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Austrobryonia (Cucurbitaceae), a New Australian Endemic Genus, is the Closest Living Relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*

Hanno Schaefer,[1,3] Ian R. H. Telford,[2] and Susanne S. Renner[1]

[1]Systematic Botany, Department of Biology, University of Munich (LMU), Menzinger Str. 67, D-80638 Munich, Germany
[2]N.C.W. Beadle Herbarium, University of New England, Armidale, NSW 2351, Australia
[3]Author for correspondence (hschaefer@lrz.uni-muenchen.de)

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Abstract*—**The Cucurbitaceae genus ***Austrobryonia, with four species endemic to Australia, is described, illustrated, and placed in a phylogenetic context based on molecular and morphological data. In the *Flora of Australia* (Telford 1982), all four species were provisionally included in *Mukia*, but not formally described. ***Austrobryonia argillicola***, ***A. centralis***, ***A. micrantha***, and ***A. pilbarensis*** are adapted to arid central regions of Australia. All species are known from several (7–27) localities, and their distributional ranges are allopatric. A phylogenetic analysis of plastid and nuclear DNA sequences that includes all four species in a family-wide context revealed that *Austrobryonia* is the closest living relative to a Eurasian and Mediterranean clade consisting of *Bryonia* L. and *Ecballium* L. An *rbcL* molecular clock, calibrated with Cucurbitaceae fossils, dates this rare biogeographic disjunction to minimally 42 my ago (with an error of ca. ± 25%), while the crown group of *Austrobryonia* may be about 8 my old.

***Keywords*—**Australia-Eurasia disjunction, molecular clock, *Mukia micrantha.*

The Australian continent is relatively poor in genera and species of Cucurbitaceae. The last comprehensive treatment (Telford 1982) lists 44 species, then assigned to 17 genera. Twelve genera and perhaps 30 species appear indigenous to the Australian continent (*Benincasa* Savi, *Cucumis* L., *Diplocyclos* (Endl.) Post & Kuntze, *Luffa* Miller, *Momordica* L., *Muellerargia* Cogn., *Neoachmandra* W. J. de Wilde & Duyfjes, *Neoalsomitra* Hutch., *Nothoalsomitra* I. Telford, *Sicyos* L., *Trichosanthes* L., and the new genus *Austrobryonia* described below), but only one genus was thought endemic to Australia. This is the monotypic *Nothoalsomitra*, a liana of SE Queensland's rain forests, originally described in Benincaseae (Telford 1982), but placing far from them in a molecular tree of Cucurbitaceae that samples 21% of the family's 800 species and 94% of its 130 genera, including species from all Australian clades (Kocyan et al. 2007).

The most problematic group in the *Flora of Australia* treatment of Cucurbitaceae was the genus *Mukia* Arnold. As previously conceived, *Mukia* was thought to include nine species, five that range from China through Indo-China southeast to Java, Borneo, and the Philippines, and west through India, Pakistan, and the Yemen into subSaharan Africa, and five that were left undescribed (Telford 1982; De Wilde and Duyfjes 2006). The type species of *Mukia*, *M. scabrella* (L.) Arn. (= *M. maderaspatana* (L.) M. Roem.), occurs from subSaharan Africa, through tropical and subtropical Asia to Australia. The pollen of the Asian *Mukia* species is so different from that of the Australian *M. micrantha* (F. Muell.) F. Muell. that Jeffrey (1969a) suspected they might not be congeneric. He thought the affinities of *M. micrantha* might lie with *Zehneria* Endl., *Kedrostis* Medik., or *Ibervillea* Greene, but could not decide about its true taxonomic position. Earlier, Ferdinand von Mueller (1854) had first included *M. micrantha* in the otherwise neotropical genus *Cucurbita*, then in the African/Asian *Zehneria* (Mueller 1858/59), and finally in *Mukia* (Mueller 1860/61), but without specifying the basis for these placements. Naudin (1859) redescribed the species as *Cucumis muelleri*, but again without providing any reasons for this generic placement. Given the extremely few collections of what appeared to be as many as five species, Telford (1982) refrained from erecting a new genus and instead provisionally discussed the new species as *Mukia* sp. A, *Mukia* sp. B, *Mukia* sp. C, *Mukia* sp. D, and *Mukia* sp. E (Telford 1982, pp. 182–187).

Over the past quarter of a century, additional flowering and fruiting collections have become available that now permit complete descriptions, including information on phenology and natural ranges. Molecular work also clarified the affinities of *Mukia*, *Zehneria*, and the five new species from Australia. Our molecular phylogenetic analyses indicate that *M. maderaspatana*, *M. javanica* (Miq.) C. Jeffrey, *Mukia* sp. A, and *Mukia* sp. B belong in the genus *Cucumis* (Renner et al. 2007), while *M. micrantha* and *Mukia* sp. C, D, and E belong in a new genus *Austrobryonia* that forms the sister clade to a Eurasian/Mediterranean clade comprising *Bryonia* L. and *Ecballium* L. We here formally describe the new genus and species, present the first molecular phylogenies that include these new taxa, and, based on a molecular clock analysis, discuss the possible origin of this unexpected Australian/Mediterranean/Eurasian disjunction.

Materials and Methods

***Morphology*—**Specimens were borrowed from BRI, CANB and NSW. Measurements were taken from rehydrated dried herbarium specimens.

***Data Generation and Retrieval*—**DNA extraction and sequencing followed standard procedures, using the *rbcL*, *matK*, *rpl20–rps12*, and *trnL* and *trnL–F* primers listed in Kocyan et al. (2007). In addition to these plastid regions, we sequenced the nuclear internal transcribed spacer region (347 nt of *ITS 1*, 162 nt of the *5.8S* gene, and 318 nt of *ITS 2*) using the ITS primers of Balthazar et al. (2000). Direct PCR amplification of ITS yielded single bands and unambiguous base calls. Thirty-six sequences were newly generated for this study (GenBank accession numbers EF 487543–EF 487578; Appendix 1).

***Sequence Alignment and Phylogenetic Analyses*—**Sequences were edited with Sequencher (4.6; Gene Codes, Ann Arbor, Michigan, USA) and aligned by eye, using MacClade 4.06 (Maddison and Maddison 2003). The aligned plastid matrix comprises 4692 nucleotides after exclusion of a poly-A run in the *trnL* intron and a TATATA microsatellite region in the *trnL–F* intergenic spacer. The aligned ITS matrix comprises 829 nucleotides. Data matrices and trees have been deposited in TreeBASE (study number S1817).

Equally weighted parsimony analyses for matrices of nucleotides were conducted using PAUP 4.0b10 (Swofford 2002). The search strategy involved 100 random addition replicates with TBR branch swapping, saving all optimal trees. For the parsimony analyses, gaps were treated as missing data, for maximum likelihood (ML) searches gaps were not removed. To assess node support, parsimony bootstrap analyses were performed using 1000 replicate heuristic searches, each with 10 random addition replicates and TBR branch swapping, saving all optimal trees.

Maximum likelihood analyses (and ML bootstrap searches) were performed using GARLI 0.94 (D. J. Zwickl, University of Texas-Austin, available at http://www.bio.utexas.edu/grad/zwickl/web/garli.html). GARLI searches relied on the GTR + G + P-invar model, the only model implemented in this program. Model parameters were estimated in GARLI over the duration of specified runs.

To identify the closest relatives of *Austrobryonia*, we added *Austrobryonia* sequences to the dataset of Kocyan et al. (2007), which includes 123 of the 130 genera of Cucurbitaceae (representing all tribes and subtribes) and 171 of the ca. 800 species. Additional sequences were obtained for the genera *Gomphogyne*, *Indomelothria*, *Pseudosicydium*, *Urceodiscus*, and *Zanonia*, which augmented our sampling to 128 of 130 genera. Parsimony and ML analyses of this dataset showed that *Austrobryonia* always forms a highly supported clade with *Bryonia* and *Ecballium*.

The two genera of Cucurbitaceae that have not yet been sequenced are *Khmeriosicyos* De Wilde & Duyfjes, a monotypic genus from Cambodia known only from the type, and *Papuasicyos* Duyfjes, a monotypic genus from Papua New Guinea. Judging from their floral morphology, both are expected to fall in Benincaseae, far from *Bryonia*, *Ecballium*, and *Austrobryonia*. In Fig. 1, we show a phylogeny based on a reduced dataset of 75 taxa, including all tribes and subtribes and all genera containing Australian species.

Molecular Clock Analyses*—rbcL* branch lengths for Cucurbitaceae were calculated under a GTR + G + I + clock model on the preferred ML topology obtained with five chloroplast loci from a 184-taxon matrix that contained *Bryonia alba*, *B. dioica*, and *Ecballium elaterium* (Kocyan et al. 2007). For the present study, we added *Austrobryonia argillicola*, *A. centralis*, *A. micrantha*, *A. pilbarensis*, and *Bryonia verrucosa* (the latter so as to span the root of *Bryonia*). The tree was rooted on Corynocarpaceae, Begoniaceae, and Datiscaceae based on Zhang et al. (2006). The resulting branch length table was saved, the distance between a calibration node (the stem lineage of *Trichosanthes*) and the present divided by the age of the calibration node to obtain a substitution rate, and this rate then used to calculate the age of divergence events of interest. Calibration came from *Trichosanthes* seeds from the uppermost Paleocene (Collinson 1986; Collinson et al. 1993, 2003). These circa 60 million year (my) old seeds are the earliest known fossils of any Cucurbitaceae.

Results

Parsimony (Fig. 1) and maximum likelihood analyses of the 128- and the 75-taxon Cucurbitaceae plastid DNA datasets resulted in topologies that showed *Austrobryonia* as sister genus to a clade of the Eurasian and Mediterranean *Ecballium* and *Bryonia*. The 20-taxon nuclear dataset (Fig. 2) also placed *Austrobryonia* closer to *Bryonia* and *Ecballium* than to *Cucumis* although without bootstrap support. In the classification of Jeffrey (2005), *Austrobryonia* should thus be placed in the tribe *Bryonieae*, which contains only *Bryonia* and *Ecballium*. No relationship to any other Australian cucurbit genus was found (Fig. 1), and *Austrobryonia* is therefore indeed a new endemic genus for the continent. Strict clock estimates yielded an age of minimally 42 my for the split between *Austrobryonia* and *Ecballium*/*Bryonia*, and of about 8 my for the crown group of *Austrobryonia* (i.e. divergence of *A. centralis* from the ancestor of the lineage leading to *A. micrantha* and its relatives; Figs. 1, 2). Compared to 191 other *rbcL* sequences of Cucurbitaceae and outgroups, the four species of *Austrobryonia* share a synapomorphic silent substitution at position 417 of the *rbcL* alpha helix 8 (GCG instead of GCC, both coding for the amino acid alanine), and *A. centralis* in addition has an autapomorphic substitution (CGA instead of AGA at position 167).

Austrobryonia H. Schaef., gen. nov.—TYPE: *Cucurbita micrantha* F. Muell., Trans. Philos. Soc. Victoria 1: 17, 1854.

A *Cucumeris* grege *Mukia* (quae floribus eiusdem sexus in unaquaque axilla numerosis (1–20) fasciculatis, fructibus rubris et seminibus plerumque turgidis foveolatis gaudet) differt floribus paucis (2–8) amborum sexuum in unaquaque axilla consociatis, fructibus flavis vel flavovirentibus nec non seminibus compressis laevibus.

Monoecious scabrid herbs; stems annual, from a perennating rootstock. Tendrils simple. Leaves petiolate; lamina more or less ovate, cordate, acute, unlobed or shallowly 3-, 5- or 7-lobed, dentate or serrate. Inflorescences of solitary flowers or fascicles, androgynous or unisexual. Flowers pedicellate, small, 3–15 mm diam.; corolla yellow-green or yellow. Male flowers: hypanthium broadly campanulate; calyx of 5 small, narrow-triangular sepals inserted on the hypanthium rim; corolla 5-lobed, more or less rotate; lobes ovate, alternating with sepals; stamens 3, inserted about the middle of the hypanthium, two 2-locular, one 1-locular; filaments short; connectives broad, appendages absent; anthers dorsifixed, curved, dehiscing longitudinally, connivent; disc absent or present, minute. Female flowers: hypanthium above constriction broadly campanulate; perianth similar to males, sometimes slightly larger; staminodes 3, inserted about the middle of the hypanthium; ovary subglobose or ellipsoidal; ovules many, horizontal; disc annular; style very short or stigma subsessile; stigma 2- or 5-lobed, the stigmatic lobes capitate or linear, papillose. Fruit a globose or ellipsoidal, 10–35 mm long, few to many-seeded berry, green to yellow. Seeds ovate, compressed, margins sometimes thickened, smooth, pale. Pollen tricolpate-oblate (Jeffrey 1969a). Chromosome number unknown.

***Distribution*—**Endemic to Australia, widespread throughout arid and semiarid parts of the mainland states.

Key to Species of *Austrobryonia*

1. Female flowers in fascicles of 2–5, mostly coaxillary with 1–3 males; fruit ellipsoidal, 10–14 mm long ..... 1. *A. micrantha*
1. Female flowers solitary, rarely 2 per fascicle, sometimes coaxillary with males 2
   2. Stigmatic lobes linear; fruit ellipsoidal, 18–35 mm long ..... 4. *A. centralis*
   2. Stigmatic lobes capitate; fruit subglobose 3
      3. Fruit 18–25 mm diam., endemic in inland northern Australia ..... 2. *A. argillicola*
      3. Fruit 12–18 mm diam., endemic to the Pilbara Region of Western Australia ..... 3. *A. pilbarensis*

1. Austrobryonia micrantha (F. Muell.) I. Telford, comb. nov. *Cucurbita micrantha* F. Muell., Trans. Philos. Soc. Victoria 1: 17, 1854. *Zehneria micrantha* (F. Muell.) F. Muell., Fragm. 1: 182, 1859. *Melothria micrantha* (F. Muell.) Cogn. in A. & C. de Candolle, Monogr. Phan. 3: 603, 1881. *Cucumis muelleri* Naudin, Ann. Sci. Nat. (Paris) ser. 4, 11: 84. 1859. *Melothria muelleri* (Naudin) Benth., Fl. Austral. 3: 320, 1866. *Mukia micrantha* (F. Muell.) F. Muell. Fragm. 2: 180. 1861 [as *Muckia*].—TYPE: AUSTRALIA. Murray River, Victoria?, *F. von Mueller s. n.* (holotype: MEL!).

Perennial herb with thickened rootstock. Stems annual, prostrate, to several m long, 0.4–2 mm diam., ribbed, sparsely scabrid to glabrescent. Tendrils simple, to 40 mm long. Leaves: petioles (10–)15–20(–50) mm long, scabrid; lamina broadly ovate, cordate, 15–60 mm × 12–45 mm, dentate or

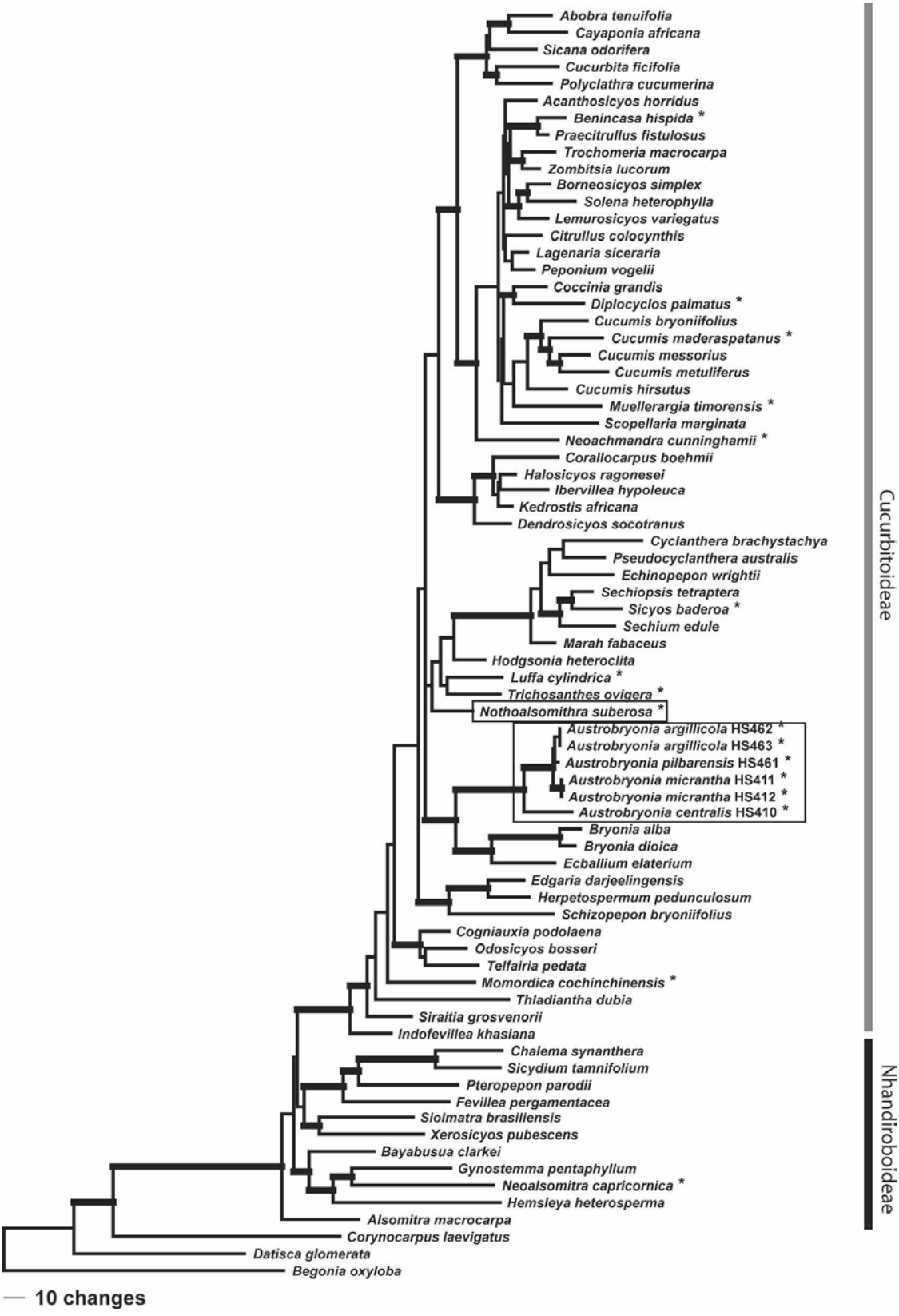

FIG. 1. Parsimony tree for *Austrobryonia* and relatives based on 4692 nucleotides from combined chloroplast loci (*rbcL* gene, *matK* gene, *trnL* intron, *trnL–F* spacer, *rpl20–rps12* spacer). Branches in bold indicate parsimony bootstrap support ≥ 80%. An asterisk (*) indicates species from Australia and genera containing Australian species. The two endemic genera are marked by rectangles. The topology obtained here, showing the Nhandiroboideae as a grade, the Cucurbitoideae as a clade, differs from that obtained under maximum likelihood for a larger taxon sample, which suggested that the two subfamilies of Cucurbitaceae are monophyletic (Kocyan et al. 2007).

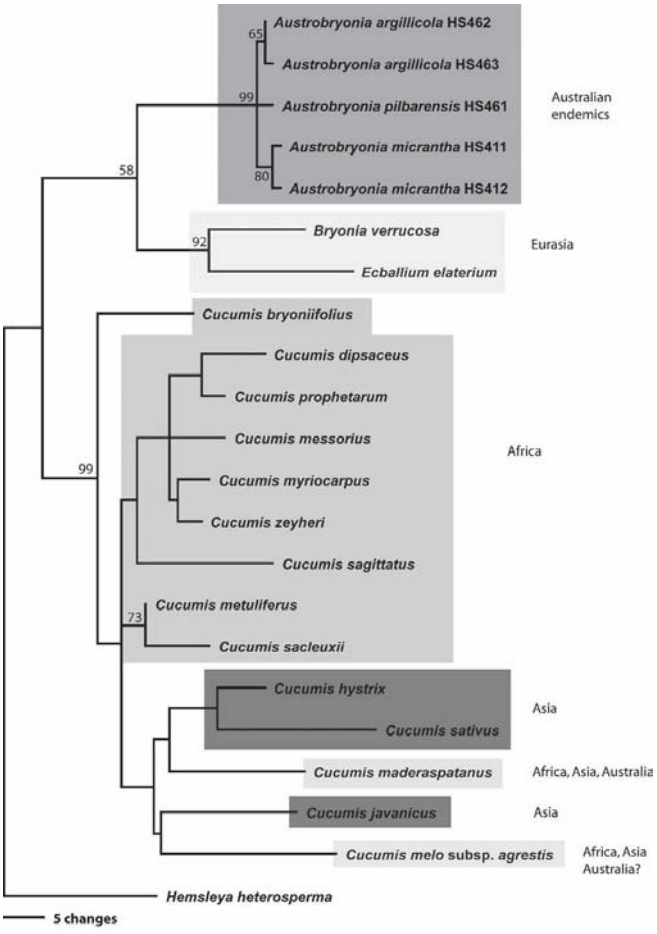

FIG. 2. Parsimony tree for *Austrobryonia* and relatives based on 829 nucleotides of the nuclear rDNA ITS 1, the 5.8S gene, and ITS 2. Parsimony bootstrap support ≥ 50% given at the nodes. Native range indicated by different grey scales.

shallowly 3–7-lobed, the lobes dentate, scabrid with tubercle-based hairs on both surfaces, more densely abaxially. Inflorescences usually androgynous fascicles. Male flowers: 1–3 per fascicle; pedicels 3–5 mm long; hypanthium broadly campanulate, 1–1.5 mm long, puberulous externally; calyx lobes narrow-triangular, 1–1.2 mm long, puberulous abaxially; corolla lobes ovate, 2–2.5 mm × 1.3–1.5 mm, obtuse, glabrous adaxially, puberulous and glandular abaxially, yellow-green; disc minute or absent; stamens 3, one 1-locular, two 2-locular; filaments 0.8–1 mm long; anthers 0.8–1.2 mm long. Female flowers: 2–5 per fascicle; pedicels 3–8 mm long; hypanthium above constriction ca. 0.5 mm long, puberulous and glandular externally; ovary ellipsoidal, 3–10 mm long; perianth similar to males; disc annular, ca. 1.6 mm diam.; staminodes minute; stigma 2-lobed, 1.5–2 mm diam., the stigmatic lobes capitate. Fruit subglobose to ellipsoidal, 10–14 mm long, 7–13 mm diam.; fruiting pedicel to 18 mm long. Seeds 5–20, ovate, 4–6.5 mm long, 2.5–3.5 mm wide, compressed, smooth, pale buff.

Illustrations—Fig. 3A; Cunningham et al. (1981) 626; Telford & Marsden (1981) Fig. 270 C–E; Telford (1982) 185, Fig. 40H–K; Jobson (1996) 384, Fig. 79 F, G.

Phenology—Flowering and fruiting from January to May.

Distribution and Habitat—*Austrobryonia micrantha* is widespread through inland Australia in the Lake Eyre and Murray-Darling Basins from the Finke River, Northern Territory, eastwards to near Boulia, Queensland and Louth, New South Wales, southwards to the Wimmera, Victoria (Fig. 4). Typically found on clay soils of river flood plains, waterhole and dam margins and swales in dunefields. Perennating by a thickened rootstock, the stems wither in the dry season with regrowth apparently initiated by heavy rainfall or flooding.

Representative Specimens Examined—AUSTRALIA. Northern Territory: Barkly Tableland: Gibson Creek, 35 miles (56 km) N of Tennant Creek, *Must 222* (CANB, NSW, NT). South Australia: Lake Eyre Basin: Edwards Creek, Old Ghan Siding, *Latz 15849* (AD, CANB, NE, NT); Birdsville Track, 16 km WSW of Ettadunna Homestead, *Jackson 1952* (AD): Murray: Lake NE of Cooltong, 28 Feb 1966, *Symon s.n.* (AD, CANB, K). Queensland: Gregory South District: 21 km from Birdsville, White Bank Dam, Adria Downs Station, *Edmunds 219* (BRI). New South Wales: North Far Western Plains: Mt Murchison Station, Wilcannia, 19 Feb. 1966, *Martensz s.n.* (CANB); South Western Plains: 35 miles N of Deniliquin, *Leigh 169* (CANB, NSW). Victoria: Wimmera: ca. 10 km SW of Watchem township, *Carr 7761* (CANB, MEL); Hattah-Kulkyne National Park, Lake Konardin, 10 km NE of Hattah, *Forbes 1427* (CANB, MEL).

2. Austrobryonia argillicola I. Telford sp. nov.—TYPE: AUSTRALIA. Northern Territory: Central Australia North: ca. 12 km SSW of Tobermorey Station Homestead, *D.E. Albrecht 6322* (holotype, NT; isotypes, CANB!, DNA).

Ab affini *Austrobryonia micrantha* differt foliorum lamina in facie adaxiali glabra, floribus foeminis plerumque solitariis et corollis minoribus.

Perennial herb with thickened rootstock. Stems annual, prostrate, to ca. 1 m long, 0.5–2.2 mm diam., ribbed, sparsely scabrid,. Tendrils simple, to 70 mm long. Leaves: petioles (8–)20–55(–100) mm long, scabrid; lamina broadly ovate, cordate, (14–)20–45(–70) mm × (12–)25–45(–60) mm, acute, unlobed or shallowly 3- or 5-lobed, serrate, glabrous or rarely very sparsely scabrid adaxially, scabrid with tubercle-based hairs abaxially. Inflorescence usually androgynous fascicles. Male flowers: 1 or 2 per fascicle; pedicels 1–3 mm long; hypanthium broadly campanulate, ca. 1 mm long; calyx lobes subulate to narrow-triangular, 0.5–0.8 mm long, green, puberulous abaxially; corolla lobes ovate, 1.3–1.8 mm × 1–1.2 mm, obtuse, glabrous adaxially, puberulous and glandular abaxially, yellow-green; stamens 3, one 1-locular, two 2-locular; filaments ca. 0.5 mm long; anthers ca. 1 mm long; disc absent. Female flowers: 1–3 per fascicle; pedicels 2.5–7 mm long; hypanthium more or less glabrous externally; ovary ellipsoidal, 2.5–4 mm long, 2–3 mm diam., perianth similar to males; staminodes 3, linear, ca. 0.5 mm long; disc annular, 1.5–2 mm diam.; style ca. 0.5 mm long; stigma 2-lobed, 1.5–1.8 mm diam., the stigmatic lobes capitate. Fruit subglobose, 18–25 mm diam., smooth, pale green to yellow with darker longitudinal stripes; fruiting pedicel 5–11 mm long. Seeds 5–15, ovate, 7.5–9 mm long, 4.5–6 mm wide, compressed, smooth, pale buff.

Illustrations—Fig. 3B; Telford (1982) 185, Fig. 40L.

Phenology—Flowering and fruiting from February to June.

Distribution and Habitat—*Austrobryonia argillicola* is endemic in inland northern Australia in the north-eastern Lake Eyre Basin of the Northern Territory and Queensland, mainly in the catchments of the Georgina, Diamantina and Thompson Rivers (Fig. 4). The species inhabits grasslands, mostly associated with Mitchell Grass (*Astrebla* spp.) on cracking clays.

Etymology—The species is named for the soil of its habitat; from the Latin *argilla* (clay) and *-cola* (dweller).

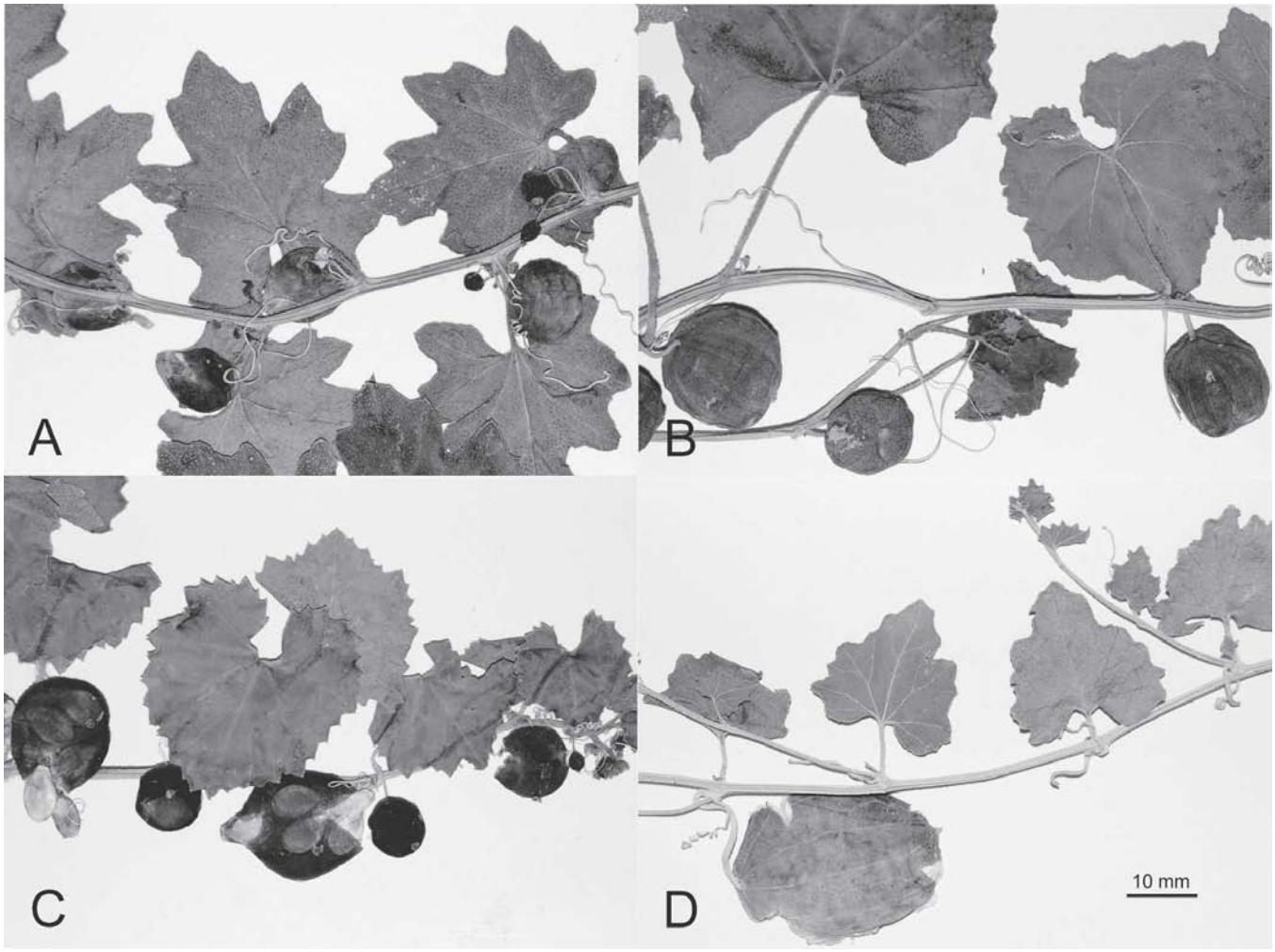

FIG. 3. Fruiting specimens of *Austrobryonia* spp.: A. *A. micrantha*, *G.W. Carr 7761* (CANB); B. *A. argillicola*, *J. Milson 330* (BRI); C. *A. pilbarensis*, *A.A. Mitchell PRP1121* (CANB); D. *A. centralis*, *D.E. Albrecht* 10205 (NE). Scale bar = 10 mm.

Representative Specimens Examined—AUSTRALIA. Northern Territory: Barkly Tableland: Brunette Downs Homestead, *Latz 14422* (CANB, DNA, NT); Central Australia North: ca. 12 km SSW of Tobermorey Station Homestead, *Albrecht 6322* (CANB, DNA, NT). Queensland: Burke District: Balcomo, near Nonda, May 1949, *Newton s.n.* (BRI); Gregory North District: 10 km S of Glenormiston Homestead, *Latz 21730* (BRI, NE, NT); Mitchell District: 30 miles (48 km) NNW of Longreach, Aug. 1952, *Davidson s.n.* (BRI); 45 km from Longreach along Capricorn Highway towards Barcaldine, *Telford 11462 & Rudd* (BRI, CANB, K).

Note—The Flora of Australia (Telford 1982) treated *A. argillicola* as *Mukia* sp. E. The species was listed under the informal phrase name, *Mukia* sp. (Longreach, D. Davidson AQ279935) in Henderson (2002).

3. Austrobryonia pilbarenis I. Telford sp. nov.—TYPE: AUSTRALIA. Western Australia: 28.2 km SSE of Warrawagine Station Homestead and 3 km N of Little River Well, *A.A. Mitchell PRP1742*; (holotype, PERTH; isotype. CANB!).

Ab affini *Austrobryonia argillicola* differt fructibus minoribus et seminibus minus numerosis minoribusque.

Perennial monoecious herb with thickened rootstock. Stems annual, prostrate or climbing, to ca. 1 m long, 0.4–2 mm diam., ribbed, sparsely scabrid. Tendrils simple, to 70 mm long. Leaves: petioles 12–40(–50) mm long, scabrid; lamina broadly ovate, cordate, (10–)20–35(–45) mm × (10–)15–40(–50) mm, acute, unlobed or shallowly 3- or 5-lobed, dentate or serrate, glabrous or very sparsely scabrid adaxially, scabrid with tubercle-based hairs abaxially. Inflorescence fasciculate, androgynous. Male flowers: 1–3 per fascicle; pedicels 2–5 mm long, sparsely scabrid; hypanthium broadly campanulate, ca. 1 mm long, sparsely scabrid externally; calyx lobes narrow-triangular, 0.5–0.8 mm long, green, puberulous abaxially; corolla lobes ovate, 1.4–1.6 mm × 1–1.2 mm, obtuse, glabrous adaxially, puberulous and glandular abaxially, yellow-green; stamens 3, one 1-locular, two 2-locular; filaments ca. 0.5 mm long; anthers 0.4–0.6 mm long; disc minute. Female flowers: 1 per fascicle; pedicels 3–7 mm long, glabrous or sparsely scabrid; ovary subglobose, 2–4 mm diam., glabrous or very sparsely scabrid; perianth similar to males; staminodes linear, ca. 0.5 mm long; disc annular, ca.1.2 mm diam.; stigma 2-lobed, 1.5–1.8 mm diam., the stigmatic lobes capitate. Fruit subglobose, 12–18 mm diam., smooth, glabrescent, yellow; fruiting pedicel 5–12 mm long. Seeds 3–8, ovate, 7–8.5 mm long, 4–5.5 mm wide, compressed, smooth, pale buff.

Illustration—Fig. 3C.

Phenology—Flowering from March to August with fruit collected from July to September

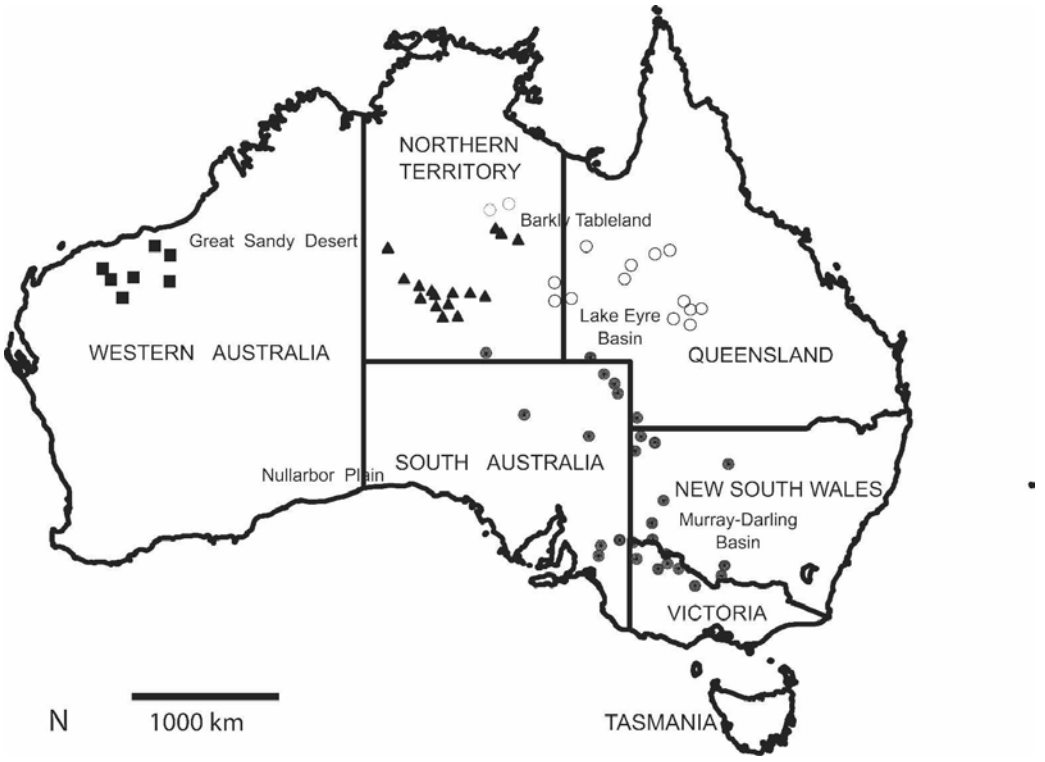

FIG. 4. Distribution map showing the natural ranges of the four species of *Austrobryonia*: ● *A. micrantha;* ○ *A. argillicola;* ■ *A. pilbarensis;* ▲ *A. centralis.*

Distribution and Habitat*—Austrobryonia pilbarensis* is endemic to the Pilbara Region of Western Australia (Fig. 4), where it grows in tussock grasslands with *Eragrostis xerophila* Domin and *Astrebla lappacea* Domin on "crabhole" clay plains, often on calcrete.

***Etymology*—**The species is named for its distribution, being endemic to the Pilbara Region of Western Australia, and the Latin *-ensis* (pertaining to).

***Representative Specimens Examined*—**AUSTRALIA. Western Australia: The Millstream, 10 Sep 1995, *Mitchell s.n.* (CANB); ca. 21 km W from Hamersley Homestead, *Mitchell PRP1050A* (CANB); Crabhole Plain, Warralong, *Burbidge 805* (PERTH); near Brady Bore, Noreena Downs Station, ca. 150 km N of Newman, *Mitchell PRP1430* (CANB, PERTH); Between the Ashburton and De Gray rivers, purchased Aug. 1900, *E. Clement s. n.* (K).

***Note*—**The Flora of Australia (Telford 1982) treated *A. pilbarensis* as *Mukia* sp. D.

4. Austrobryonia centralis I. Telford, sp. nov.—TYPE: AUSTRALIA. Northern Territory: Barkly Tableland: 2 km NNW of Brunchilly Homestead, *D.E. Albrecht 7500 & P.K. Latz* (holotype, NT; isotypes: CANB!, DNA).

Ab affini *Austrobryonia micrantha* differt floribus foeminis et masculis solitariis, lobis stigmaticis linearibus, fructibus maioribus et seminibus magis numerosis.

Perennial, monoecious herb with thickened rootstock. Stems annual, prostrate or climbing to 2.5 m long, 0.6–2 mm diam., densely scabrid. Tendrils simple, to 50 mm long. Leaves: petioles 4–15 mm long, scabrid; lamina ovate to subtriangular, (12–)25–35(–50) mm × (13–)20–30(–50) mm, cordate, acute, dentate, usually shallowly 3- or 5-lobed, scabrid with tubercle-based hairs on both surfaces. Inflorescence of solitary flowers, the males distal. Male flowers: pedicels 8–12 mm long; hypanthium campanulate, 2–3.5 mm long, puberulous externally; calyx lobes narrow-triangular, ca. 1.5 mm long, puberulous abaxially; corolla lobes ovate, 2.5–3 mm × 1.8–2 mm, obtuse, glabrous adaxially, puberulous abaxially, yellow; stamens 3, two 2-locular, one 1-locular; filaments ca. 1 mm long; anthers 2–2.3 mm long; corolla tube pubescent internally; disc absent. Female flowers: pedicels 15–27 mm long; hypanthium puberulous externally, 2–3.5 mm long above constriction; calyx lobes narrow-triangular, 1.8–2 mm long, puberulous abaxially; corolla lobes ovate, 4–6 mm × 3.5–4 mm, obtuse, puberulous abaxially, yellow; staminodes 3, ca. 2 mm long; corolla tube pubescent inside; disc annular, ca. 3.5 mm diam.; ovary narrow-ellipsoidal to fusiform, 5.5–12 mm long, 1.5–4 mm diam.; style 2–3 mm long; stigma 5-lobed, the stigmatic lobes linear, 2–2.5 mm long, papillose. Fruit ellipsoidal, 18–35 mm long, 15–20 mm diam., slightly longitudinally ribbed, sparsely puberulous to glabrescent, yellow-green; fruiting pedicel to 35 mm long. Seeds 10–ca. 40, ovate, 5.5–8 mm long, 4–6 mm wide, compressed, smooth, pale buff.

***Illustrations*—**Fig. 3D; Telford & Marsden (1981) Fig. 270 A–B.

***Phenology*—**Flowering mainly from April to May, with single records from November and January. Fruit collected from April to July.

Distribution and Habitat*—Austrobryonia centralis* is endemic in the inland Northern Territory, Australia, from near Tanami eastwards to the southern Barkly Tableland, southwards to the Macdonnell Range (Fig. 4). It grows mainly in *Acacia kempeana* F. Muell. and *A. aneura* F. Muell. s.l. grassy woodlands on red earths. Other habitats recorded are woodland with *Corymbia opaca* (D.J. Carr & S.G.M. Carr) K.D. Hill & L.A.S. Johnson, grasslands on black clay soils with *Astrebla* and with a single record from rocky quartzite hills with *Acacia maidenii* F. Muell.

Etymology—The species is named for its distribution, being endemic to Central Australia.

Representative Specimens Examined—AUSTRALIA. Northern Territory: Barkly Tableland: 22 km E of Banka Banka, *Albrecht 7471* (BRI, CANB, DNA, NT); Central Australia North: S of Tanami, *Maconochie 2549* (AD, B, CBG, K, MO, NT); Walkabout Bore, Epenarra Station, *Latz 14425* (CANB, DNA, NT); intersection of Stuart Hwy and Plenty Hwy ca. 70 km N of Alice Springs, *Albrecht 10205* (M, NE, NT); 4 km ESE of Muranji Rockhole, *Latz 10620* (CANB, DNA, NT).

Notes—The Flora of Australia (Telford 1982) treated *A. centralis* as *Mukia* sp. C. Some specimens were annotated as *Mukia* sp. A50961 Glen Helen Station (DNA, NT) or *Mukia* sp. (Haast Bluff Stn, P.K. Latz 7624 in BRI), and some as *Mukia centralis* I. Telford (B and K), a *nomen nudum*.

Discussion

Austrobryonia is remarkable because of its close relationship to a Mediterranean and southern Eurasian clade (*Bryonia* and *Ecballium*). This is unique among the native Australian cucurbits (Australian taxa are marked in Fig. 1), which typically have close relatives or even conspecifics among tropical Asian lineages, as is the case in *Cucumis, Diplocyclos, Neoalsomitra*, *Neoachmandra*, and *Trichosanthes* (Telford 1982; De Wilde and Duyfjes 2003, 2006). The sole indigenous Australian *Momordica*, *M. cochinchinensis* (Lour.) Spreng., is widespread from New Guinea to China and India (De Wilde and Duyfjes 2002), and the Australian *Muellerargia timorensis* also occurs in the rainforests of East Timor. (The second species of *Muellerargia* is endemic to Madagascar, and has not yet been sequenced.) *Sicyos* is represented in the south-western Pacific by *S. australis* Endl., and two unnamed species, one of which is endemic to New Zealand, while the remaining 40 species of *Sicyos* occur in the neotropics and on Hawaii. Because of this disjunct distribution *Sicyos* is suspected to have reached the south-western Pacific via ancient long distance dispersal (M. Nee, New York Botanical Garden, personal communication, 2003). Another suspected case of ancient long distance dispersal concerns the indigenous *Luffa aegyptiaca* Mill., which is sister to the Central American *L. operculata* L. (Kocyan et al. 2007). The second Australian *Luffa*, *L. graveolens* Roxb., has not yet been sequenced. *Benincasa hispida* (Thunb.) Cogn., finally, is sister to the Indian genus *Praecitrullus* (Kocyan et al. 2007), and the endemic *Nothoalsomitra suberosa* forms an isolated branch, and its relationships are still unclear (Kocyan et al. 2007; our Fig. 1).

Unlike all these genera, the closest relatives of *Austrobryonia* occur in the Mediterranean and southern Eurasia. There, the species of *Bryonia* and *Ecballium* have similar habitat preferences as the four species of *Austrobryonia*, but we know of no morphological synapomorphies. *Ecballium* includes only *E. elaterium* L. and is native in xeric and mesic regions of the Mediterranean. Most of the twelve species of *Bryonia* occur in dry areas in Turkey, Syria, Iran, Iraq, Afghanistan, and Pakistan, with at least four adapted to sand deserts in Afghanistan, Turkmenistan, Uzbekistan, and Kazakhstan; a few species occur in northern Europe and west to the Canary Islands (Jeffrey 1969b).

The fruits of *Austrobryonia* are pale green, apparently with longitudinal striping when young and ripening to yellow. They are recorded as fleshy, the flesh "very tart" (specimen label information). There is one record of macropods (no species given on the herbarium specimen label) eating the rinds of *A. centralis*, but the major dispersers are probably large terrestrial birds of the grasslands, such as the Emu (*Dromaius novaehollandiae* Latham) and Australian Bustard (*Ardeotis australis* Gray), that eat whole fruits. The fruits of *Cucumis* are similar to those of *Austrobryonia*, and large quantities of *Cucumis melo* (subsp. *agrestis*) seeds have been recorded from the stomachs of bustards in the northern Australian savannas (Mark Ziembicki, Biodiversity Conservation Unit, Dept. of Natural Resources, Palmerston, personal communication, 21 March 2007). Bustards are mainly African (21 of 25 extant species), with only four species in Australasia (Broders et al. 2003). One predominantly African species, the Houbara Bustard, has reached the Canary Islands, a larger distance than that between Sumatra/Java and New Guinea/Australia in the Upper Miocene (10–8 my ago), when sea levels were low. The oldest fossils of bustards are from the late Eocene/early Oligocene of France (38 my ago) but the oldest known bustard fossils from Asia are only 5 my old (Broders et al. 2003). The oldest fossils of emus are from the Upper Oligocene of Australia (Boles 2001). Given the molecular age estimate of the stem lineage of *Austrobryonia* (some 42 my), a possible scenario for its arrival in Australia is ocean dispersal with Tethyan ocean currents during the Eocene, combined with or followed by bird dispersal between Sumatra/Java and Australia during the Miocene. Plant taxa with similar disjunction are species of *Scleranthus, Ceratocephala,* and *Gypsophila* (Caryophyllaceae; Smissen et al. 2003), *Hebe/Veronica* (Wagstaff et al. 2002), and a few Chenopodiaceae (Kadereit et al. 2005).

The estimated age of about 8 my (with an error of ca. ± 2 my) of the *Austrobryonia* crown group fits well with other findings from molecular-clock dating, which suggest that rapid climatic change during the MidCenozoic (25–10 my ago) resulted in radiations of sclerophyll taxa, such as *Banksia*, eucalypts, pea-flowered legumes and *Allocasuarina* (Crisp et al. 2004), with at least 23 plant lineages in southern Australia including taxon pairs that diverged from each other 13–14 my ago, at a time when the arid treeless Nullarbor Plain acquired much of its current vegetation (Crisp and Cook 2007). Severe aridification at about 3 my then caused the rapid opening of the central Australian arid zone (today with less than 250 mm of rainfall per year; Barlow 1981). The Quaternary dune field of the Great Sandy Desert and the Tanami sand plain separate the clay habitats of *Austrobryonia argillicola* and *A. pilbarensis*, and their gradual development may have caused the divergence of these two species (although their precise relationships are not resolved by our data). The only other Cucurbitaceae found in these habitats are *Mukia maderaspatana* (*Cucumis maderaspatanus* L., Schaefer 2007), *Cucumis melo*, the introduced *Citrullus colocynthis*, and the likewise introduced *Ecballium elaterium*, the latter from the sister clade of *Austrobryonia*.

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APPENDIX 1. Species and loci sequenced newly for this study, voucher specimen (collection locality, collector and numbr, and herbaria where deposited), and GenBank accession numbers, in the order: *rbcL, matK, trnL* intron + *trnL–F* spacer, *rpl20–rps12* spacer, and ITS spacer (partial) + 5.8S ribosomal RNA gene + ITS2 spacer (partial). Herbarium acronyms follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Austrobryonia argillicola I. Telford - Australia. Near Northern Territory - Queensland border. *P. K. Latz 21730* (NT) EF 487549, EF 487556, EF 487572, EF 487564, EF 487543. ***Austrobryonia argillicola*** I. Telford - Australia. Queensland, near Barcaldine. *A. R. Bean 14908* (BRI) EF 487548, EF 487555, EF 487571, EF 487563, EF 487544. ***Austrobryonia centralis*** I. Telford - Australia. Northern Territory, Central Australia, Alice Springs. *D. E. Albrecht 10205* (NT) EF 487550, EF 487557, EF 487573, EF 487565, -. ***Austrobryonia micrantha*** (F. Muell.) I. Telford - Australia. South Australia. Lake Eyre Basin, Edwards Creek. *P. K. Latz 15849* (NT) EF 487551, EF 487558, EF 487574, EF 487566, EF 487545. ***Austrobryonia micrantha*** (F. Muell.) I. Telford - Australia. Cultivated from seeds collected at New South Wales, Yarriarraburra Swamp. *I. R. Telford 8173* (CANB) EF 487552, EF 487559, EF 487575, EF 487567, EF 487546. ***Austrobryonia pilbarensis*** I. Telford - Australia. Western Australia, Pilbara region. *A. R. Mitchell* PRP 1742 (PERTH) EF 487553, EF 487560, EF 487576, EF 487568, EF 487547. ***Momordica cochinchinensis*** (Lour.) Spreng. - PR China, Guangdong Province. *H. Schaefer* 05/075 (M) EF 487554, EF 487561, EF 487577, EF 487569, -. ***Neoachmandra cunninghamii*** (F. Muell.) W. J. de Wilde & Duyfjes - NE Australia, Queensland. *H. Schaefer* 07/025 (M) -, EF 487562, EF 487578, EF 487570, -.