1) Is the genus *Muehlenbeckia* monophyletic? (2) What is its relationship to other genera in Polygonoideae with particular reference to *Fallopia* and *Reynoutria*? (3) Do the recovered relationships of species of *Muehlenbeckia* reflect any morphological or geographical patterns?

## Material and Methods

### Taxon Sampling

Eighty-seven species were included in this study, with an emphasis on *Muehlenbeckia* and related genera in the Polygonoideae (app. A). Taxon sampling was based on the results of previous large-scale studies in Polygonaceae that indicated relationships of *Muehlenbeckia* to *Atraphaxis*, *Fallopia*, *Polygonella*, and *Polygonum* s.s. (Lamb-Frye and Kron 2003; Sanchez et al. 2009). Twenty-two of the 30 currently recognized species of *Muehlenbeckia* were included in this study. Eight species were unavailable at the time of this project due to the lack of suitable material: *M. andina* Brandbyge, *M. fruticulosa* (Walp.) Standl., *M. bastulata* (Sm.) I.M.Johnst., *M. monticola* Pulle, *M. nummularia* H.Gross, *M. polybotrya* Meisn., *M. sagittifolia* (Ortega) Meisn., and *M. triloba* Danser. Ten species of *Fallopia* s.l. were sampled (Holub 1970; Tzvelev 1987; Li et al. 2003), because previous analyses have shown *Fallopia* to be sister to *Muehlenbeckia* (e.g., Sanchez et al. 2009). Representatives of all sections of *Fallopia* that are found in the literature were included (sections *Fallopia*, *Pleuropterus* (Turcz.) Haraldson, *Reynoutria* (Houtt.) Ronse Decr., and *Sarmentosae* (Grinz.) Holub) except section *Parogonum* Haraldson. Species of *Fallopia* that could not be included in this study are *F. cilinodis* (Michx.) Holub, *F. cynanchoides* (Hemsl.) Haraldson, *F. denticulata* (Huang) Holub, *F. filipes* (Hara) Holub, *F. pterocarpa* (Meisn.) Holub, and *F. schischkinii* Tzvelev.

Additional representatives from the other tribes in Polygonoideae (Calligoneae C.A. Mey., Fagopyreae Yonek., Persicarieae Dumort., Rumiceae) and Eriogonoideae were included as well. Plumbaginaceae (*Armeria splendens*, *Limonium delicatum*, *Limonium narbonense*, and *Plumbago auriculata*) were designated as the outgroup.

### DNA Extraction, Amplification, Cloning, and Sequencing

Total genomic DNA was isolated from freshly collected silica-gel-dried leaves or herbarium material with the DNeasy Plant

Table 2

Position of *Reynoutria* by Selected Authors

	Meisner (1856)	Bentham and Hooker (1880)	Nakai (1926)	Holub (1970)	Haraldson (1978)	Ronse Decraene and Akeroyd (1988)	Bailey and Stace (1992)	Brandbyge (1993)
<i>Fallopia</i>	<i>Polygonum</i> sect. <i>Tiniaria</i>	<i>Polygonum</i> sect. <i>Tiniaria</i>	<i>Bilderdykia</i>	<i>Fallopia</i>	<i>Fallopia</i>	<i>Fallopia</i>	<i>Fallopia</i>	<i>Fallopia</i>
<i>Reynoutria</i>		<i>Polygonum</i> sect. <i>Pleuropterus</i>	<i>Reynoutria</i>	<i>Reynoutria</i>	<i>Reynoutria</i>			<i>Reynoutria</i>

Mini Kit from Qiagen (Valencia, CA). Seven gene regions were amplified, including the two chloroplast (cp) genes *matK* and *ndbF*, three cp intergenic spacer regions *3'rps16-5'trnK*, *trnL-trnF*, *3'trnV-ndbC* and two nuclear (nr) markers, the 2nd intron of *LEAFY* (*lfy2*) and nuclear ribosomal ITS. These markers were chosen because they are fast-evolving regions and have been shown to be useful for phylogenetic analyses of Polygonaceae in previous studies addressing infraspecific relationships (e.g., Shaw et al. 2007; Kim and Donoghue 2008b; Kim et al. 2008; Sanchez and Kron 2008).

GoTaq Colorless Master Mix from Promega (Madison, WI) was used for all PCR reactions, since the dye in GoTaq Green Master Mix interferes with ExoSAP-IT (USB, Cleveland, OH) cleanup. A final primer concentration of 0.6  $\mu$ M was used for all 25- $\mu$ L PCR reactions with the addition of 0.01% of DMSO per reaction. See appendix B in the online edition of the *International Journal of Plant Sciences* for primer pair names, oligonucleotide sequences, PCR program settings, and associated publications. Sequences of primers newly designed for this study (with the primer design tool in Geneious v.5.0.4; Drummond et al. 2009) and cycling conditions are as follows: *lfy2*-forward (MLFYI2-1543F): 5' TGY GGA GAC AAG TGC CCC MCT A 3'; *lfy2*-reverse (MLFYI2-2385R): 5' TGC GTA YCT GAA CAC TTG GTT YGT 3'; step-down cycling conditions of  $-3^{\circ}\text{C}$  from  $68^{\circ}$  to  $47^{\circ}\text{C}$ ; *ndbF*-forward (ndhF-110F): 5' GCG TAT GTG GGC TTT TCC TA 3'; *ndbF*-reverse (ndhF-1248R): 5' TGG ACC AAA AAC AAG CAA GA 3'; cycling conditions:  $94^{\circ}\text{C}/01:00 + 25 \times (94^{\circ}\text{C}/00:30, 64^{\circ}\text{C}/00:30, 72^{\circ}\text{C}/01:00) + 72^{\circ}\text{C}/10:00$ . The reference used for numbering the *ndbF* primer is *Fagopyrum esculentum* subsp. *ancestrale* (GenBank, EU254477). When little product was generated during the first PCR, a second round was done with a 1- $\mu$ L template of the first reaction using the same primers and PCR program settings.

Both nuclear regions were cloned with the Invitrogen (Carlsbad, CA) TOPO TA cloning kit for sequencing. All cloning reactions were done by using one-third of the volumes given in the manufacturer's manual. In addition, the protocol was modified to use a 15-s heat shock period instead of 30 s. Ten colonies per taxon were picked, diluted in 10  $\mu$ L nuclease-free ddH<sub>2</sub>O, and visualized via electrophoresis (0.8% agarose) after PCR amplification with the universal M13 primers. One  $\mu$ L of the colony in ddH<sub>2</sub>O was used as template for the M13 PCR reaction, and the remaining volume was stored at  $-20^{\circ}\text{C}$ . For ITS, M13 primer PCRs did not amplify product for most species of the ingroup (likely due to complications involving secondary structures), and in that case Templiphi rolling circle amplification (GE Healthcare, Piscataway, NJ) was used for amplification. Cleanup of

all PCR products was done with Qiagen's QIAquick purification columns or ExoSAP-IT. Sequencing was carried out on an ABI-3100 genetic analyzer (Applied Biosystems, Carlsbad, CA) at the Wake Forest University School of Medicine, Winston-Salem, NC. Three clones of a subset of four species were sequenced for ITS for both the forward and reverse reaction of the M13 PCR products after cloning. *LEAFY* is single copy in all Polygonaceae sampled to date, and therefore, one clone per species was sequenced. Sequences for both directions were also obtained for the directly sequenced cp fragments.

## Alignment and Phylogenetic Analyses

PeakTrace Basecaller v.10.8 (Nucleics 2010) was used on all sequences to obtain read extensions (up to 300 bp). Sequences were edited with Geneious v.5.0.4 (Drummond et al. 2009), blasted against GenBank entries to check gene region and taxon matches, aligned with the automatic alignment software MAFFT v.6 (Katoh et al. 2002, 2005, 2008), and checked by eye. Data columns missing  $\geq 80\%$  data were excluded for all regions utilizing the python language script Chargap.py (available from Sabrina Setaro by e-mail at [sabrina.setaro@gmail.com](mailto:sabrina.setaro@gmail.com)). Insertion/deletion information was not included in the analyses, and these sites were treated as missing data. An analysis of congruence assessment among ultrametric distance matrices (CADM; Legendre and Lapointe 2004; Campbell et al. 2009) was used to check for incongruence between the data partitions. In addition, bootstrap support (BS) values (Felsenstein 1985, 1988) for separate analyses of each gene region and the combined nr versus the concatenated cp data sets were compared in a search for strongly supported conflicting clades. An incongruence length difference (ILD) test (Farris et al. 1994) was not performed due to criticisms of the test documented in the literature (Cunningham 1997; Dolphin et al. 2000; Yoder et al. 2001; Barker and Lutzoni 2002).

The molecular data were analyzed under maximum likelihood (ML) and maximum parsimony (MP) criteria. Maximum parsimony analyses were conducted in PAUP\* v.4.0b.10 (Swofford 2002) with the following settings: parsimony informative characters were treated as unordered and unweighted and gaps as missing data. Heuristic searches employed tree-bisection-reconnection branch swapping and 1000 replicates of random stepwise additions. For ML, the general time reversible (GTR) substitution model (option GTRMIX and partitioned data mode using seven distinct models per partitions with joint branch length optimization) was used in RAxML where the likelihood of the final tree is evaluated and optimized under GAMMA (Stamatakis et al.

2005; Stamatakis 2006). Analyses were run for each gene region separately, for all five chloroplast regions combined, for the two nuclear regions combined, and for total combined data (app. C data matrix file, available in the online edition of the *International Journal of Plant Sciences*). Bootstrap values were generated with each program using the rapid bootstrap option in RAxML, which is not comparable to fast bootstrap in PAUP (Stamatakis et al. 2008), and parametric bootstrap for MP. One thousand bootstrap repetitions were done for both ML and MP. References in the text to level of BS are low, 50%–69%; moderate, 70%–89%; and high, 90%–100%.

## Results

All sequences generated for this study are available from the National Center for Biotechnology Information (app. A). Data were combined in a total evidence approach, since no strongly supported conflicts were detected by the CADM analysis or by the comparison of BS values of analyses performed for each separate gene region (each highly unresolved) and combined cp versus combined nr data sets. We consider the total evidence tree (ML) as the best estimate of the phylogenetic relationships in this study (figs. 1, B1 in the online edition of the *International Journal of Plant Sciences*). Statistics of the results such as length in bp of each aligned gene region, number of parsimony informative characters (%), CI, RI, and number of missing species (%) for each data partition are presented in table 3. Note that only ML clade support is referenced in the text (both ML and MP BS values are shown in figs. 1, B1).

### Phylogenetic Analyses

As in previous molecular studies, our results recover two well-supported clades within Polygonaceae that correspond to the most recent circumscription of subfamilies Eriogonoideae and Polygonoideae. In Polygonoideae, relationships of tribes obtained with this taxon sampling (fig. 1) are summed up as follows: Persicarieae (Fagopyreae (Calligoneae (Rumiceae + Polygoneae))). The backbone of the tree is well supported and there are no extremely long branches.

Within tribe Polygoneae (fig. 1), *Knorringia sibirica* is sister to the remaining species sampled. These are contained in two strongly supported subclades: *Atraphaxis-Duma-Polygonum* + *Polygonella* (ADP clade) and *Reynoutria-Muehlenbeckia* + *Fallopia* (RMF clade). The ADP clade contains three species previously placed in *Muehlenbeckia* (*M. coccoloboides*, *M. florulenta*, and *M. horrida*). These form a well-supported clade (*Duma*) that is sister to the *Polygonum* + *Polygonella* clade (fig. 1). This result indicates that, as currently recognized, *Muehlenbeckia* is polyphyletic.

The RMF clade contains three well-supported subclades (fig. 1). The *Reynoutria* clade (98% BS) includes *R. forbesii*, *R. japonica*, *R. multiflora*, and *R. sachalinensis*. *Reynoutria* is the sister group to *Fallopia* + *Muehlenbeckia*. The *Fallopia* clade (97% BS) contains *F. baldschuanica*, *F. convolvulus*, *F. dentatoalata*, *F. dumetorum*, *F. koreana*, and *F. scandens*.

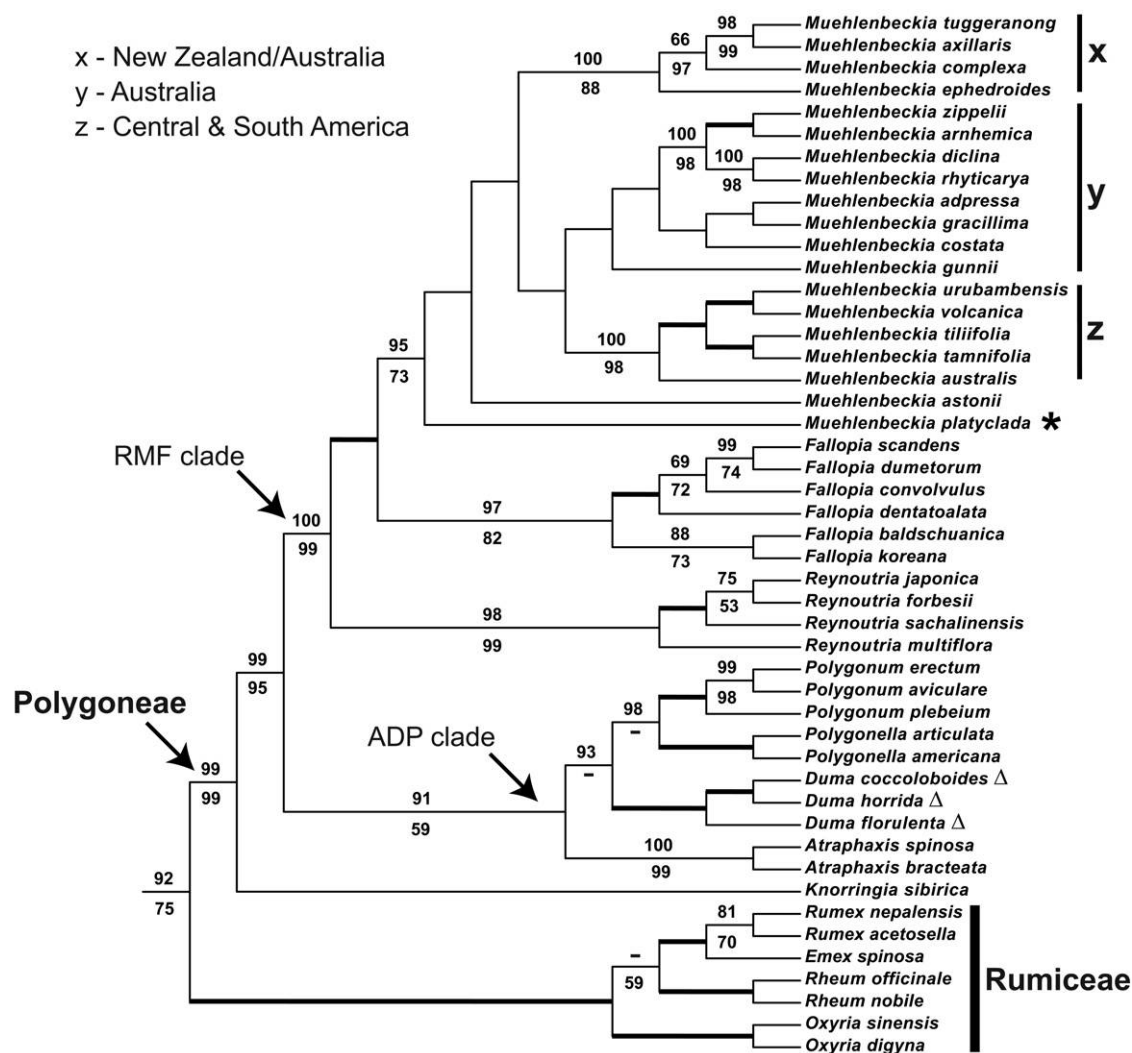
Within *Muehlenbeckia* there are three subclades (x, y, and z), but their relationships to each other are not well sup-

ported (fig. 1). There is strong support for clade x containing *M. axillaris*, *M. complexa*, *M. ephedroides*, and *M. tuggeranong*. Within this clade, a relationship of *M. axillaris* as sister to *M. uggeranong* has strong support. Within clade y, *M. arnhemica* is sister to *M. zippelii*, and this strongly supported pair is sister to another strongly supported pair, *M. diclina* and *M. rhyticarya*. All relationships are strongly supported in clade z that contains *M. australis*, *M. tiliifolia*, *M. urubambensis*, and *M. volcanica*. In this clade *M. australis* is sister to *M. tamnifolia* + *M. tiliifolia* and *M. urubambensis* + *M. volcanica*. *Muehlenbeckia astonii* is placed as sister to clades x + y + z, and *M. platyclada* is sister to this group. Support for the branching order in this region of the tree is poor, and therefore, relationships among *M. platyclada*, *M. astonii*, and clades x, y, and z are uncertain.

## Discussion

It is surprising that three species of *Muehlenbeckia* (*M. coccoloboides*, *M. florulenta*, and *M. horrida*) form a clade (*Duma*) that is separate from the remaining species of *Muehlenbeckia* (fig. 1). Previous workers never questioned the inclusion of *M. coccoloboides*, *M. florulenta*, and *M. horrida* within *Muehlenbeckia*, but our results indicate that the genus as presently circumscribed is polyphyletic. The position of *Duma* as a member of the ADP clade and as sister to the *Polygonum* + *Polygonella* clade (93% BS) is strongly supported. Based on the findings of this study and because there is no legitimate generic name for the *M. coccoloboides* + *M. florulenta* + *M. horrida* clade (*M. australis* is the type for *Muehlenbeckia* and is placed in the main *Muehlenbeckia* clade; fig. 1), it receives the new name *Duma* T.M.Schust. (see “Taxonomic Treatment”).

*Duma* is a group of erect, sometimes scrambling rhizomatous shrubs with thornlike branch tips. The latter are not found in any *Muehlenbeckia* s.s. (as recognized in this study). In addition, *Duma* has glaucous or whitish stems that often have a corky or stringy texture at maturity. This characteristic is also present in *Atraphaxis* and some *Polygonella*. *Duma* does not have extrafloral nectaries at the petiole base, which are present in most species of *Muehlenbeckia*, *Fallopia*, and *Reynoutria*. *Atraphaxis*, *Polygonum aviculare*, and *Polygonella* also lack extrafloral nectaries (T. M. Schuster, personal observation). In *Duma*, the leaves are linear, (sub)sessile, and often deciduous. *Duma coccoloboides* and *D. horrida* have flowers that are clustered at the nodes of the stems. *Duma florulenta* also has sessile flower clusters at the nodes but can have axillary or terminal fascicles of spikelike, interrupted inflorescences as well. The stigmas of *Duma* are fimbriate. The accrescent tepals develop into a thick, spongy tissue that completely encloses the achenes in *Duma* and may be fleshy in *D. coccoloboides*. In terms of distribution, *D. florulenta* is widely distributed throughout inland Australia and associated with floodplains and other seasonally wet situations, while *D. coccoloboides* is a desert plant. The latter is restricted to sand dunes in the Lake Eyre, Strzelecki Creek region of northeastern South Australia and to the southern area of the Northern Territory. *Duma horrida* subsp. *horrida* is known from scattered populations mainly in the Murray-Darling river



**Fig. 1** Enlarged portion of the total evidence tree found by the maximum likelihood (ML) analysis using molecular data (nrITS, *lfiy2*, *matK*, *ndhF*, *3' rps16-5' trnK*, *trnL-trnF*, and *3' trnV-ndhC*) showing the Polygonaceae and sister group Rumiceae. The entire tree is available as figure B1 in the online edition of the *International Journal of Plant Sciences*. Bootstrap support (BS) values (above 49%) shown above (ML) and below (maximum parsimony [MP]) the branches. Heavy branches designate 100% BS values for both ML and MP analyses. MP analysis results: number of trees = 13,  $L = 8440$ ,  $CI = 0.51$ , and  $RI = 0.65$ . ADP = *Atraphaxis-Duma-Polygonum-Polygonella* clade; RMF = *Reynoutria-Muehlenbeckia-Fallopa* clade. The species of *Duma* designated by a triangle were previously included in *Muehlenbeckia*, and the position of *Muehlenbeckia platyclada* that has been treated as *Homalocladium platycladum* is noted with an asterisk. Clades x, y, and z within *Muehlenbeckia* correspond to the geographic areas shown next to the tree.

system in eastern Australia and is often found in saline situations, while subsp. *abdita* occurs only on and around three ephemeral freshwater lakes in southwestern Western Australia (Wilson 1996) and is critically endangered (Phillimore 2008).

Among the remaining species of *Muehlenbeckia* (fig. 1), evolutionary relationships generally correspond to geographic distribution. Two clades (x and y) contain most of the Australian/New Zealand species, and one clade (z) contains all the sampled Central and South American species. All of the species that occur at higher altitude or latitude, mainly in New Zealand, fall within clade x except *M. australis*, which is sister to the American clade with strong support. Within clade x, *M. ephedroides* is endemic to New Zealand. *Muehlenbeckia complexa* is native to New Zealand and Lord Howe Island.

Most populations of *M. axillaris* occur in New Zealand, but scattered populations are found at higher elevations in southeastern Australia (New South Wales, Victoria, and Tasmania). The one strictly Australian species in this clade (*M. tuggeranong*) is a narrow endemic found in the Canberra area of southeastern Australia. The remaining Australian species form clade y. Two of these are tropical (*M. arnhemica* + *M. zippelii*) that also occurs in New Guinea, and they are closely related to the more temperate *M. diclina* + *M. rhyticarya* of eastern Australia. The widespread *M. adpressa* (mainly coastal in southeastern Australia but extending well inland in the southwest) and *M. gunnii* (mainly coastal, in southeastern Australia and Tasmania) are also part of this clade, as are *M. gracillima* and *M. costata*. *Muehlenbeckia costata* has recently been seg-

Table 3

Statistical Values for Analyzed Data Partitions

Partition/statistic	Aligned bp L	No. p.i.c. (%)	CI/RI	No. (%) missing species
<i>matK</i>	801	259 (32.3)	.66/.83	18 (20.7)
<i>ndbF</i>	1170	209 (17.9)	.67/.84	26 (29.8)
<i>trnL-trnF</i>	1023	451 (44.1)	.64/.74	28 (32.2)
<i>3'trnV-ndbC</i>	655	127 (19.4)	.82/.71	59 (67.8)
<i>3'rps16-5'trnK</i>	1031	101 (9.8)	.89/.76	64 (73.6)
nrITS	768	447 (58.2)	.31/.53	6 (6.9)
<i>LEAFY2</i>	995	103 (10.4)	.88/.84	53 (60.9)
cp combined	4680	1147 (24.5)	.68/.76	1 (1.1)
nr combined	1763	550 (31.2)	.34/.54	7 (8.0)
Total combined	6443	1697 (25.0)	.51/.65	

Note. bp L = base pair length, p.i.c. = parsimony informative characters (p.i.c. as % of total characters), CI = consistency index, RI = rescaled consistency index values, cp = chloroplast, and nr = nuclear. No. (%) of missing species versus a total of 87 species.

regated from *M. rhyticarya* (Wilson 1990). This study confirms that *M. costata* is not closely related to *M. rhyticarya* and should be recognized as a distinct species (fig. 1). The oblong-ovate, yellow-green leaves of *M. costata* have a reddish hue that is pronounced at the leaf margin. This differs from *M. rhyticarya*, which has glaucous, semicircular leaves. In addition, the achene is subglobose with six ridges in *M. costata* versus trigonous or triquetrous in *M. rhyticarya*. Both *M. costata* (Hunter et al. 1998) and *M. rhyticarya* are fire ephemerals. Other fire weeds in this clade are *M. diclina*, *M. gracillima*, *M. zippelii*, and *M. arnhemica*. This would suggest that fire adaptation has played an important role in the evolution of Australian *Muehlenbeckia*. Other forms of disturbance may also be important in the evolution of the genus. For example, *M. zippelii* flourishes after cyclonic disturbance as well as wildfires.

*Muehlenbeckia australis* occurs in New Zealand and on Norfolk Island, and this species is sister to a clade that contains Central and South American species. The widespread *M. volcanica*, a nontwining subshrub that forms large tangled mats, is closely related to the erect suffruticose *M. urubambensis*, which is a narrow endemic from Peru. This species pair is sister to the twining *M. tannifolia* + *M. tiliifolia*, which are widespread. Five of the nine *Muehlenbeckia* that could not be included in this study are from Central and South America. Based on the strong BS for the clade formed by the American species in clade z (fig. 1), it would be surprising if the other five species fell outside of this clade. To confirm this, a future study should include all of the American species. It would also be important to include *M. monticola*, endemic to New Guinea, and *M. polybotrya*, endemic to Western Australia, since the addition of these species may provide increased support for the position of *M. astonii* and *M. platyclada* within *Muehlenbeckia*.

The current results support previous authors who included the monotypic *Homalocladium platycladum* in *Muehlenbeckia* (Meisner 1865; Brandbyge 1992; Sanchez et al. 2009). Bailey (1929) emphasized the distinctive morphology of this species that has phylloclades and so recognized it as a separate genus. However, this species, native to the Solomon Islands and New Guinea and commonly cultivated, should be re-

ferred to as *Muehlenbeckia platyclada* (fig. 1; app. D in the online edition of the *International Journal of Plant Sciences*).

The close relationship of the *Muehlenbeckia* clade to the *Fallopia* clade agrees with the results of previous molecular studies that used more limited sampling (Galasso et al. 2009; Sanchez et al. 2009). This is different from the traditional placement of *Muehlenbeckia* in Coccolebeae along with *Antigonon*, *Brunnichia*, *Coccoloba*, *Fallopia*, *Reynoutria*, and others (Haraldson 1978). Within the tribe, *Muehlenbeckia* was considered most closely related to *Coccoloba* due to its fleshy fruits. *Antigonon* and *Brunnichia* were also included in Coccolebeae because they share a woody habit and similarities in petiole anatomy with *Coccoloba* and *Muehlenbeckia*. Jaretzky (1925) and Edman (1929) recognized the similarities of secondary chemistry and endosperm morphology between *Muehlenbeckia* and their concept of *Polygonum* sect. *Pleuropterus*, which included *Reynoutria japonica* and *Reynoutria sachalinensis* (as recognized in this study). They considered *Muehlenbeckia* derived from within *P.* section *Pleuropterus* because of the presence of anthraquinones and the lack of a ruminant endosperm in both. Haraldson (1978) agreed with Jaretzky (1925) and Edman (1929) and also placed *Reynoutria* and *Fallopia* as distinct genera within Coccolebeae. She considered *Fallopia* to be derived from within *Reynoutria*.

The distinctions between *Reynoutria* and *Fallopia* that have been made by Haraldson (1978) and several other authors (table 2) include, for example, habit (erect in *Reynoutria* and twining in *Fallopia*), stigma characters (fimbriate in *Reynoutria* and smooth or papillate in *Fallopia*), and anatomical differences in the petiole. Ronse Decraene and Akeroyd (1988) stress the inconsistency and heterogeneity of these characters and use similarities of the stamens, outer tepals, and tepal vasculature as supporting evidence to merge *Reynoutria* into *Fallopia*. The Ronse Decraene and Akeroyd (1988) classification based on floral characters was supported by Bailey and Stace (1992), who mainly used habit to delimit four sections in *Fallopia* (including *Reynoutria*): (1) annual vines—section *Fallopia* (*F. convolvulus*, *F. dumetorum*, and *F. scandens*); (2) perennial climbers—section *Parogonum* (*F. cilinodis*, *F. cyanchooides*); (3) woody climbers—section *Sarmentosae* (*F. baldschuanica*, *F. multiflora*); and (4) robust,



rhizomatous perennial herbs with an erect habit—section *Reynoutria* (*F. japonica* and *F. sachalinensis*). However, most studies of the characters used to merge or separate *Reynoutria* and *Fallopia* did not consider *Muehlenbeckia* because they were not addressed from a phylogenetic perspective. In addition, there are few anatomical observations on *Muehlenbeckia* (but see Carlquist 2003). The complex history of generic limits between *Fallopia/Reynoutria* and *Polygonum* s.l. is likely due to the use of plesiomorphic characters to define each genus. The consideration of some characters as more important than others has also contributed to the confusion and resulted in numerous overlapping classifications (table 2).

*Fallopia* and *Reynoutria* should be recognized as separate genera (app. D), based on the results of this study (fig. 1) and as indicated in previous molecular studies with more limited sampling (Sanchez et al. 2009; Burke et al. 2010). Although the sampling in these clades is not complete, nearly all of the recognized species have been included. It is expected that most if not all six species of *Fallopia* that could not be included in this study will fall into the *Fallopia* clade. *Fallopia denticulata* may be included in the *Fallopia* clade, based on the presence of partially fused styles and capitate and papillate stigmas. Little is known about *F. filipes* except that it has racemose rather than the paniculate inflorescences of *Reynoutria*. *Fallopia pterocarpa* is a twining vine with winged fruits and racemose inflorescences from the Himalayas (Wood 1902), and it was also considered to belong to *Fallopia* by Holub (1970). Tzvelev (1987) described *F. schischkii* in *F.* section *Fallopia* as distinct from *Reynoutria*, based on the fruiting perianth not being winged or being “*subapterum*” (presumably meaning keeled). It is the only *Fallopia* aside from *F. convolvulus* that does not have pronounced wings on the mature perianth. In *F. cynanchoides* the stems and leaves are covered with distinctive multicellular hairs with an acute apex. This character, in addition to the paniculate inflorescences and the long distinct styles, indicates that *F. cynanchoides* may belong to the *Reynoutria* clade. *Reynoutria sachalinensis* has similar hairs on the leaves and this trichome type is not common in *Fallopia*. *Fallopia cynanchoides* has a twining habit similar to the other species of *Fallopia*, but this character has not proved useful for delimiting evolutionary relationships in *Fallopia* and *Reynoutria*. For example, the twining *R. multiflora* (= *F. multiflora*) is included in *Reynoutria* (fig. 1), which was previously thought to include only erect, rhizomatous herbs. Now that *Reynoutria* and *Fallopia* are placed in separate clades, previously studied or newly discovered characters may be useful in investigating each genus in more detail.

In this study, the clades of *Fallopia* and *Reynoutria* (fig. 1) correspond to different chromosome base numbers. *Fallopia* and *Muehlenbeckia* have  $x=10$  (Jaretsky 1928) and these genera are sister to *Reynoutria*, which has  $x=11$  (Bailey and Stace 1992). Kim et al. (2000b) investigated chromosome numbers and flavonoid chemistry in *Fallopia* section *Pleuropterus* and suggested a split between species characterized by  $x=10$  (*F. baldschuanica* and *F. koreana*) and those with  $x=11$  (*F. multiflora*). This is supported by this study, with *F. multiflora* falling within the *Reynoutria* clade ( $x=11$ ). Chromosome counts for *R. forbesii*, *R. japonica*, and *R. sachalinensis*

are also  $x=11$  (Jaretsky 1928; Kim and Park 2000). The base chromosome number for species included in the *Fallopia* clade is  $x=10$  (Bailey and Stace 1992; Kim et al. 2000b). However, chromosome counts for *F. cilinodis* are variable, with  $x=10$  or 11 (Bailey and Stace 1992), and the significance of this requires further investigation. *Fallopia baldschuanica* and *F. koreana* are diploids, and *F. convolvulus* is tetraploid. Some populations of *Fallopia scandens* and *F. dentatoalata* have been reported as  $2n=40$  or 60 (Kim 1999). Polyploidy also occurs in *Reynoutria*. For example, *R. japonica* can be tetraploid, hexaploid, or octaploid, and in *R. sachalinensis* there are also counts of  $2n=102$  and 132 (Holub 1970; Bailey and Stace 1992; Beerling et al. 1994; Kim and Park 2000; Tiébré et al. 2007; Bailey et al. 2007). The polyploid species form a clade within *Reynoutria* and have erect, robust and vigorous growth. *Reynoutria multiflora* is sister to the polyploid clade and has a twining habit unlike the polyploids. It was considered closely related to *R. japonica* and *R. sachalinensis* by Bentham and Hooker (1880), who placed these three species in *Polygonum* section *Pleuropterus*. Of the three varieties sometimes recognized within *R. multiflora* (Yonekura and Ohashi 1997), two (var. *angulata* and var. *ciliinervis*) are diploid ( $2n=22$ ) and one (var. *hypoleuca*) is a tetraploid ( $2n=44$ ; Kim et al. 2000a). These varieties have been recognized as species by some authors (Nakai 1926; Ohwi 1937; Moldenke 1941). In the case of *R. multiflora* var. *hypoleuca*, recognition as a species may be appropriate based on its ploidy level. The phylogenetic relationships found in this study indicate that polyploidy may have played an important role in the evolution of *Fallopia* and *Reynoutria*.

In addition to genetic data, some chemical and morphological traits have been used to support a split between *Reynoutria* and *Fallopia*. According to Kim et al.'s (2000a, 2000b) studies, *Fallopia* has both flavonols and flavones but lacks kaempferol and myricetin glycosides. *Reynoutria*, however, has the flavonol glycosides of kaempferol, myricetin, and quercetin but lacks flavones. Several morphological characters support the recognition of *Reynoutria* as distinct from *Fallopia*. Both *Fallopia* and *Reynoutria* have bisexual flowers, but they are functionally dioecious in *Reynoutria* (Kim and Park 2000; Barney et al. 2006), and some populations also have pistillate flowers (Beerling et al. 1994; Freeman and Hinds 2005). *Fallopia* has short, partially fused styles with capitate stigmas that are either smooth or papillate. The styles in *Reynoutria* are long and distinct with triangular stigmas that are fimbriate. Some of these characters are also present in *Muehlenbeckia* (e.g., fimbriate stigmas), and further investigation of morphological and other traits may provide additional insight into the evolution of morphology and anatomy in these genera. For example, the evolution of tepal vasculature or stamen insertion may be reevaluated now that there is a phylogenetic context for Ronse Decraene and Akeroyd's (1988) study.

In contrast to *Fallopia* and *Reynoutria*, *Muehlenbeckia* is dioecious to polygamous. The thickened and often succulent fruiting perianth of *Muehlenbeckia* also distinguishes it from these two genera that have a papery dry perianth at maturity. The presence of extrafloral pit nectaries at the base of the articulated petiole may be a synapomorphy for the RMF clade (*Reynoutria*, *Muehlenbeckia*, and *Fallopia*). The extrafloral

nectaries have either been thought to function as hydathodes regulating water pressure in the immature unfolding leaves (Salisbury 1909; Graham and Wood 1965) or to attract insect pollinators or defenders (Kawano et al. 1999). It is more likely that in *Muehlenbeckia* the extrafloral nectaries function to attract pollinators, because the nectar is sweet (T. M. Schuster, personal observation in *M. platyclada*), and hydathodes usually secrete water (Esau 1960). T. M. Schuster has observed ants and ladybeetles actively searching for the nectar and crawling all over the stems and flowers in *M. platyclada* and *M. axillaris* and apparently pollinating the plants in the process. Although in *Muehlenbeckia* the ants do not exhibit defensive behavior, in *R. japonica* and *R. sachalinensis* ants appear to protect seedlings from herbivores (Sukopp and Schick 1991), as in some other Polygonaceae such as *Triplaris* (Ward 1991).

In summary, neither *Fallopia* or *Muehlenbeckia* is monophyletic as previously recognized. One clade of *Fallopia*, previously characterized as *Polygonum* section *Pleuropterus* by Bentham and Hooker (1880) and now treated as *Reynoutria* ( $x=11$ ), is sister to the rest of *Fallopia* + most *Muehlenbeckia* ( $x=10$ ; fig. 1). This suggests that  $x=10$  may be a synapomorphy for the *Fallopia* + *Muehlenbeckia* clade. *Duma* contains three Australian species segregated from *Muehlenbeckia*. *Duma* falls outside the main clade formed by all other species of *Muehlenbeckia* and is characterized by the presence of thornlike branch tips, sessile, linear leaves, clusters of sessile flowers at the nodes, and the lack of extrafloral nectaries. This study provides new insights into the historically complicated relationships of some of the species in Polygonaceae by using molecular phylogenetic analyses. The identification of the clade that corresponds to *Duma* will help to clarify relationships within tribe Polygoneae. These analyses have confirmed significant patterns of chromosome number in *Reynoutria*, *Fallopia*, and *Muehlenbeckia*, and geographic patterns among species within *Muehlenbeckia*. Though much work remains to be done, the results of this study will promote the reevaluation of morphological characters and will enable a more informed approach in understanding the evolution of Polygonaceae and Polygoneae.

### Taxonomic Treatment

*Duma* T.M.Schust., *genus novum*.

*Typus*: *Duma florulenta* Meisn., in DC., Prodr 14: 146. 1856.

*Fallopiae* Adans., *Reynoutriae* Houtt. et *Muehlenbeckiae* Meisn. affinis, sed a quibus spinis armatum et sine nectariis extrafloralibus petiolo insidentibus differt. Frutices dioici ramosissimi, ramis flexuosis spinescentibus teretibus striatis; *cortex* eburneus vel glaucus; *ocreae* hyalinae membranaceae deciduae; *folia* decidua, lanceolata vel linearia, (sub)sessilia basi obtusa vel attenuata, apice spina minuta recurvata; *flores* sessiles, fasciculatae ad nodos vel in racemis spiciformibus interruptis axillaribus terminalibusque dispositae; flores staminatae staminibus octo, pistillo rudimentario vel nullo; flores pistillatae staminibus rudimentariis, stigmata fimbriata; *perianthium* fructiferum accrescens carnosumque vel incrassatum siccatumque; *achenia* ovoidea vel trigona, apice acuto,

laevia, nitida, in perianthio inclusa; *endospermium* abusque plisicis intrusis.

*Plants* dioecious, much-branched shrubs; branches flexuous, terete, striate, with thorn-like branch tips; bark whitish or glaucous. *Ocreae* deciduous, hyaline, membranaceous. *Leaves* deciduous, lanceolate or linear, (sub)sessile, obtuse or attenuate at the base, with a minute recurved spine at the apex. *Flowers* sessile, fasciculate at the nodes, or in axillary or terminal interrupted spiciform racemes; tepals 5(–6), accrescent and fleshy, or thickened and dry when fruiting, staminate flowers, with eight stamens, pistillodes present or entire ovary lacking; pistillate flowers with staminodes; stigmas fimbriate. *Achenes* ovoid or trigonous, acute at the apex, smooth, shiny, and included in the perianth; endosperm without intruding folds (neither ruminant or lobed).

The name *Duma* was chosen in reference to thorn-bush (Latin). One species, *Duma florulenta*, is widely distributed throughout inland Australia; the other two species (*D. coccoloboides* and *D. horrida*) have more restricted distributions (see discussion above).

*Duma coccoloboides* (J.M.Black) T.M.Schust. *comb. nov.*

*Basionym*. *Muehlenbeckia coccoloboides* J.M.Black, Trans. & Proc. Roy. Soc. South Australia 42:170, pl. XV. 1918.

*Duma florulenta* (Meisn.) T.M.Schust. *comb. nov.*

*Basionym*. *Muehlenbeckia florulenta* Meisn., in DC., Prodr 14:146. 1856.

*Polygonum cunninghamii* Meisn., in DC., Prodr 14:85. 1856.

*Polygonum junceum* T.Mitch., *nom. inval.*, *nom. nud.*, *nom. illeg.* [non *Polygonum junceum* Ledeb.] T.Mitch., J. Exped. Trop. Australia. 2:85. 1848.

*Muehlenbeckia cunninghamii* (Meisn.) F.Muell., Fragm 5:91. 1865.

*Duma horrida* (H.Gross) T.M.Schust. *comb. nov.*

*Basionym*. *Muehlenbeckia horrida* H.Gross, Bot. Jahrb. Syst. 49:347. 1913.

*Duma horrida* H.Gross subsp. *abdita* (K.L.Wilson) T.M.Schust. *comb. nov.*

*Basionym*. *Muehlenbeckia horrida* H.Gross subsp. *abdita* K.L.Wilson, Nuytsia 11:136. 1996.

### Key to the Genera *Duma*, *Fallopia*, *Muehlenbeckia*, and *Reynoutria*

1. Shrubs with thornlike branch tips; extrafloral nectaries absent [with trigonous achenes enclosed in thickened mature perianth].....*Duma*
  1. Vines, lianas or (sub)shrubs without thornlike branch tips; extrafloral nectaries present ..... 2.
  2. Plants dioecious-polygamous; perianth thickened, dry or fleshy when mature; achenes with rounded edges (trigonous-subglobose) ..... *Muehlenbeckia*
    2. Plants monoecious, rarely dioecious; perianth dry and papery when mature, winged or keeled; achenes with sharp edges (triquetrous)..... 3.
    3. Inflorescence paniculate; flowers bisexual with some unisexual flowers; styles long, divided; stigmas distinct, triangular, fimbriate ..... *Reynoutria*
      3. Inflorescence racemose; flowers bisexual; styles short to subsessile; stigmas partially fused, capitate, smooth or papillate ..... *Fallopia*

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Lim (Australian Tropical Herbarium, Cairns), Bob Makinson (Royal Botanic Gardens, Sydney), Dave Mallinson (Centre for Plant Conservation, Canberra), Benjamin Øllgaard (University of Aarhus), Jo Palmer (Centre for Plant Conservation, Canberra), Miles Silman (Wake Forest University), Leonie Stanberg (Royal Botanic Gardens, Sydney), and Helen Vonow (State Herbarium of South Australia, Adelaide) are thanked for help with field trips, for collecting silica gel material, or for other aspects of the project. We also wish to thank staff at herbaria AD, ATH, BRI, CANB, MO, NSW, and NY for help with locality information for field trips or for allowing DNA extraction of materials in their collections. T. M. Schuster received financial support from the Richter Scholarship Program and the Vecellio Graduate Student Fund, Wake Forest University.

## Appendix A

### Voucher Information

Voucher information and GenBank accession numbers for sequences generated for this study. The following information is given for each sequence: **species** — **collector(s)** and **collection number** (herbarium code), gene marker designation (I = nrITS, L = *lfy2*, M = *matK*, N = *ndhF*, F = *trnL-trnF*, V = *3'trnV-ndhC*, K = *3'rps16-5'trnK*, and NA = not available), and GenBank accession number. Herbarium codes follow Index Herbariorum and are only given if a specimen is not located at the Herbarium of Wake Forest University (WFU). AAU = Herbarium of the University of Aarhus, AD = State Herbarium of South Australia, ANBG = National Botanic Garden Canberra, BRI = Queensland Herbarium, K = Royal Botanic Gardens Kew, MO = Missouri Botanical Garden, NSW = National Herbarium of New South Wales, Royal Botanic Gardens Sydney, and NY = New York Botanical Garden. Information for accessions obtained from GenBank follow the same format, excluding the collector's and herbarium information.

1) **Sequences generated for this study.** (27 species — 153 sequences: I-18, L-21, M-19, N-21, F-25, V-25, K-24). Extractions that were isolated from herbarium material are marked by an asterisk. POLYGONACEAE: *Atraphaxis spinosa* L. — K.A. Kron s.n., F-JF831296, V-JF831321, K-JF831272; \**Duma coccoloboides* (J.M.Black) T.M.Schust. [*Muehlenbeckia coccoloboides* J.M.Black] — P.K. Latz 23583 (AD), I-JF831204, L-NA, M-JF831264, N-JF831243, F-JF831297, V-JF831322, K-JF831273; *Duma florulenta* (Meisn.) T.M.Schust. [*Muehlenbeckia florulenta* Meisn.] — K.L. Wilson 10552 (NSW), I-JF831205, L-JF831346, M-JF831265, N-JF831244, F-JF831298, V-JF831323, K-JF831274; *Duma horrida* (H.Gross) T.M.Schust. subsp. *horrida* [*Muehlenbeckia horrida* H.Gross] — K.L. Wilson 10555 (NSW), I-JF831206, L-NA, M-JF831232, N-JF831245, F-JF831299, V-JF831324, K-JF831275; *Fallopia convolvulus* (L.) Á.Löve — K.L. Wilson 10568 (NSW), L-JF831222, N-NA, V-JF831325, K-JF831276; *Muehlenbeckia adpressa* (Labill.) Meisn. — K.L. Wilson 10487 (NSW), I-JF831207, L-JF831347, M-JF831233, N-JF831246, F-JF831300, V-JF831326, K-JF831277; \**Muehlenbeckia arnhemica* (*Muehlenbeckia* sp. Mt Brockman) K.L.Wilson & R.O.Makinson ms — Russell-Smith 8040 & Lucas (NSW), I-JF831221, L-NA, M-JF831234, N-JF831247, F-JF831301, V-JF831327, K-JF831278; *Muehlenbeckia astonii* Petrie — K.L. Wilson 10567 (NSW), L-NA, M-JF831266, N-JF831248, F-JF831302, V-JF831328, K-JF831279; *Muehlenbeckia australis* (G.Forst.) Meisn. — W.R. Barker 8995 & R.M. Barker (AD), I-JF831208, L-JF831348, M-JF831267, N-JF831249, F-JF831303, V-JF831329, K-JF831280; *Muehlenbeckia axillaris* (Hook.f.) Endl. — K.L. Wilson 10562 (NSW), I-JF831209, L-JF831349, M-JF831268, N-JF831250, F-JF831304, V-JF831330, K-JF831281; *Muehlenbeckia complexa* (A.Cunn.) Meisn. — K.L. Wilson 10677 (NSW), L-JF831223, N-JF831251, F-JF831305, V-JF831331, K-JF831282; *Muehlenbeckia costata* (*Muehlenbeckia* sp. Mt Norman) K.L.Wilson & R.O.Makinson ms — J.J. Bruhl 2680 (NE), I-JF831210, L-JF831350, M-JF831269, N-JF831252, F-JF831306, V-JF831332, K-JF831283; *Muehlenbeckia diclina* subsp. *stenophylla* (*Muehlenbeckia* subsp. Gippsland) (F.Muell.) K.L.Wilson & R.O.Makinson — K.L. Wilson 10497 (NSW), I-JF831211, L-JF831224, M-JF831235, N-JF831253, F-JF831308, V-JF831333, K-JF831284; \**Muehlenbeckia ephedroides* Hook.f. — T.R.N. Lothian s.n. (AD), I-JF831212, L-JF831225, M-JF831236, N-JF831254, F-JF831307, V-JF831334, K-JF831285; \**Muehlenbeckia gracillima* Meisn. — R. Johnstone 2022 & E.A. Orme (NSW), I-JF831213, L-JF831226, M-JF831237, N-JF831255, F-JF831309, V-JF831335, K-JF831287; *Muehlenbeckia gunnii* (Hook.f.) Endl. — K.L. Wilson 10493 (NSW), I-JF831214, L-JF831351, M-JF831238, N-JF831256, F-JF831310, V-JF831336, K-JF831286; *Muehlenbeckia platyclada* (F.Muell.) Meisn. [*Homalocladium platycladum* (F.Muell.) L.H.Bailey] — K.L. Wilson 10678 (NSW), L-JF831227, M-JF831239, N-JF831257, F-JF831311, V-JF831337, K-JF831288; \**Muehlenbeckia rhyticarya* F.Muell. ex Benth. — S.P. Phillips 1978 (BRI), L-JF831228, M-NA, N-NA, F-JF831312, V-JF831338, K-NA; *Muehlenbeckia tammifolia* (Kunth) Meisn. — S.D. Setaro s.n., L-JF831229, F-JF831313, V-NA, K-JF831289; *Muehlenbeckia tiliifolia* Wedd. — B. Øllgaard 87EC63051 (AAU), I-JF831215, L-JF831230, M-JF831270, N-JF831258, F-JF831314, V-JF831339, K-JF831290; *Mueb-*

*lenbeckia tuggeranong* Mallinson — K.L. Wilson 10540 & D.J. Mallinson (ANBG), I-JF831216, L-JF831352, M-JF831271, N-JF831259, F-JF831315, V-JF831340, K-JF831291; \**Muehlenbeckia urubambensis* Brandbyge — A. Tupayachi 1003 (NY), I-JF831217, L-JF831353, M-JF831240, N-JF831260, F-JF831316, V-JF831341, K-NA; *Muehlenbeckia volcanica* (Benth.) Endl. — M. Silman s.n., I-JF831218, L-JF831354, M-JF831241, N-JF831261, F-JF831317, V-JF831342, K-NA; \**Muehlenbeckia zippelii* (Meisn.) Danser — G.N. Batianoff 0704352 & R. Booth (BRI), I-JF831219, L-JF831355, M-JF831242, N-JF831262, F-JF831318, V-JF831343, K-JF831292; *Reynoutria japonica* Houtt. [*Fallopia japonica* (Houtt.) RonseDecr.] — M. Chase 898 (K), L-JF831231, V-JF831344, K-JF831293; *Reynoutria sachalinensis* (F.Schmidt) Nakai [*Fallopia sachalinensis* (F.Schmidt) RonseDecr.]— M. Chase 896 (K), L-JF831356, N-JF831263, F-JF831320, V-JF831345, K-JF831294.

PLUMBAGINACEAE: *Plumbago auriculata* Lam. — T. Schuster 1, I-JF831220, L-NA, M-NA, N-NA, F-JF831319, V-NA, K-JF831295.

2) Sequences downloaded from GenBank. (69 species – 179 sequences; no data were available for 3'*trnV-ndbC* and 3'*rps16-5'trnK*). Many of these sequences (61%) were generated in the Kron Lab for previous projects. POLYGONACEAE: *Aconogonon alpinum* (All.) Schur I-NA, L-NA, M-AF204858, N-NA, F-NA; *Aconogonon molle* (D.Don) H.Hara I-EF653687, L-NA, M-GQ206190, N-GQ206271, F-EF653790; *Aconogonon songoricum* I-NA, L-NA, M-EU02773, N-NA, F-NA; *Antigonon guatemalense* Meisn. I-FJ154461, L-NA, M-FJ1591, N-FJ154503, F-NA; *Antigonon leptopus* Hook. & Arn. I-FJ154462, L-NA, M-EF37988, N-EF438027, F-NA; *Atraphaxis bracteata* Losinsk. I-NA, L-NA, M-NA, N-NA, F-EU109601; *Atraphaxis spinosa* L. I-FJ154463, L-NA, M-EF37989, N-EF438028; *Bistorta tenuicaulis* (Bisset et S.Moore) Nakai I-GQ206240, L-NA, M-NA, N-GQ206274, F-NA; *Bistorta vivipara* var. *vivipara* (L.) Delarbre I-DQ372903, L-NA, M-NA, N-NA, F-EU024776; *Brunnichia ovata* (Walter) Shinnars I-FJ154465, L-NA, M-AY042561, N-EF438029, F-NA; *Calligonum aphyllum* (Pall.) Gürke I-GQ206241, L-NA, M-GQ206192, N-GQ206275, F-NA; *Calligonum junceum* (Fisch. & C.A.Mey.) Litv. I-GQ206243, L-NA, M-GQ206194, N-GQ206277, F-EU109590; *Chorizanthe brevicornu* Torr. var. *brevicornu* I-FJ154466, L-NA, M-EF437991, N-EF438030, F-NA; *Chorizanthe rigida* (Torr.) Torr. & A.Gray I-NA, L-NA, M-EF437993, N-EF438032, F-NA; *Coccoloba swartzii* Meisn. I-FJ154469, L-NA, M-EF437995, N-EF438034, F-NA; *Coccoloba uvifera* (L.) L. I-GQ206246, L-NA, M-EF437996, N-NA, F-AJ312249; *Dedeckera eurekaensis* Reveal & J.T.Howell I-FJ154470, L-NA, M-EF437997, N-EF438036, F-NA; *Emex spinosa* (L.) Campd. I-FJ154471, L-NA, M-AY042582, N-EF438037, F-NA; *Eriogonum alatum* Torr. var. *alatum* I-FJ154472, L-NA, M-EF437998, N-EF438038, F-NA; *Eriogonum clavellatum* Small I-GQ206247, L-NA, M-EF438000, N-EF438040, F-NA; *Fagopyrum gracilipes* (Hemsl.) Dammer ex Diels I-AB000332, L-NA, M-AB026314, N-NA, F-EU024787; *Fagopyrum tataricum* subsp. *pontanicum* (L.) Gaertn. I-AB000339, L-NA, M-AB089712, N-NA, F-EU109606; *Fallopia baldschuanica* (Regel) Holub I-AF040063, L-NA, M-NA, N-NA, F-EU024779; *Fallopia convolvulus* (L.) Á. Löve I-AF040064, M-EU024770, F-EU024782; *Fallopia dentatoalata* (F.Schmidt apud Maxim.) Holub I-AF040066, L-NA, M-EU024769, N-NA, F-EU024775; *Fallopia dumetorum* (L.) Holub I-AF040068, L-NA, M-AM503813, N-NA, F-EU024785; *Fallopia koreana* B.U.Oh & J.G.Kim I-AF040061, L-NA, M-NA, N-NA, F-AF189740; *Fallopia scandens* (L.) Holub I-AF040069, L-NA, M-NA, N-NA, F-EF653785; *Gilmania luteola* (Coville) Coville I-GQ206250, L-NA, M-EF438010, N-EF438049, F-NA; *Gymnopodium floribundum* Rolfe I-GQ206251, L-NA, M-GQ206197, N-GQ206282, F-NA; *Johanneshowellia crateriorum* Reveal I-GQ206252, L-NA, M-EF438011, N-EF438050, F-NA; *Knorringia sibirica* (Laxm.) S.P.Hong I-GQ206253, L-NA, M-EU024771, N-GQ206284, F-EU024789; *Koenigia forrestii* (Diels) Měsíček and Soják I-GQ206254, L-NA, M-EF438012, N-EF438051, F-FJ821779; *Koenigia islandica* L. I-DQ406625, L-NA, M-NA, N-NA, F-EF653789; *Leptogonum buchii* Urb. I-GQ206256, L-NA, M-GQ206199, N-GQ206285, F-NA; *Muehlenbeckia astonii* Petrie I-EF635479; *Muehlenbeckia complexa* (A.Cunn.) Meisn. I-GQ206224, M-GQ206200; *Muehlenbeckia platyclada* (F.Muell.) Meisn. [*Homalocladium platycladum* (F.Muell.) L.H.Bailey] I-AF189738; *Muehlenbeckia rhyticarya* F.Muell. ex Benth. I-AF189739; *Muehlenbeckia tamnifolia* I-FJ154473, M-FJ154499, N-FJ154511; *Neomillspaughia emarginata* (H.Gross) S.F.Blake I-GQ206257, L-NA, M-GQ206201, N-GQ206287, F-NA; *Oxyria digyna* (L.) Hill I-FJ154474, L-NA, M-FJ154500, N-FJ154512, F-AY566466; *Oxyria sinensis* Hemsl. I-GQ206258, L-NA, M-EF438013, N-EF438053, F-NA; *Parapteropyrum tibeticum* A.J.Li I-NA, L-NA, M-NA, N-NA, F-EU109589; *Persicaria sagittata* (L.) H.Gross I-FJ154476, L-NA, M-EF438018, N-GQ206288, F-EF653799; *Persicaria virginiana* (L.) Gaertn. I-EU410358, L-NA, M-EF438019, N-EF438058, F-EF653801; *Podopterus cordifolius* Rose & Standl. I-FJ154479, L-NA, M-FJ154494, N-FJ154505, F-NA; *Polygonella americana* (Fisch. & C.A.Mey.) Small I-GQ206259, M-GQ206202, N-GQ206289, F-NA; *Polygonella articulata* I-NA, M-NA, N-GQ206290, F-EF653786; *Polygonum aviculare* L. I-FJ493492, L-NA, M-EF438020, N-EF438059, F-FJ627271; *Polygonum erectum* L. I-GQ206260, L-NA, M-GQ206203, N-NA, F-NA; *Polygonum plebeium* L.Br. I-GQ339946, L-NA, M-NA, N-NA, F-EU109598; *Pteropyrum aucheri* Jaub. & Spach I-GQ206261, L-NA, M-GQ206205, N-GQ206292, F-NA; *Pteropyrum olivierii* Jaub. & Spach I-GQ206262, L-NA, M-NA, N-GQ206293, F-NA; *Pterostegia drymaroides* Fisch. and C.A.Mey. I-GQ206263, L-NA, M-GQ206206, N-GQ206294, F-NA; *Pteroxygonum giraldii* Dammer & Diels I-DQ406627, L-NA, M-GQ206207, N-GQ206295, F-EU402464; *Reynoutria forbesii* (Hance) T.Yamaz. [*Fallopia forbesii* (Hance) Yonekura & Ohashi] I-AF040072, L-NA, M-NA, N-NA, F-NA; *Reynoutria japonica* Houtt. [*Fallopia japonica* (Houtt.) RonseDecr.] I-AF040070, M-EU024772, N-EF438048, F-EU024786; *Reynoutria multiflora* (Thunb.) Moldenke [*Fallopia multiflora* (Thunb.) Czerep.] I-AF040053, L-NA, M-EF153684, N-NA, F-EU402461; *Reynoutria sachalinensis* (F.Schmidt) Nakai [*Fallopia sachalinensis* (F.Schmidt) RonseDecr.] I-AF040073, M-EF438009; *Rheum nobile* Hook.f. & Thomson I-GQ206264, L-NA, M-EF438021, N-EF438060, F-AY566465; *Rheum officinale* Baill. I-FJ503007, L-NA, M-AB115684, N-NA, F-AF303431; *Rumex acetosella*

L. I-AF189730, L-NA, M-EF438022, N-GQ206298, F-GQ245413; *Rumex nepalensis* Spreng. I-AF338219, L-NA, M-NA, N-NA, F-EU326091; *Triplaris americana* L. I-FJ154486, L-NA, M-AY042668, N-FJ154508, F-AJ312251; *Triplaris cumingiana* Fisch. & C.A.Mey. ex C.A.Mey I-NA, L-NA, M-NA, N-GQ206301, F-NA.

PLUMBAGINACEAE: *Armeria splendens* Webb I-AY444093, L-NA, M-NA, N-NA, F-AJ391316; *Limonium delicatulum* Kuntze I-AJ222851, L-NA, M-NA, N-NA, F-AJ391324; *Limonium narbonense* Mill. I-AJ222838, L-NA, M-AF204853, N-NA, F-AJ391327.

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