

# Classification of the early-divergent angiosperm family Hydatellaceae: one genus instead of two, four new species and sexual dimorphism in dioecious taxa

Dmitry D. Sokoloff<sup>1</sup>, Margarita V. Remizowa<sup>1</sup>, Terry D. Macfarlane<sup>2</sup> & Paula J. Rudall<sup>3</sup>

<sup>1</sup> Department of Higher Plants, Biological Faculty, Moscow State University, 119992 Moscow, Russia

<sup>2</sup> Western Australian Herbarium, Science Division, Department of Environment & Conservation, Brain Street, 6258 Manjimup, WA, Australia

<sup>3</sup> Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, U.K. p.rudall@kew.org (author for correspondence)

Hydatellaceae was traditionally circumscribed as a family of two genera, *Hydatella* and *Trithuria*. We place *Hydatella* in synonymy of *Trithuria*. We describe three new species of Hydatellaceae from northern Australia and one new species from south-western Western Australia. Thus, we distinguish a single genus of Hydatellaceae, *Trithuria*, which consists of one species in New Zealand, ten species in Australia and one species in India. Two main characters were formerly used to distinguish between *Hydatella* and *Trithuria*: (1) reproductive units unisexual (homogamous) vs. bisexual (heterogamous) and (2) fruits with three prominent ribs, typically dehiscent vs. without prominent ribs, indehiscent. New evidence shows that the type of reproductive unit does not correlate with fruit morphology in Hydatellaceae. We hypothesize that two south-western Australian endemics known as *Hydatella dioica* and *Trithuria occidentalis* represent male and female individuals, respectively, of the same biological species. All four dioecious species of Hydatellaceae show similar sexual dimorphism. Male plants have reproductive units with longer and fewer involucre bracts than female ones. Anthers are much longer in dioecious species than in the morphologically closest cosexual species. The evolutionary significance of dioecy in Hydatellaceae is discussed.

**KEYWORDS:** Australia, dioecy, evolution, flower, Hydatellaceae, *Hydatella*, inflorescence, morphology, taxonomy, *Trithuria*

## INTRODUCTION

Hydatellaceae is a small family of tiny aquatic or semi-aquatic, mostly annual, herbs with a centre of species diversity in Australia. Interest in this formerly obscure family has increased exponentially following the discovery that Hydatellaceae belong to the ANITA grade of early-divergent angiosperms, as close relatives of Nymphaeaceae and Cabombaceae (Saarela & al., 2007) (Fig. 1). Traditionally, Hydatellaceae was placed among monocots, either in an order of its own or within Poales. Since molecular data on placement of Hydatellaceae became available, significant morphological similarities between Hydatellaceae, Nymphaeaceae and Cabombaceae have been identified (Rudall & al., 2007; Saarela & al., 2007). While investigating the developmental morphology of Australian Hydatellaceae we realized a need to clarify some problematic issues of species-level taxonomy and generic delimitation in the family.

**Terminology.** — We use the neutral term “reproductive units” for the structures that Cooke (1987) termed capitula, because their morphological nature merits further investigation. Reproductive units of Hydatellaceae

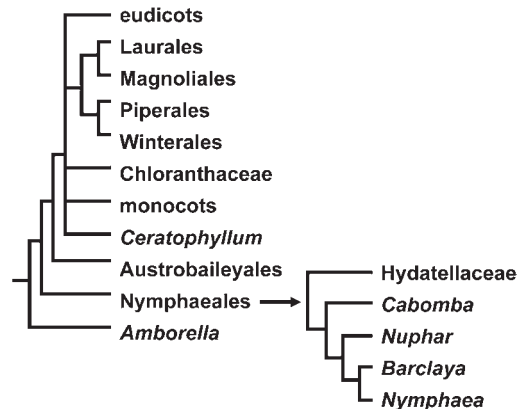


Fig. 1. Diagram showing hypothesis of relationships of Hydatellaceae (based on Saarela & al., 2007). Although Saarela & al. (2007) did not formally assign the family Hydatellaceae to the order Nymphaeales, we consider structural similarities between Hydatellaceae, Nymphaeaceae and Cabombaceae as sufficient for placing all three families in the same order.

combine features of inflorescences with some features of “true” flowers (Rudall & al., 2007 and in prep.). Structures combining features of flowers and vegetative shoots are

described in *Nymphaea* spp. (Grob & al., 2006), but these differ from reproductive units of Hydatellaceae. If the traditional pseudanthial interpretation of the reproductive units in Hydatellaceae proves correct, the term “(partial) inflorescence” could be applied. A capitulum is a racemose inflorescence of sessile flowers, which is not the case in Hydatellaceae (Rudall & al., 2007). On similar grounds we use a more neutral term “cosexual” instead of “monoecious” because the latter term implies co-occurrence of male and female flowers on the same individual.

**Taxonomic background.** — Prior to our study, two genera and nine or ten species were recognized within the family Hydatellaceae. The genus *Trithuria* Hook. f. was characterized by bisexual reproductive units and usually dehiscent fruits that always possess three prominent longitudinal ribs (Cooke, 1987; Hamann, 1998; see also Hooker, 1858; Cheeseman, 1907; Edgar, 1966; Hamann & al., 1979). Three species of *Trithuria* were recognized in Australia, including Tasmania (Cooke, 1987), and a fourth species was recently discovered in India (Yadav & Janarthanam, 1994, 1995; see also Gaikwad & Yadav 2003). Among the Australian species, *T. lanterna* D.A. Cooke occurs in the northern part of the continent, while *T. bibracteata* D.A. Cooke is restricted to the south-west of Western Australia. According to Cooke (1987), these two species are characterized by possession of reproductive units with two involucre bracts, and *T. lanterna* is reportedly remarkable in possessing indehiscent fruits, though Rudall & al. (2007) demonstrated fruit dehiscence at least in one accession of *T. lanterna*. Finally, *T. submersa* Hook. f. has a disjunct distribution between the south-west of Western Australia, the south-east of Australia and Tasmania. Some plants from Western Australia have been segregated as *T. occidentalis* Benth. (Bentham, 1878) on the basis of more numerous involucre bracts (up to eight instead of usually four) and narrower fruits, but Cooke (1987) reported that *T. occidentalis* intergrades with typical *T. submersa* and did not recognize *T. occidentalis* as a distinct species.

The genus *Hydatella* Diels (here synonymized with *Trithuria*) was characterized by unisexual reproductive units and indehiscent fruits without distinct ribs (Cooke, 1987; Hamann, 1998). *Hydatella inconspicua* (Cheesem.) Cheesem. is restricted to New Zealand, where it is now known from both North Island and South Island (Wells & al., 1998). In natural populations of the species most plants are female but very few are bisexual with both female and male reproductive units (Edgar, 1966; Pledge, 1974). Three cosexual species (with male and female reproductive units on the same plant) are recognized in Australia (Cooke, 1987): *H. filamentosa* (Rodway) W.M. Curtis (Tasmania), *H. australis* Diels (W. Australia) and *H. leptogyne* Diels (W. Australia). There are reports of occasional (possibly teratological) bisexual reproductive units in *H. inconspicua* and *H. filamentosa* (Edgar, 1966;

Cooke, 1987). Finally, for many years the rare Western Australian *Hydatella dioica* D.A. Cooke was known only from male plants (Cooke, 1983, 1987).

## MATERIAL AND METHODS

**Plant material.** — Herbarium specimens from B, BM, BRI, CHR, DNA, E, HO, K, MEL, NSW, NT, PERTH were examined, listed in the Appendix. Considering the amount of material studied, note that most samples contain several or many individuals (each with many reproductive units) of these tiny plants. For example, the three samples used to describe the new species *Trithuria cowieana* contain in total 34 plants.

**Methods.** — Fruit morphology was examined using a Leica dissecting microscope and scanning electron microscope. Loose, herbarium-dried fruits and reproductive units were temporarily removed from packets on herbarium sheets and mounted onto specimen stubs using thick double-sided tape, coated with platinum using an Emitech K550 sputter coater, and examined using a Hitachi cold field emission SEM S-4700-II at RBGK. After SEM examination, the entire mounting tape was removed from stubs together with the plant material and returned in packets on herbarium sheets.

## SEXUAL DIMORPHISM IN DIOECIOUS TAXA, AND FOUR NEW SPECIES

Analysis of herbarium material leads us to support the hypothesis that plants described as *Hydatella dioica* (Fig. 2A) represent male individuals of plants that were described as *Trithuria occidentalis* (Fig. 2D–F). This idea was already suggested by an unsigned annotation on a sheet in the Kew herbarium (the annotation probably dates from the 1970s, as its author clearly had not read Cooke’s publications of the 1980s), but it has apparently not been published elsewhere.

Examination of type material of *T. occidentalis* (Western Australia, Swan River, *J. Drummond* 689), which contains many reproductive units, showed that all these units are female. In contrast, type material of *T. submersa* (Tasmania, Macquarie River, *R.C. Gunn* 2014) and all other (ample) material available from Tasmania and south-eastern Australia constantly possess bisexual reproductive units. To our knowledge, this difference has not previously been discussed in the literature, though in 1903 Otto Stapf drew excellent images (unpublished, held at K) that clearly show that *T. occidentalis* is female. Closer examination of the Western Australian material identified in collections as *T. submersa* s.l. showed that it can readily be divided



Fig. 2. SEM reproductive units (A, D–F) and anthers (B, C) of *Trithuria* spp. from south-west Western Australia. A, male reproductive unit of *Trithuria occidentalis* = “*Hydatella dioica*” (Morrison s.n., 22 Nov. 1899, K); B, anther of *T. austinensis* (Keighery & Gibson s.n., 23 Feb. 1999, PERTH); C, anther of *T. bibracteata* (Fitzgerald s.n., Oct. 1903, K); D–F, female reproductive units of *T. occidentalis*. A–D are at same magnification (scale bar = 1 mm). In E and F female reproductive units are enlarged to show details of their morphology; arrowheads indicate bracts of the innermost whorl of the involucre; scale bars = 1 mm in E and 500  $\mu$ m in F.

(without intermediates) into plants with bisexual reproductive units and plants with entirely female reproductive units. The majority of Western Australian collections contain bisexual plants; these are assignable to *T. submersa* s.str., together with material from south-eastern Australia and Tasmania. All available collections of female plants are restricted to a small area around Midland in the neighbourhood of Perth. The female plants have eight (rarely nine) involucre bracts per reproductive unit (Fig. 2E–F). In bisexual units of *T. submersa* s.str., bract number varies from four to eight, but typically there are four bracts.

Finally, female plants produce highly characteristic elongate fruits with maximum width in the upper part of the fruit (Fig. 3A–B), and elongate seeds with very regular sculpturing of the exotestal cells, which are rectangular in outline and pronouncedly transversely elongated (Fig. 3H). This represents a different morphology from fruits (Fig. 3C–D) and especially from seeds (Fig. 3I) produced by bisexual plants (i.e., *T. submersa* s.str.). Seeds of *T. submersa* s.str. (i.e., bisexual plants) display a honeycomb surface pattern, and are morphologically closer to seeds of south-western Australian *T. bibracteata* (Fig. 3G) than



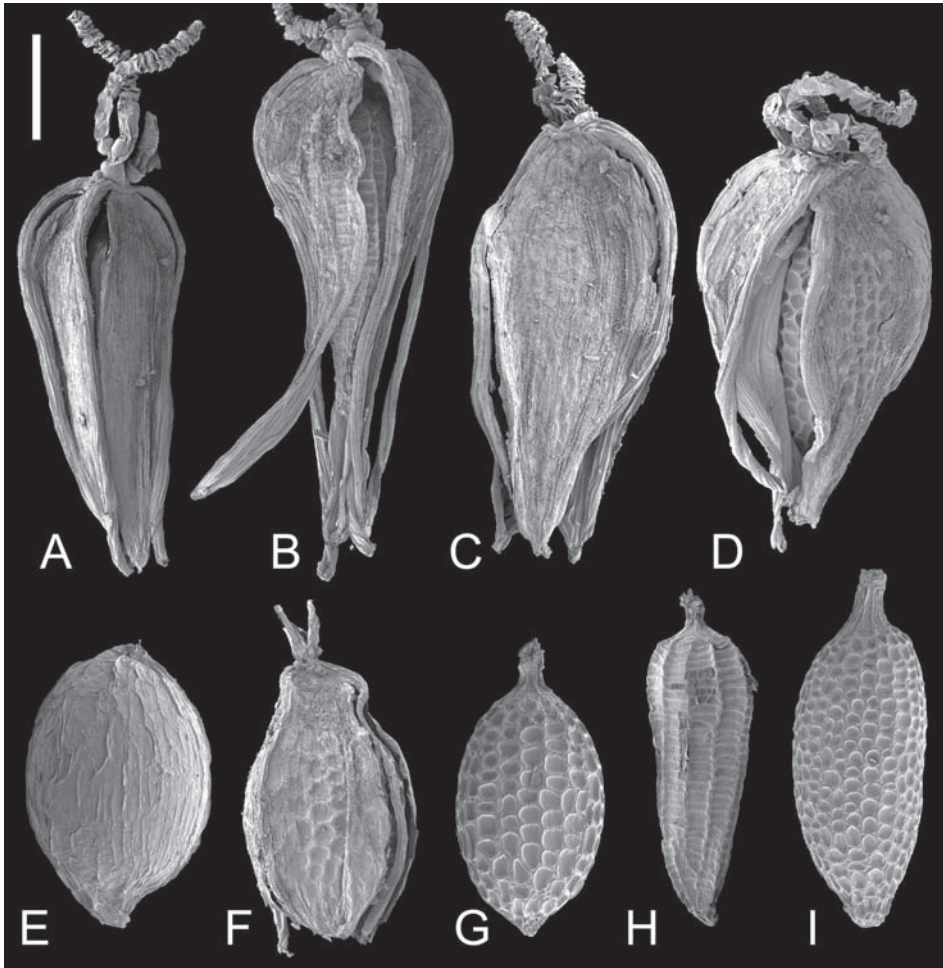


Fig. 3. SEM fruits (A–F) and seeds (G–I) of *Trithuria* spp. from south-west Western Australia. Fruits: A–B, *Trithuria occidentalis* (Morrison s.n., 22 Nov. 1899, K); C–D, *T. submersa* (McCallum Webster 640, 21 Nov. 1980, PERTH); E, *T. austinensis* (Keighery & Gibson s.n., 23 Feb. 1999, PERTH); F, *T. bibracteata* (Kelly 90/50, 31 Oct. 1990, PERTH). Seeds (vouchers as before): G, *T. bibracteata*; H, *T. occidentalis*; I, *T. submersa*. All images are at the same magnification. Scale bar = 200  $\mu$ m.

to seeds produced by female plants (i.e., *T. occidentalis*). Since female plants occupy a restricted area, and differ from bisexual plants in morphological characters other than unisexuality, we believe that *T. occidentalis* cannot be synonymized with *T. submersa*. Cooke (1987) concluded that *T. occidentalis* intergrades with *T. submersa*, but he apparently focused mainly on bract number per reproductive unit, which is indeed variable. However, other characters are sufficiently stable to readily distinguish the two taxa.

*Hydatella dioica* (male plants) was described from the same locality (Midland Junction), where *T. occidentalis* has been repeatedly collected. Similar male plants, also identified by Cooke as *H. dioica*, have been collected in this area. One specimen at Kew actually represents a mixed collection of *H. dioica* (male) and *T. submersa* s.l. (female). It is scarcely credible that two closely related dioecious plant species with annual growth form and abun-

dant seed production on female plants occur together in the same localities, and that only male specimens have been collected from one species and only female from the other. One possible interpretation is that *T. occidentalis* is apomictic, like the New Zealand species *Hydatella inconspicua*, which consists mostly of female individuals (Hamann, 1976). However, this explanation is unlikely because we found normal pollen and pollen tubes growing on stigmas of *T. occidentalis*. Unfortunately, pollen morphology is so uniform in Hydatellaceae that comparison with pollen of *H. dioica* provides no helpful interspecific information.

Finally, we found two individuals of *H. dioica* (male plants) still remaining in organic association with the seed coat of the seeds that gave rise to them. The seed coat has sculpturing that is typical of *T. occidentalis* and unique to this species, at least among Australian Hydatellaceae. Thus, we consider *H. dioica* to represent male plants of *T.*

*occidentalis*. The name *T. occidentalis* has nomenclatural priority against *H. dioica*. *Hydatella dioica* is therefore a synonym of *T. occidentalis*. If we accept this synonymization, then the species shows unusually strong sexual dimorphism. Female reproductive units of *T. occidentalis* typically possess eight involucre bracts, whereas male reproductive units (of plants formerly classified as *H. dioica*) possess only two bracts. The bracts of male plants (*H. dioica*, Fig. 2A) are significantly longer than those of females (Fig. 2D). Incidentally, anthers of *T. occidentalis* (= *H. dioica*) are ca six times longer than those of *T. submersa* s.str., making the differences between these species significant. Another closely related species, *T. bibracteata*, which like *T. occidentalis* and *T. submersa* has dehiscent fruits (Fig. 3F) and sculptured seeds (Fig. 3G), also possesses very short anthers (Fig. 2C).

Apart from *T. occidentalis* (syn. *H. dioica*), we recognize another dioecious species endemic to south-west of Western Australia, *T. austinensis* sp. nov. (Figs 3E, 4). Its male plants are extremely similar to male plants of *T. occidentalis* (*H. dioica*), so some specimens were previously identified as *Hydatella dioica*. However, female plants of *T. austinensis* are very different from those of *T. occidentalis*. Female reproductive units have four (rarely three) bracts in *T. austinensis* (Fig. 4C) and eight bracts in *T. occidentalis* (Fig. 2E–F). Also, *T. austinensis* has indehiscent fruits (Fig. 3E) and unsculptured seeds. A combination of characters such as dioecy, indehiscent

fruits, and male reproductive units with typically two bracts (Fig. 4, right) distinguishes *T. austinensis* from all other members of Hydatellaceae discovered so far. This identification of the male plants of *T. austinensis* is supported by the co-occurrence of male and female plants of this morphology in several localities and by field observations (T.D. Macfarlane). It could be suggested that male plants that we assign to *T. austinensis* in fact belong to *T. occidentalis* (*H. dioica*). However, *T. austinensis* has a much wider distribution range than *T. occidentalis*, and occurs in several localities where female plants of *T. occidentalis* have not been recorded (e.g., Austin Bay). Seed germination experiments at Kew showed that seeds from female plants of *T. austinensis* can produce male plants of the morphology discussed above (P.J. Rudall, M.M. Ramsay, D.D. Sokoloff, work in progress).

Indirect support for our concepts of *T. occidentalis* and *T. austinensis* (i.e., pronounced dimorphism between male and female units of the same species and close similarity between male plants of different species) comes from examination of two herbarium collections from the northern part of Australia (Figs 5–6): *I.D. Cowie* 5934 (DNA-D0125097) and *J.H. Willis* s.n. (MEL-1586286). Both collections were annotated by D.A. Cooke as *Trithuria polybracteata*, but this name was not published. The samples are remarkable in having female reproductive units with numerous (always more than eight) narrow involucre bracts (Figs 5C, 6E–F). This feature has not

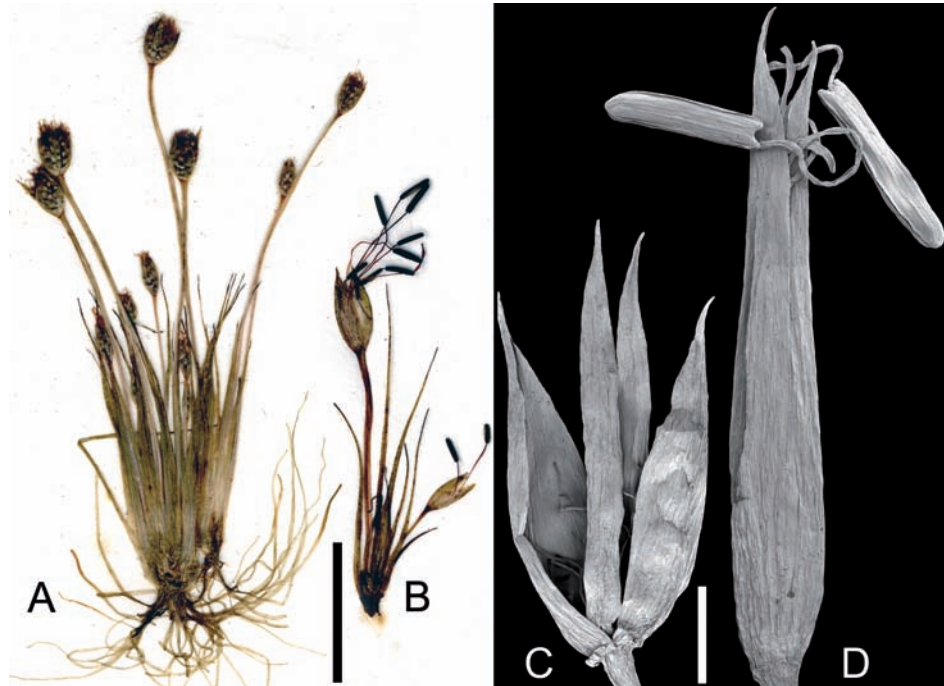


Fig. 4. *Trithuria austinensis* (A, B — from holotype, Gibson & Lyons 2387, PERTH; C, D — from Keighery & Gibson s.n., 23 Feb. 1999, PERTH). A, entire female plant; B, entire male plant. Common scale bar for A and B = 1 cm. C, involucre of female reproductive unit (SEM); D, male reproductive unit, some anthers abscised (SEM). Common scale bar for C and D = 1 mm.

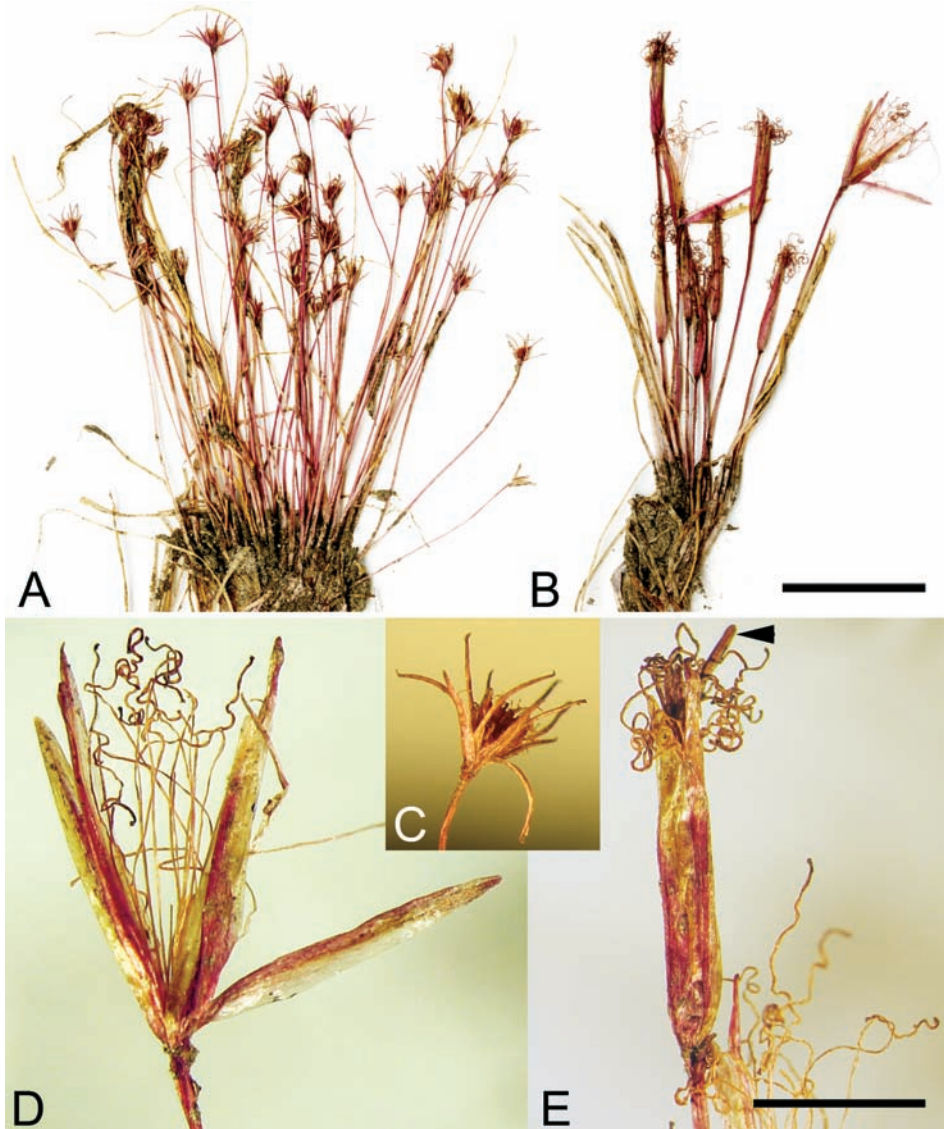


Fig. 5. *Trithuria cookeana* (Cowie 5934, DNA). A, entire female plant; B, entire male plant; C, female reproductive unit; D, postanthetic male reproductive unit, all anthers abscised; E, male reproductive unit with anther of last-formed stamen exposed (arrowhead), while all other anthers are abscised. A and B at same magnification, scale bar = 1 cm. C–E at same magnification, scale bar = 3 mm.

been recorded previously in any other member of Hydatellaceae. Even more interestingly, both samples contain only unisexual plants. Each sample has several female (Figs 5A, 6A–C) and one or several male (Figs 5B, 6D) individuals. (One sample is annotated by the collector as having a plant with male and female inflorescences, but our closer inspection clearly showed the absence of an organic connection between male and female plants; these were merely cemented together by dried soil.) Male and female plants of each sample show similar differences. The involucral bracts of the male reproductive units (Figs 5D, 6G) are much longer, wider, and fewer in number than the female reproductive units. This is the same type of dif-

ference as observed between *H. dioica* and *T. occidentalis* (Fig. 2A, D), which gives indirect support to taxonomic identity between *H. dioica* and *T. occidentalis*.

The collections *I.D. Cowie 5934* and *J.H. Willis s.n.* cannot be assigned to any species of Hydatellaceae thus far described. They clearly differ from the dioecious *T. occidentalis* and *T. austinensis* in possessing more numerous involucral bracts (5–9 rather than 2[–4] in male reproductive units and 10–30 instead of [3]4–9[–12] in female reproductive units). Smooth or almost smooth seeds (Figs 7E, 8D) are strongly dissimilar to the regularly sculptured seeds of *T. occidentalis* (Fig. 3H). In addition, *I.D. Cowie 5934* and *J.H. Willis s.n.* differ from each other in fruit morphology.



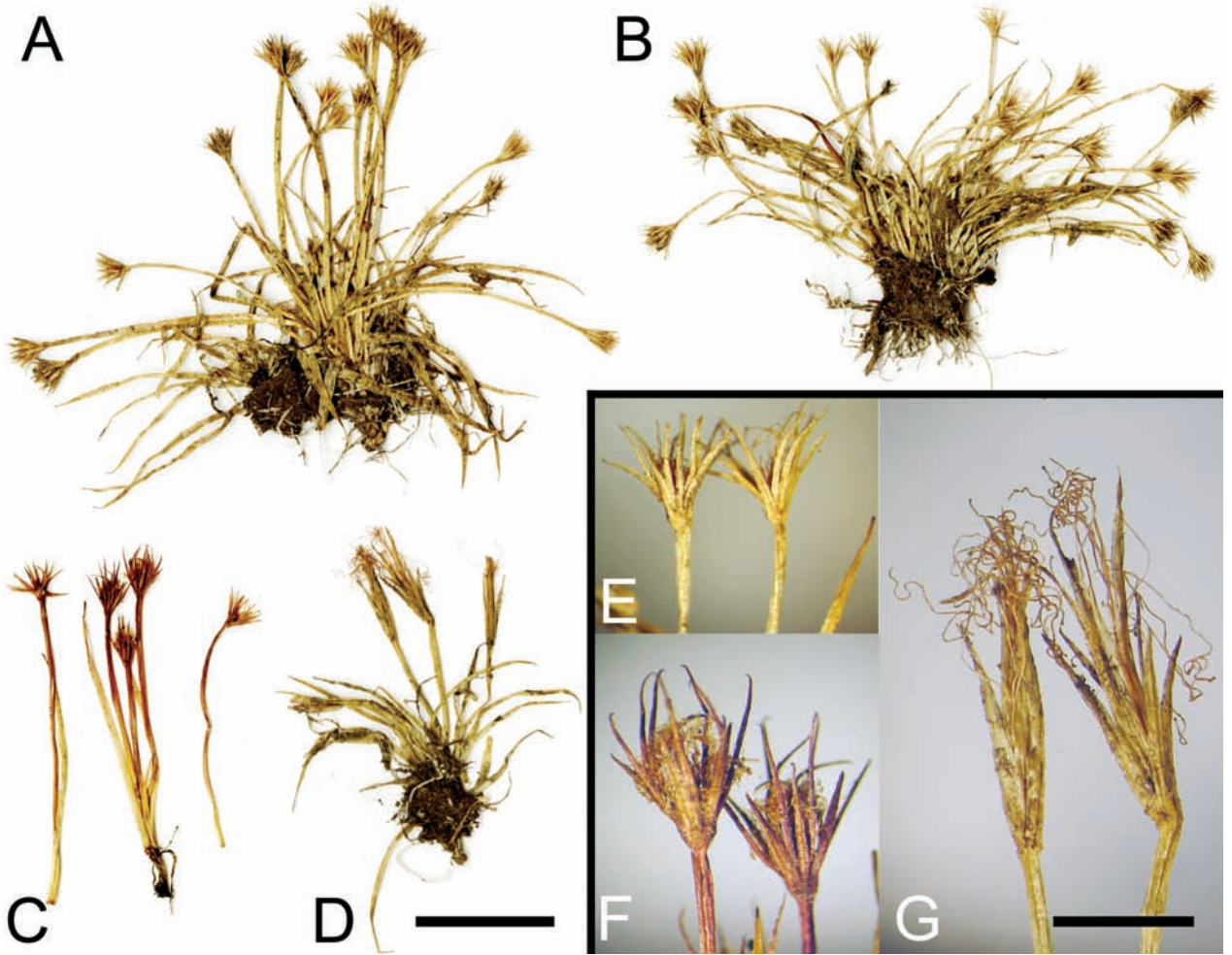


Fig. 6. *Trithuria polybracteata* (Willis s.n., 26 May 1984 — MEL). A–C, female plants; D, male plant; E–F, female reproductive units; G, male reproductive units, the anthers abscised. A–D at same magnification, scale bar = 1 cm. E–G at same magnification, scale bar = 3 mm.

Fruits of *I.D. Cowie 5934* are indehiscent, with a papillate surface and without distinct longitudinal ribs (Fig. 7). Fruits of *J.H. Willis s.n.* lack papillae, but possess three clearly defined longitudinal ribs that separate from the rest of the pericarp when the fruit dries up (Fig. 8). We consider these to be important differences and regard *I.D. Cowie 5934* and *J.H. Willis s.n.* as two new species. We describe *J.H. Willis s.n.* under the name *Trithuria polybracteata* and *I.D. Cowie 5934* as *Trithuria cookeana* sp. nov.

The most recent monographic revision of Australian Hydatellaceae (Cooke, 1987) recognized only one species—*Trithuria lanterna*—for the northern part of Australia. *Trithuria lanterna* is a species with bisexual reproductive units. According to Cooke (1987), the species can be mainly recognized by indehiscent fruits and reproductive units that always bear two involucre bracts. However, our data show that many specimens of *T. lanterna* (including the holotype) possess three or four involucre

bracts (Fig. 9A) while others have two bracts (as illustrated in Rudall & al., 2007). This difference is probably not of great taxonomic significance because plants with different bract number are similar in other respects. In some specimens (e.g., *Johnson 4597*, DNA) bract number was variable between two and four. Note also the bract number was indicated as “about four” in the first description by Cooke (1981), but changed to two in the later revision (Cooke, 1987). Rudall & al. (2007) demonstrated fruit dehiscence in one accession of *T. lanterna*. Now we have been able to examine fruits from many accessions of this species (Appendix). They all possess dehiscent fruits. Fruits and seeds of *T. lanterna* are generally similar to those of *T. polybracteata*, though seeds of *T. lanterna* are completely smooth (Fig. 9B–E) while those of *T. polybracteata* are slightly sculptured (Fig. 8D). More SEM data is certainly needed to understand whether the difference in sculpturing is stable.

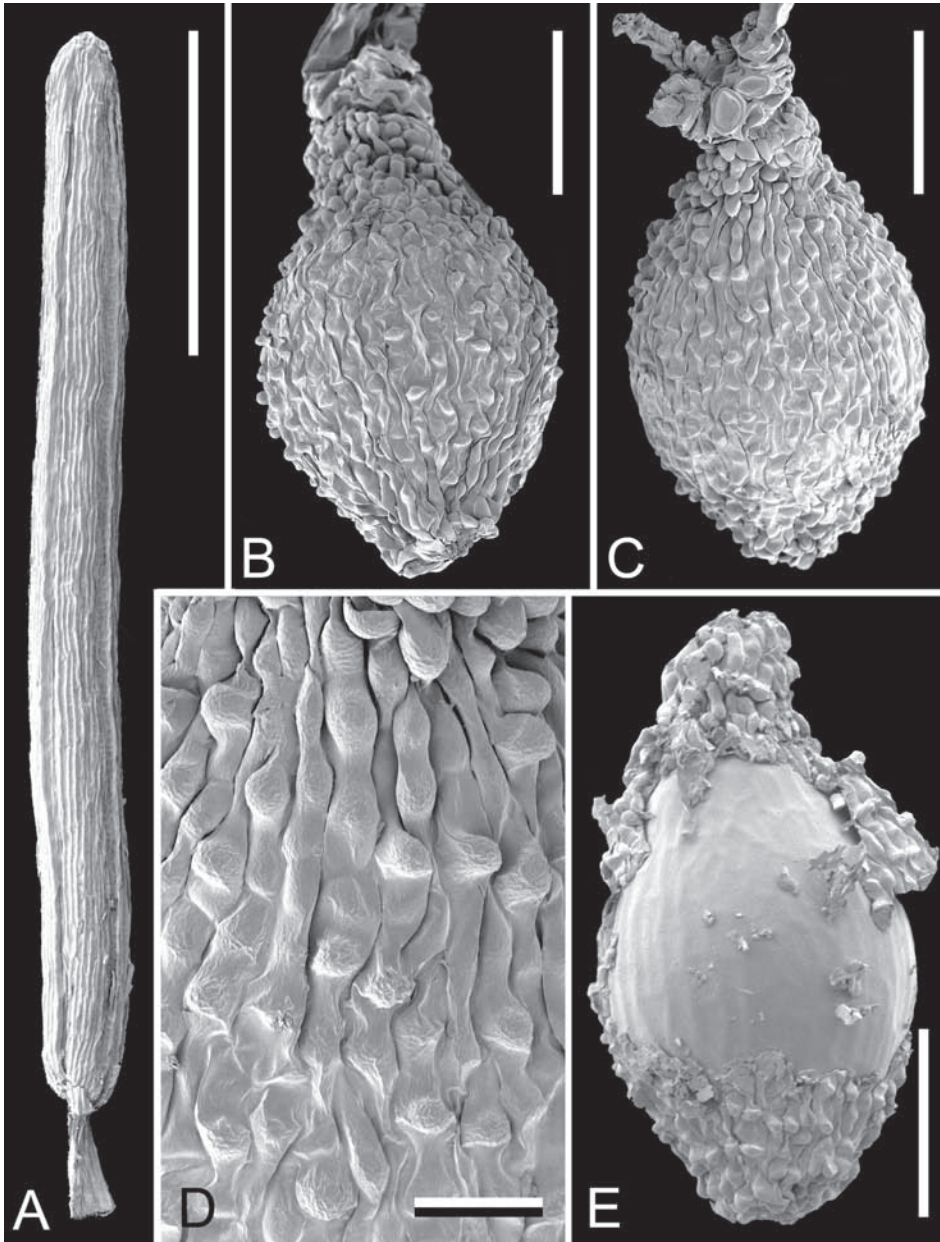


Fig. 7. *Trithuria cookeana* (Cowie 5934, DNA), SEM. A, anther; B–C, fruits; D, detail of pericarp surface; E, fruit with pericarp partly removed to show smooth seed surface. Scale bars: A = 500  $\mu$ m; B, C, E = 100  $\mu$ m; D = 25  $\mu$ m.

Examination of herbarium collections from the northern part of Australia has revealed a fourth species of Hydatellaceae in this region. We describe this species as *Trithuria cowieana* sp. nov. It is known to us from three different localities, all in the north-western part of the Northern Territory. *Trithuria cowieana* is a cosexual plant with always bisexual reproductive units and four to seven involucre bracts (Fig. 10). It usually possesses long peduncles of reproductive units, being different in this respect from *T. lanterna*. In general appearance, plants of *T. cowieana* resemble the most common form of *T. submersa*

(there is also a less common form of *T. submersa* with very short peduncles). However, they have entirely different fruit morphology. Fruits of *T. cowieana* are indehiscent, bear no longitudinal ribs, and are densely covered by papillae (Fig. 10). In fruit structure, *T. cowieana* resembles *T. cookeana* (Fig. 7), though papillae are less dense in *T. cookeana*. The presence of papillae is so far a unique feature of these two species, not previously reported in any species of Hydatellaceae; we have confirmed absence of papillae in ample material of all other collections listed in the Appendix. In spite of the similarity in fruit structure, *T. cowieana* differs



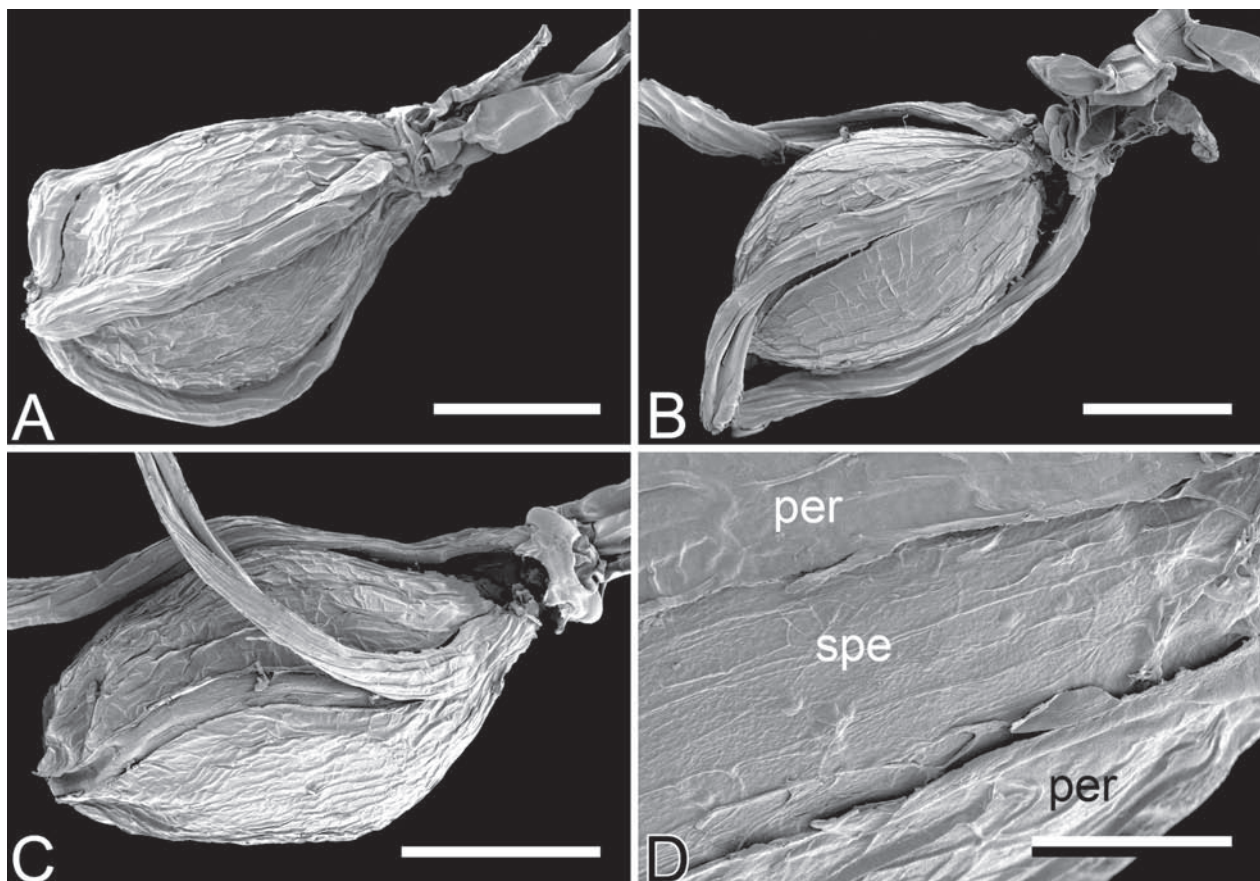


Fig. 8. *Trithuria polybracteata* (Willis s.n., 26 May 1984, MEL), SEM. A, fruit before dehiscence; B–C, dehiscent fruits; D, details of pericarp (per) and spermoderm (spe) surface. Scale bars: A–C = 100  $\mu$ m, D = 20  $\mu$ m.

sufficiently from *T. cookeana* to be recognized as a distinct species. The main diagnostic differences of *T. cowieana* are that reproductive units are always bisexual, with involucre bracts less numerous than in female units of *T. cookeana* and much shorter than in male units of *T. cookeana*. Besides, *T. cowieana* has obtuse or almost obtuse (not acute) involucre bracts and shorter anthers (0.4–0.5 mm long in *T. cowieana*; 1.7–1.9 mm in *T. cookeana*, Fig. 7A).

## ONE GENUS INSTEAD OF TWO

Recognizing four new species of Hydatellaceae and the revised concept of *T. occidentalis* reveals considerable problems in distinguishing *Hydatella* as a genus separate from *Trithuria*. As outlined in the Introduction, *Trithuria* was traditionally characterized by fruits with three longitudinal ribs, usually dehiscent, and by bisexual reproductive units. *Hydatella* was characterized by indehiscent non-ribbed fruits and unisexual reproductive units. Our current data show absence of a correlation between these two characters (Table 1). *Trithuria occidentalis* and *T. submersa* possess generally similar fruits and seeds but

differ in the sexuality of their reproductive units. *Trithuria cookeana* and *T. cowieana* share generally similar fruit structure but again differ in sexuality of reproductive units. The third species pair in this equation is *T. lanterna* and *T. polybracteata*. We therefore combine all species of Hydatellaceae into a single genus, *Trithuria*, which has nomenclatural priority over *Hydatella*.

An alternative would be to recognize two genera based on fruit structure alone, and place all species with indehiscent fruits in *Hydatella*. However, we consider this to be an unnatural distinction, though ultimately molecular phylogenetic studies of Hydatellaceae will show whether *Hydatella* is monophyletic in such a circumscription. Even if all the species with indehiscent fruits formed a clade, we do not recommend their segregation as a distinct genus because there is no clear correlation between this and any other morphological character. Cooke (1981, 1983, 1987) nicely demonstrated a morphological series from typical indehiscent fruits of *Hydatella* to actively dehiscent fruits of *Trithuria submersa*. Fruits of *T. submersa* (and *T. occidentalis*) dehisce to release the seed, which is therefore a diaspore in these species. This correlates with the regularly sculptured seeds of *T. occidentalis* and *T. submersa*.



Fig. 9. *Trithuria lanterna* (Johnson 4555, DNA), SEM. A, entire reproductive unit with four involucral bracts (one bract on reverse side not visible; arrowheads = visible bracts); B, reproductive unit with two of four bracts removed; C, stipitate fruits; D, dehiscent fruit, side view; E, dehiscent fruit, view from base. Scale bars: A = 1 mm, B = 500  $\mu$ m, C = 250  $\mu$ m, D–E = 100  $\mu$ m.

Fruits of *T. bibracteata* are less actively dehiscent, though its seeds are also sculptured. Fruits of *T. lanterna* are only tardily dehiscent (Cooke considered them indehiscent), and do not release seeds because only longitudinal ribs separate from the fruit during dehiscence, while the rest of the pericarp remains with the seed. The same feature occurs in *T. polybracteata*. Seed of *T. lanterna* is smooth, closely resembling the *Hydatella* seed type. Thus, *T. lanterna* and *T. polybracteata* provide a morphological (and ecological) link between fruit and seed character set of typical *Hydatella* and typical *Trithuria*. We consider it unnatural to distinguish *Hydatella* from *Trithuria* on the basis of fruit structure alone.

Hutchinson (1959) earlier proposed combining *Hydatella* with *Trithuria* under the name *Juncella* F. Muell. ex Hieron. However, the name *Juncella* is a later synonym of *Trithuria* and thus illegitimate. His proposal was subsequently rejected partly on this basis, and partly on the basis of the perceived distribution of the two critical characters, fruit structure and sexuality of reproductive units (e.g., Edgar, 1966). Our data, based on examination of a broader species range, now uphold this combination.

Edgar (1966) reported that *Hydatella* has 5–10 unequal, undivided “styles” (i.e., stigmatic hairs), but that *Trithuria* has 2–3 equal, often bifid “styles”. However, we have never seen bifid stigmatic hairs in Hydatellaceae.



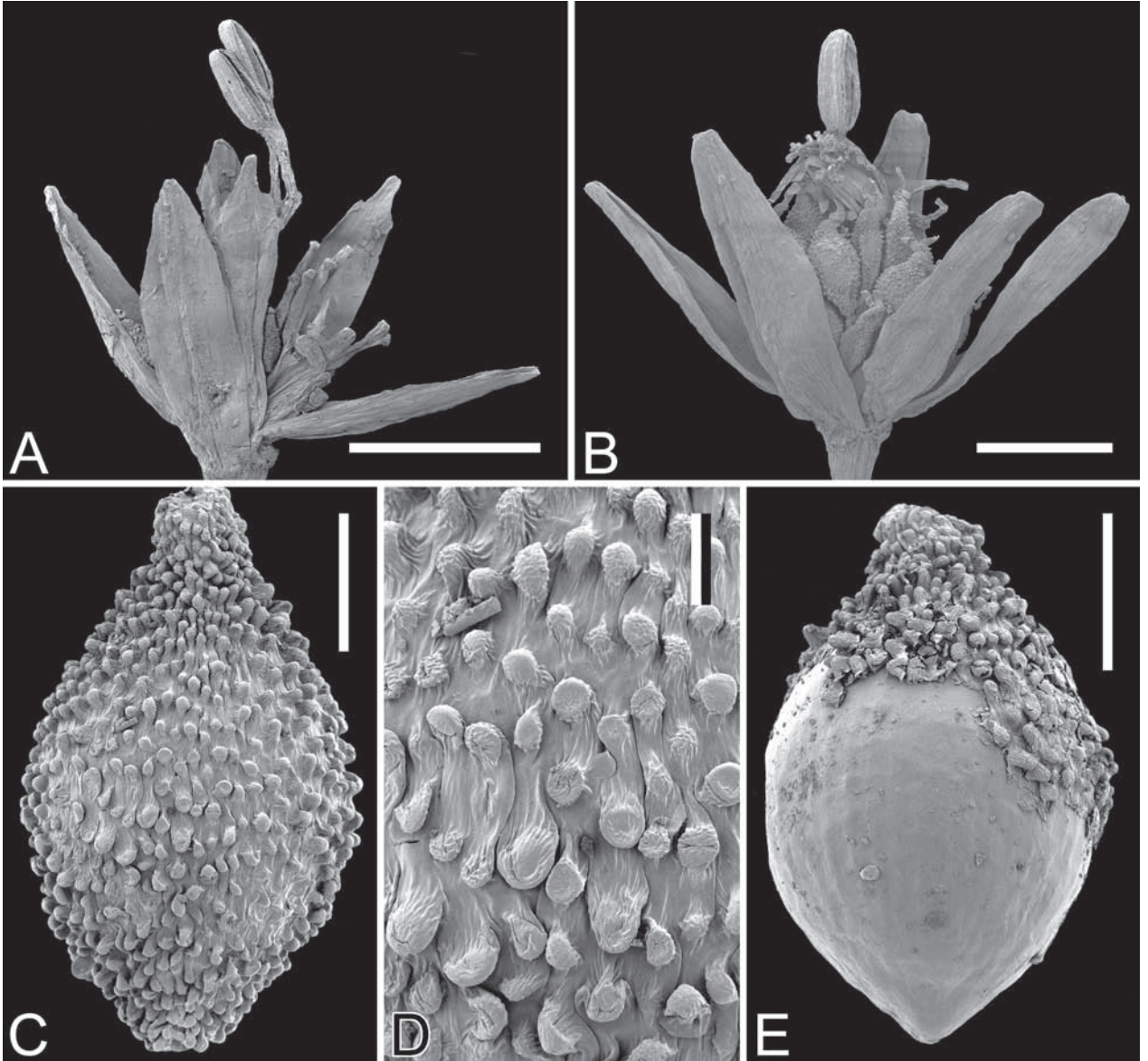


Fig. 10. *Trithuria cowieana* (SEM). A, postanthetic reproductive unit with two stamens, most fruits abscised; B, reproductive unit with one stamen; C, fruit; D, detail of pericarp surface; E, fruit with pericarp partially removed to show smooth seed surface. All figures except A from *Michell 3863* (DNA); A from *Cowie & Jacka 9995* (DNA). Scale bars: A = 1 mm, B = 500  $\mu\text{m}$ , C, E = 100  $\mu\text{m}$ , D = 25  $\mu\text{m}$ .

Other authors (e.g., Mabberley, 1987) highlighted a difference in the number of stigmatic hairs between the two genera, but Cooke (1987) indicated overlapping ranges of stigmatic hairs (4–10 in *Hydatella* versus 2–6 in *Trithuria*). Rudall & al. (2007) demonstrated the absence of substantial differences in stigma morphology between *Trithuria* and *Hydatella*.

To summarise, we distinguish a single genus of Hydatellaceae, *Trithuria*, which consists of one species in New Zealand, ten species in Australia (Fig. 11) and one species in India.

## EVOLUTIONARY SIGNIFICANCE OF DIOECY IN HYDATELLACEAE

Current knowledge on phylogenetic placement of Hydatellaceae does not clarify whether the ancestors of the family were dioecious or cosexual. The molecular analysis of Saarela & al. (2007) indicated Cabombaceae and Nymphaeaceae as the closest relatives of Hydatellaceae. However, it is not clear whether *Amborella* is sister to all other angiosperms (Fig. 1), or sister to the clade that contains Hydatellaceae, Nymphaeaceae and Cabombaceae. Sev-



Table 1. Main diagnostic characters of *Trithuria* species.

Characters	<i>Trithuria filamentosa</i>	<i>Trithuria inconspicua</i>	<i>Trithuria australis</i>	<i>Trithuria austinensis</i>	<i>Trithuria lanterna</i>	<i>Trithuria polybracteata</i>	<i>Trithuria cowieana</i>	<i>Trithuria cookeana</i>	<i>Trithuria bibracteata</i>	<i>Trithuria occidentalis</i>	<i>Trithuria submersa</i>
Growth form: Annuals / Perennials	P	P	A	A	A	A	A	A	A	A	A
Reproductive units: Unisexual / Bisexual	U (B)	U (B)	U	U	B	U	B	U	B	U	B
Plants Cosexual / Dioecious / Female only	C (F)	F (C)	C	D	C	D	C	D	C	D	C
Bract number											
– in male reprod. units	2–4	2–4	2(–4)	2(–4)		5–9		5–7		2	
– in female units	2–4(5)	2–4	2(–4)	(3)4		12–25(–30)		10–16(–21)		8–9(–12)	
– in bisexual units	4	4			2–4		4–8(–10)		2(4)		(2–)4–8
Outer whorl bract length in female or bisexual reprod. units, mm	2.1–3.8	2.5–4.0	1.8–3.5	2.3–3.9	2.8–6.0	2.0–2.5	1.4–1.8	1.5–1.8	2.5–3.3	1.5–2.2	1.8–2.4
Outer whorl bract length in male reprod. units, mm	3.0–5.0	3.1–5.2	1.8–4.3	4.2–7.2		4.0–4.6		6.0–7.5		5.5–10.1	
Stamen number in repro- ductive unit	4–6	2–8	3–8	3–15	1–2	9–11	1–3	14–17	1–2	8–10	1–5
Anther length, mm	1.5–2.0	1.0–1.2	0.5–1.0	1.6–2.8	0.6–1.0	1.5–2.0	0.4–0.6	1.7–1.9	0.3–0.6	1.7–4.3	0.3–0.6
Stigmatic hair length, mm (max.)	5	1	3	2	2.2	1.5	1.2	1	2	2	1
Papillae on ovary and pericarp	–	–	–	–	–	–	+	+	–	–	–
Fruit dehiscence	–	–	–	–	+	+	–	–	+/-	+	+
Seed release from fruit	–	–	–	–	–	–	–	–	+	+	+
Seed surface: Smooth (or just slightly sculptured) / Clearly Sculptured	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sc	Sc	Sc

eral recent molecular analyses place *Amborella* as sister to Nymphaeaceae plus Cabombaceae, although Hydatellaceae were not included (Soltis & al. 2005, 2007; Qiu & al. 2006). Cabombaceae and Nymphaeaceae have bisexual flowers. *Amborella* is dioecious; one sex bears male flowers, and the other sex bears functionally female flowers and possesses sterile stamens that closely resemble fertile stamens. Thien & al. (2003) suggested that in *Amborella*, functionally female flowers mimic male flowers to attract pollen-feeding insects (see also Endress, 2004). Inflorescence structure is similar in male and female plants of *Amborella* (Buzgo & al., 2004). By contrast, dioecious Hydatellaceae possess strongly dissimilar male and female reproductive units; neither sterile stamens nor sterile carpels were observed.

We have revealed three species pairs that resemble each other in fruit and seed morphology but differ in possessing bisexual reproductive units and cosexuality vs. unisexual reproductive units and dioecy: (1) *T. submersa* and *T. occidentalis*; (2) *T. cowieana* and *T. cookeana*; (3) *T. lanterna* and *T. polybracteata*. A fourth pair of species, which share closely similar fruits, is the cosexual *T. australis*, with unisexual reproductive units, and the dioecious *T. austinensis*. All four dioecious species (*T. occidentalis*, *T. austinensis*, *T. cookeana*, *T. polybracteata*) show a similar range of differences between male and female plants. Several evolutionary scenarios can be hypothesized to explain these data, including the following:

(1) Multiple losses of dioecy: *T. occidentalis*, *T. austinensis*, *T. cookeana* and *T. polybracteata* are derived

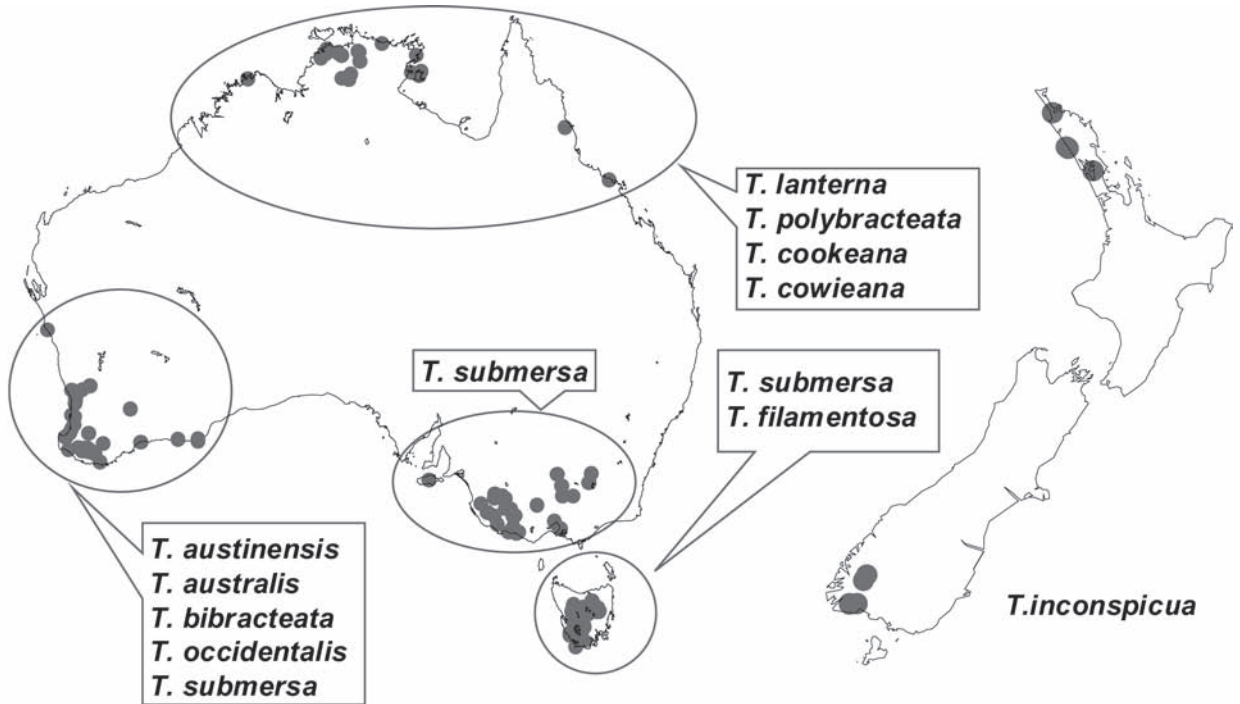


Fig. 11. Distribution maps of *Trithuria* in Australia and New Zealand, based on specimens examined. There is only one species in New Zealand, *T. inconspicua*. Ten species (all endemic) occur in Australia plus Tasmania. The map of Australia and Tasmania shows all examined collections of *Trithuria*.

from a shared dioecious ancestor, and the transition to bisexuality of reproductive units occurred at least three times within Hydatellaceae.

(2) Multiple gains of dioecy: Dioecy is a derived condition within Hydatellaceae, with at least four independent origins of this character state (in *T. occidentalis*, *T. austinensis*, *T. cookeana* and *T. polybracteata*).

(3) Single origin of dioecy, with no reversals. Thus, the similarity between fruits and seeds in the pairs of species resulted from parallel evolution as an adaptation for similar ecological and climatic conditions and/or reproductive strategies.

(4) Single loss of dioecy, with no reversals; dioecy is ancestral for Hydatellaceae. Like the previous scenario, this implies homoplasy in fruit/seed evolution.

These hypotheses are at least partly testable using a more detailed phylogeny of species relationships within Hydatellaceae (see Renner & Won, 2001, for a similar case study). If the species pairs outlined above form sisters on phylogenetic trees, this will reject hypotheses (3) and (4), but additional evidence will be needed to distinguish between hypotheses (1) and (2). Other interpretations may be possible if hybridization proves to be common within Hydatellaceae.

The fact that the differences between male and female plants are similar in all three dioecious taxa (and, in addition, dioecious species have much longer anthers than

their paired cosexual species) means that switches between dioecy and cosexuality implied by hypotheses (1) and (2) occurred due to a similar genetic change. The genetic background of the origin of dioecy in various angiosperm groups has been extensively discussed in the literature (reviewed in Grant, 1999; Renner & Won, 2001; Barrett, 2002). Several scenarios of the evolutionary origins of dioecy have been proposed by various authors, including from gynodioecy, and from monoecy via paradioecy. The “dioecy is primitive” concept has not been sufficiently explored in genetic studies of angiosperms (Meagher, 2007), because the most parsimonious interpretation is that the monoclinal condition is a primitive condition in most angiosperm groups, and probably in angiosperms in general. Endress (2004) pointed out that although many early-divergent angiosperms have functionally unisexual flowers, sterile organs of the opposite sex normally occur in the appropriate location (e.g., staminodes of *Amborella* surrounding carpels), supporting the view that primitive angiosperm flowers were probably bisexual. However, Hydatellaceae do not fit this scheme, if their bisexual reproductive units are compared with flowers. Since Hydatellaceae are close to the root of angiosperms, primitiveness of dioecy in Hydatellaceae cannot be ruled out at this stage. Most extant gymnosperms have unisexual, often dioecious, reproductive units, though some extinct Mesozoic bennettites such as *Williamsoniella* possessed flower-like bisexual cones. Genetic studies of sex

determination in Hydatellaceae will help to resolve these questions.

Regardless of which scenario proves correct, Hydatellaceae offer an excellent model system for studying evolutionary transformations (and their molecular genetic background) between unisexual and bisexual reproductive structures in early-divergent angiosperms. This is highly significant because bisexuality of reproductive structures (flowers) is a key innovation of angiosperms, and the origin of bisexuality has been the focus of discussion for more than a century of research on the origin of angiosperms. Several genetically-based hypotheses on the evolutionary origins of bisexual angiosperm flowers have recently been proposed (e.g., Fröhlich & Parker, 2000; Theißen & Becker, 2004; Baum & Hileman, 2006); the bisexual *flower* is derived from unisexual *nonflowers* in these theories. The “nonflowers” (reproductive units) of Hydatellaceae could usefully inform this discussion. The precise morphological nature of reproductive units of Hydatellaceae merits further investigation (Rudall & al., 2007). In any case, the reproductive units of Hydatellaceae resemble “normal” flowers of other early-divergent angiosperms at the level of morphological synorganization of parts.

## TAXONOMIC TREATMENT OF HYDATELLACEAE IN AUSTRALIA AND NEW ZEALAND

*Trithuria* Hook. f., Fl. Tasman. 2: 78. 1858 – Type: *Trithuria submersa* Hook. f. ≡ *Juncella* F. Muell. ex Hieron. in Engl. & Prantl, Pflanzenfam. 2(4): 15. 1888 (nom. illeg.).  
= *Hydatella* Diels in Bot. Jahrb. Syst. 35: 93. 1904 – Lectotype (designated here): *Hydatella australis* Diels. (≡ *T. australis* (Diels) D.D. Sokoloff & al.).

*Trithuria austinensis* D.D. Sokoloff, Remizowa, T.D. Macfarl. & Rudall, **sp. nov.** – Holotype: AUSTRALIA. Western Australia, Austin Bay Nature Reserve, wetland in NW corner immediately behind beach dune, 32°37' S, 115°47' E, seasonally inundated wetland, grey-brown clay, saline aquatic herbland, wetland connected to sea, 16 Nov. 1995, *N. Gibson & M. Lyons* 2387 (PERTH-04278208) – Paratypes: AUSTRALIA. Western Australia, Cape Arid National Park, ca 1 km SW of summit of Mt Arid, 3 Oct. 2006, *Hopper & Yates* 8681 (K, PERTH); S of Lake Unicup, 17 Jan. 2006, *Hearn s.n.* (K, PERTH); S of Lake Unicup, 20 Dec. 2005, *Hearn s.n.* (K, PERTH); central wetland in Austin Bay Nature Reserve, N of track, ca 3 km S of Yunderup, 32°36'56" S, 115°46'39" E, low lying seasonal wetland, brown sandy clay, species rich

herbland, 29 Oct. 1993, *B.J. Keighery & Gibson* 85 (PERTH); Lake Pindicup, Pindicup Nature Reserve, 34°24'14" S, 116°43'60" E, lake bed and edges, white clay, sedgeland, 23 Feb. 1999, *B.J. Keighery & Gibson s.n.* (PERTH); Tagon Road, Cape Arid National Park, 2 Nov. 1989, *G.J. Keighery* 11926 (PERTH); NE corner junction Old Plains & Mount Roads, Drummond Nature Reserve, ca 10 km W of Bolgart, 31°18'27" S, 116°24'60" E, claypan, shallow water, clay, low shrubland over sedges and aquatic herbs, 9 Nov. 1998, *G.J. Keighery* 15707 (PERTH); Tolkerlup Swamp, Unicup Nature Reserve, ca 64 km ESE of Manjimup, 26 Oct. 2006, *Macfarlane & Tuckett* 3988 (PERTH).

Planta annua, caespitosa, dioica. Unitates reproductivae masculinae bracteis 2(–4) involucretae. Unitates reproductivae foeminae bracteis 4(3) involucretae. Fructus indehiscens pericarpio papillis nullis. *Trithuriae australis* similis, sed planta dioica.

Dioecious annual herb. All leaves basal, linear. Stems and leaves green or reddish. Leaves (10)20–40 mm long. Peduncles (5)10–50 mm long, shorter than, as long as, or longer than leaves. Male plants with two to eight peduncles. Male reproductive units with two (rarely up to four) involucreta bracts and 3–15 stamens. Bracts 4.2–7.2 mm long and 1.0–3.0 mm wide, lanceolate, acuminate, membranous on margin, with a distinct midvein, with base exceeding half of peduncle circumference, so that the first bract encloses the second bract in aestivation. Stamens develop and open in centrifugal succession. Anthers yellow to purple, 1.6–2.8 mm long, with two distinct small appendages at the base (so that the filament is attached to a sinus at the base of the anther). Postanthetic stamen filaments persistent, longer than involucreta bracts, distally often twisted. Female plants with up to 30 peduncles. Female reproductive units mostly with four (rarely three) acuminate, membranous on margin involucreta bracts with a distinct midvein. Two outer bracts different in shape from two (rarely one) inner bracts. The two outer bracts opposite, 2.3–3.9 mm long and 1.0–1.9 mm wide, concave (boat-shaped), base of each bract covers a half of peduncle circumference. Inner bracts (2.3)3–4 mm long and 0.4–0.8 mm wide, flat, narrower and typically longer than the outer bracts; bract base much narrower than a half of peduncle circumference. Seventeen to twenty-two pistils develop in centrifugal succession. Pistils sessile or very shortly stipitate when young (preanthetic?), later developing a very narrow, but long stalk (sometimes almost as long as involucreta bracts). Ovary ca. 0.3 mm long. Stigmatic hairs 1–2 mm long. Fruit 0.4–0.6 mm long, deciduous from persistent stalks, with abscising stigmatic hairs, indehiscent, without distinct ribs, without papillae. Seed with a smooth surface, completely filling fruit.

The species is similar to *T. australis* in annual habit, unisexual reproductive units and indehiscent fruits without



papillae. However, it clearly differs from *T. australis* in being dioecious, and in possessing female reproductive units typically with four involucre bracts (of which the two inner ones are much narrower and slightly longer than the outer ones), and longer anthers (1.6–2.8 mm long, compared to 0.5–1 mm long in *T. australis*). *Trithuria austinensis* can be distinguished from other dioecious species of *Trithuria* in fruit structure and in the morphology of the involucre of female reproductive units. Other dioecious species have either dehiscent or papillose fruits and always more than four involucre bracts in female reproductive units.

*Etymology*: the name *austinensis* is given because some of the collections were made in the Austin Bay Nature Reserve, and because the informal phrase name “*Hydatella* sp. Austin Bay (N. Gibson & M. Lyons 2387)” has been in use in Western Australia (Paczkowska & Chapman, 2000).

*Distribution* map: Fig. 12.

*Trithuria australis* (Diels) D.D. Sokoloff, Remizowa, T.D. Macfarl. & Rudall, **comb. nov.**  $\equiv$  *Hydatella australis* Diels in Bot. Jahrb. Syst. 35: 93. 1904 – Holotype: AUSTRALIA. Western Australia, Hamersley River, Oct. 1901, L. Diels 4983 (B-100258867!; isotype PERTH-01050672!).

= *Hydatella leptogyne* Diels in Bot. Jahrb. Syst. 35: 93. 1904 – Holotype: AUSTRALIA. Western Australia, Hutt River, ca 75 m ü. M., L. Diels 5698 (B-100258859!).

*Distribution*: western and south-western parts of Western Australia (Fig. 12).

*Note*: The name *Hydatella leptogyne* was introduced using a single specimen (holotype). Diels & Pritzel (1904) reported that *H. leptogyne* differs from *H. australis* in possessing longer leaves, female reproductive units fewer than male on a single individual (in contrast, *H. australis* was described as having numerous female reproductive units), purple (rather than pale) anthers and narrow pyriform ovaries. In our experience, leaf length and anther colour are taxonomically relatively uninformative within Hydatellaceae. Our examination of ovary shape in the type specimen of *H. leptogyne* revealed absence of significant differences from *T. australis* (Fig. 13). Since it closely resembles *T. australis* in most available characters, we follow Cooke’s (1987) suggestion, and place *H. leptogyne* in synonymy with *T. australis*. There has been no other collection of Hydatellaceae in the type locality of *H. leptogyne* or its neighbourhood. Since the type specimen of *T. leptogyne* lacks mature fruits and because fruit morphology is so taxonomically important, the identity of *T. leptogyne* will remain tentative until fruiting material is collected.

We assign a recent collection in PERTH that was identified as *H. leptogyne* (Keighery 15707) to *T. austinensis*.

*Trithuria bibracteata* Stapf ex D.A. Cooke in Muelleria 5: 123. 1983 – Type: AUSTRALIA. Western Australia, Boyanup, 15 Oct. 1947, R.D. Royce 2265 (PERTH-01051091!).

*Distribution*: south-western Western Australia (Fig. 12).

*Trithuria cookeana* D.D. Sokoloff, Remizowa, T.D. Macfarl. & Rudall, **sp. nov.** – Holotype: AUSTRALIA. Northern Territory, 24 km SE of Maningrida, 12°07' S, 134°26' E, with *Utricularia* on damp sand at edge of drying swamp, *Melaleuca viridifolia* over story, 22 Aug. 1995, I.D. Cowie 5934 (DNA-D0125097) (see Fig. 14).

Planta annua, caespitosa, dioica. Unitates reproductivae masculinae bracteis 5–7 hyalinis acutis involucretae. Unitates reproductivae foeminae bracteis 10–16(21) acutis involucretae. Fructus indehiscens pericarpio papilloso. Testa laevis nitens.

Habitu *T. polybracteatae* valde similis, sed fructibus indehiscensibus papillosis bene differt.

Dioecious annual herb. All leaves basal, linear. Stems, leaves and bracts of postanthetic plants reddish. The only available male plant has ten peduncles 13–30 mm long. Male reproductive units with five to seven involucre bracts and 14–17 stamens. Bracts 6.0–8.2 mm long (two outer bracts often slightly shorter than inner ones) and 1–1.5 mm wide, without distinct veins or a midrib, with wide base exceeding half of peduncle circumference, so that the first and the second bract enclose other bracts in aestivation. Stamens developing and opening in centrifugal succession. Anthers (measured only in oldest developed stamens) 1.7–1.9 mm long, abscising after anthesis. Postanthetic stamen filaments persistent, longer than involucre bracts, distally twisted. Female plants with numerous (up to 45) peduncles 12–40 mm long. Female reproductive units mostly with 10–16 involucre bracts, but a unit on one specimen examined with 21 bracts. Bracts 1.5–2.6 mm long and 0.15–0.45 mm wide; four (five) outermost bracts slightly wider and shorter than inner ones. Bract base much narrower than in male units (a third to a quarter of the peduncle circumference). Bract apex usually apiculate. Numerous (more than 40) stipitate pistils develop in centrifugal succession. Ovary 0.2 mm long (measured only in latest developed pistils). Stigmatic hairs 3–5, up to 1 mm long (or longer?). Fruits about 0.3 mm long, deciduous from persistent stalks, with abscising stigmatic hairs, indehiscens, without distinct ribs, densely papillose, with papillae more pronounced in the apical part of the fruit. Seed completely filling the fruit, with smooth surface (cells of exotesta not recognizable from the surface).

The species is very similar to *T. polybracteata* in habit, but clearly differs from it in indehiscens papillose fruits.

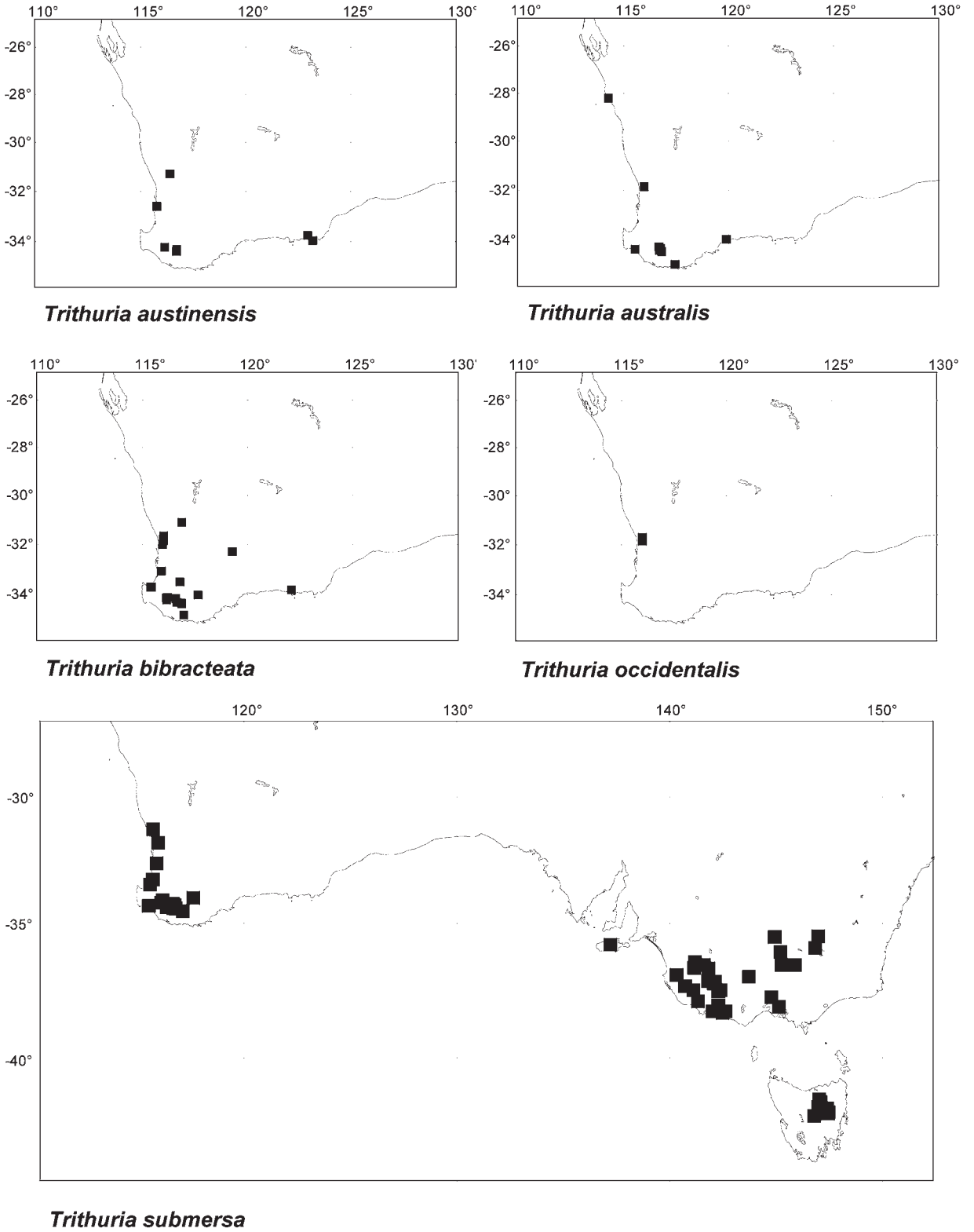


Fig. 12. Distribution ranges of *Trithuria* spp. occurring in the south-west of Western Australia. Of five species that occur in this region, four are endemic.



Fig. 13. Pistils of *Trithuria australis*. Left: SEM of two pistils from *Macfarlane & Hearn TDM 3357* (alcohol-fixed and critical-point dried material). Right: LM of two pistils from type specimen of *Hydatella leptogyne*. Scale bar (common to all images) = 500  $\mu$ m.

*Etymology*: the species is named in honour of David A. Cooke (Adelaide), whose publications opened a new era in understanding species-level diversity of Hydatellaceae.

***Trithuria cowieana*** D.D. Sokoloff, Remizowa, T.D. Macfarl. & Rudall, **sp. nov.** – Holotype: AUSTRALIA. Northern Territory, Nitmiluk National Park, Northern Marrawal Plateau, 13°55'13" S, 132°33'04" E, on seepage flat at edge of swamp, 28 Mar. 2002, *C.R. Michell* 3863 (DNA-D0156956) – Paratypes: AUSTRALIA. Northern Territory, Kakadu NP, just W of Wildman River Creek, 12°49'13" S, 132°00'46" E, in sedge-land/grassland, drainage flat on damp loam, 08 Apr. 2003, *Cowie & Dixon s.n.* (DNA); Wangi Road, near Finnis River crossing, 12°58'41" S, 130°45'35" E, in grassland, seasonally shallowly flooded drainage flat near river, grey loam, 11 May 2004, *Cowie & Jacka* 9995 (DNA).

Planta annua, caespitosa, monoica. Unitates reproductivae omnes hermaphroditae bracteis 4–8(10) obtusis vel subobtusis involucretae. Fructus indehiscens pericarpio papilloso. Testa laevis nitens.

Fructu *T. cookeanae* valde similis, sed unitatibus reproductivis semper hermaphroditis, bracteis obtusis vel subobtusis (nec acutis) antheribusque brevioribus (0.4–0.5 mm nec 1.7–1.9 mm) bene differt.

Cosexual annual herb. All leaves basal, linear, about 0.3–0.4 mm wide and up to 75 mm long. Stems, leaves and bracts of postanthetic plants reddish. Peduncles of reproductive units 2–110 mm long. Reproductive units bisexual, with four to eight (ten) involucreta bracts, 20–40 stipitate pistils and one to three central stamens. Involucreta bracts 1.2–2.3 mm long and (0.2)0.3–0.5(0.6) mm wide, without distinct veins, usually with obtuse apex. When there are

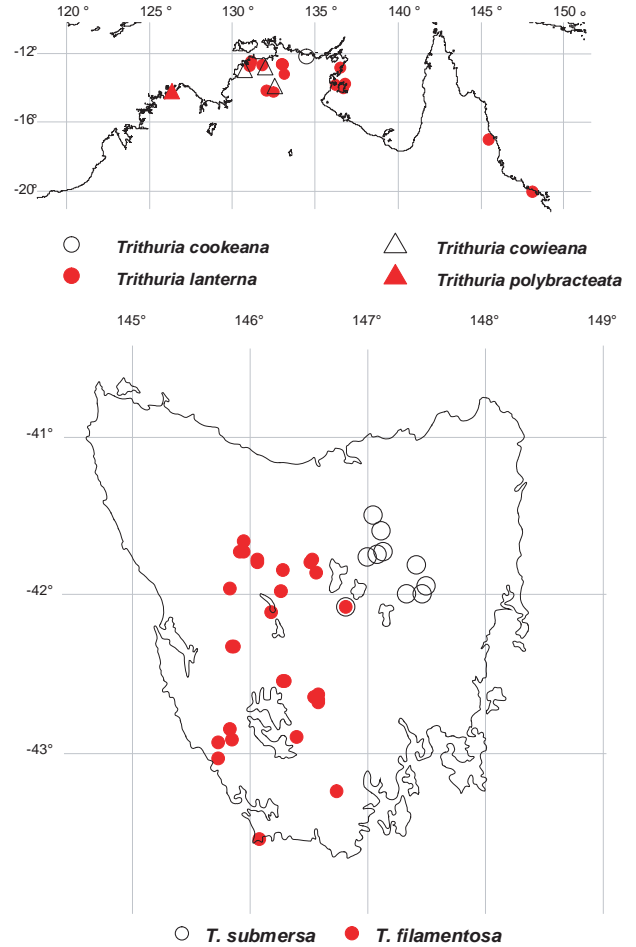


Fig. 14. Above: map of *Trithuria* collections examined from northern Australia. Below: map of *Trithuria* collections examined from Tasmania.

eight involucreta bracts they appear to form two alternating tetramerous whorls, with inner whorl bracts narrower than the outer whorl ones. When there are four involucreta bracts, they can form two dimerous whorls with two inner bracts narrower and longer than the two outer bracts. Stamens filaments red, as long as, slightly shorter or slightly longer than involucreta bracts, persistent after anthesis, distally not twisted. Anthers 0.4–0.6 mm long, yellow with purple strips, abscising or persisting after anthesis. Pistils develop in centrifugal succession. Pistil stalk 0.2–0.3 mm at anthesis, ovary 0.2–0.3 mm long, with 3–6 stigmatic hairs up to 1.2 mm long. Fruits 0.3–0.4 mm long, deciduous from persistent stalks (those as long as or much shorter than involucreta bracts), with abscising stigmatic hairs, indehiscent, without distinct ribs, densely papillose. Seed completely filling the fruit, with smooth surface (cells of exotesta not recognizable from the surface).

The species is similar to *T. cookeana* in fruit morphology, but clearly differs in possessing invariably bisexual reproductive units, obtuse or almost obtuse (not acute) in-



volucral bracts and shorter anthers (0.4–0.5 mm long in *T. cowieana*, compared with 1.7–1.9 mm in *T. cookeana*).

*Etymology*: the species is named in honour of Ian D. Cowie (Palmerston, Northern Territory, Australia), who made many significant collections of Hydatellaceae in Northern Territory.

*Distribution* map: Fig. 14.

***Trithuria filamentosa*** Rodway in Pap. & Proc. Roy. Soc. Tasmania 1897: 48. 1898 ≡ *Hydatella filamentosa* (Rodway) W.M. Curtis in Rec. Queen Victoria Mus. (Tasmania) 50: 5. 1974 – Lectotype (designated here): AUSTRALIA. Tasmania, Broad River, Dec. 1896, *L. Rodway s.n.* (NSW-148485!; isolectotype MEL!).  
*Distribution*: Tasmania (Fig. 14).

***Trithuria inconspicua*** Cheesem., Man. N. Zeal. Fl.: 756. 1906 ≡ *Hydatella inconspicua* (Cheesem.) Cheesem. in Trans. New Zealand Inst. 39: 434. 1906 – Lectotype (designated by Edgar in New Zealand J. Bot. 4: 154. 1966): NEW ZEALAND. Lake Ngatu, 1 Jan. 1902, *H. Carse s.n.* (CANTY).  
*Distribution*: New Zealand (Fig. 11).

***Trithuria lanterna*** D.A. Cooke in Muelleria 4: 301. 1981 – Holotype: AUSTRALIA. Northern Territory, South Bay, Bickerton Island, in the Gulf of Carpentaria, 14 June 1948, *R.L. Specht 566* (MEL-1517931!; isotypes BRI-256564!, PERTH-01051105!).

*Distribution*: Australia: Queensland, Northern Territory (Fig. 14) and probably northern part of Western Australia.

***Trithuria occidentalis*** Benth., Fl. Austral. 7: 199. 1878 ≡ *Juncella occidentalis* (Benth.) F. Muell. ex Hieron. in Engl. & Prantl, Pflanzenfam. 2(4): 14. 1888 – Lectotype (designated here): AUSTRALIA. Western Australia, Swan River, *J. Drummond 689* (K!; isotypes: B! BM!, K!, MEL-536061!, PERTH-01051113!).

= *Hydatella dioica* D.A. Cooke in Muelleria 5: 124. 1983 – Holotype: AUSTRALIA. Western Australia, Midland Junction, 16 Nov. 1898, *A. Morrison s.n.* (PERTH-01051075!; isotype E!).

*Distribution*: Western Australia, apparently restricted to small area to north-east from Perth (Fig. 12).

***Trithuria polybracteata*** D.A. Cooke ex D.D. Sokoloff, Remizowa, T.D. Macfarl. & Rudall, **sp. nov.** – Holotype: AUSTRALIA. Western Australia, North Kimberley, Vansittart Bay, Pauline Bay Area, west side of mangrove inlet, 1.5 km N from camp at mouth of freshwater creek, 14°12'30" S, 126°22' E, in spring with *Utricularia*, 26 May 1984, *J.H. Willis s.n.* (MEL-1586286) (see Fig. 14).

Planta annua, caespitosa, dioica. Unitates reproductivae masculinae bracteis 5–9 hyalinis acutis involucretae. Unitates reproductivae foeminae bracteis 12–25(30) acutis involucretae. Fructus dehiscens pericarpio papillis nullis. Testa sublaevis.

Fructu *T. lanternae* similis, sed unitatibus reproductivis homogamis dioicis (nec hermaphroditis), bracteis multioribus (5–30 nec 2–4) scapis longioribus antheribusque longioribus bene differt.

Dioecious annual herb. All leaves basal, linear, about 0.5 mm wide and up to 15 mm long. The only available complete male plant with four peduncles 10–11 mm long (also one incomplete male plant and separate male peduncles were available). Male reproductive units with five to nine lanceolate involucre bracts and 9–11 stamens. Outer bracts shorter than inner ones. In reproductive unit with nine bracts, the bracts apparently forming three alternating trimerous whorls; outer whorl bracts 4.0–4.6 mm long, middle whorl bracts 4.5–5.0 mm long and inner whorl bracts 6.8–7.2 mm long. In other available male reproductive units, the first whorl also apparently trimerous. Postanthetic stamen filaments persistent, longer than involucre bracts, distally twisted. Anthers 1.5–2 mm long, abscising after anthesis. Female plants with up to 20 peduncles 15–27 mm long. Female reproductive units with 12–25(30) narrowly lanceolate acute involucre bracts and numerous stipitate pistils. Outer bracts shorter than inner ones. Outermost bracts (usually three or four) 2–2.5 mm long and 0.3–0.4 mm wide, innermost bracts 2.2–4 mm long and 0.1–0.3 mm wide. Anthetic pistils not observed. Fruits 0.2–0.25 mm long, deciduous from persistent stalks, with more or less abscising stigmatic hairs up to 1.5 mm long. Pericarp with three equally-spaced narrow longitudinal ribs, which twist and separate from the rest of mature fruit. The remaining part of the pericarp very thin, membranous, with margins of epidermal cells more or less recognizable from the surface, remaining appressed to the seed and not separating from it; outer anticlinal walls of epidermal cells smooth, papillae absent. Seed completely filling fruit, with seed surface similar to that of pericarp, though anticlinal walls of exotesta cells very slightly sculptured.

*T. polybracteata* is similar in fruit morphology to *T. lanterna*, but clearly differs from it in unisexual (rather than bisexual) reproductive units, dioecy, more numerous bracts (5–30 in *T. polybracteata*, 2–4 in *T. lanterna*), longer anthers and longer peduncles.

***Trithuria submersa*** Hook. f., Fl. Tasmania 2: 79. 1858 ≡ *Juncella occidentalis* (Benth.) F. Muell. ex Hieron. in Engl. & Prantl, Pflanzenfam. 2(4): 14. 1888 – Lectotype (designated here): AUSTRALIA. Tasmania, Macquarie River, bottom of lagoon, 6 Nov. 1845, *R.C. Gunn 2014* (K!).

*Distribution:* south-eastern part of South Australia, southern part of New South Wales, Victoria, Tasmania, south-western Western Australia (Fig. 12). This is the only species occurring in New South Wales, Victoria and South Australia. It co-occurs with *T. filamentosa* in Tasmania, but it seems that areas of the two species are only slightly overlapping here (Fig. 14). In south-west of Western Australia, *T. submersa* co-occurs with four other species, often growing in the same localities.

**KEY TO TRITHURIA SPECIES IN AUSTRALIA AND NEW ZEALAND**

- 1 Perennial plants with rhizomes growing in permanent lakes or permanently wet habitats. Reproductive units mostly unisexual (only very rarely bisexual), with two to five (typically four) involucral bracts. Fruits indehiscent, without papillae. Seeds not sculptured. Anthers 1–2 mm long . . . . . 2
- 1 Annuals without rhizomes, often growing in seasonally wet habitats or temporary pools . . . . . 3
- 2 Plants often (not always) with some male reproductive units. Anthers 1.5–2 mm long. Stigmatic hairs long (longer ones 2–5 mm long), well recognizable at anthesis but abscising in fruits . . . *T. filamentosa*
- 2 Most plants entirely female, without male reproductive units. Stamens, when present, with anthers 1–1.2 mm long. Stigmatic hairs short (less than 1 mm long) . . . . . *T. inconspicua*
- 3(1) Reproductive units unisexual . . . . . 4
- 3 Reproductive units bisexual . . . . . 8
- 4 Plants dioecious . . . . . 5
- 4 Plants cosexual, with male and female reproductive units on the same plant. Reproductive units (both male and female) usually with two bracts, rarely some units with up to four bracts. Anthers 0.5–1 mm long. Fruits indehiscent, without papillae. . . . . *T. australis*
- 5 Male reproductive units with more than four bracts. Female reproductive units with more than nine bracts. . . . . 6
- 5 Male reproductive units with two or sometimes up to four bracts. Female reproductive units with (two) three to eight, rarely up to 12 bracts<sup>1</sup> . . . . . 7
- 6 Fruits indehiscent, papillose, with papillae more developed in the upper part of the fruit . . . . . *T. cookeana* (Figs 5, 7)

<sup>1</sup> The presence of more than eight bracts was observed only in some reproductive units of some specimens of *T. occidentalis*. Fruit and seed morphology helps in identification of such specimens, as fruits of *T. occidentalis* are always dehiscent (in contrast to *T. cookeana*) and seeds are clearly sculptured with exotesta cells transversally elongated (in contrast to both *T. cookeana* and *T. polybracteata*).

- 6 Dry mature fruits dehisce by separating three narrow longitudinal ribs; fruit surface without papillae. . . . . *T. polybracteata* (Figs 6, 8)
- 7(5) Fruits with maximum width in their middle part, without prominent longitudinal ribs, indehiscent; seeds smooth. Male reproductive units usually with two (rarely up to four) bracts 4.2–7.2 mm long; female reproductive units usually with four (rarely three) bracts. . . . . *T. austinensis* (Figs 2B, 3E, 4)
- 7 Fruits with maximum width in their distal part; dry mature fruits dehisce by separating three longitudinal ribs; dry mature seeds clearly sculptured (cells of exotesta well recognizable from surface with dissecting microscope). Male reproductive units with two bracts 5.5–10.1 mm long; female reproductive units usually with eight bracts . . . . . *T. occidentalis* (Figs 2A, D–F, 3A–B, H)
- 8(3) Fruits indehiscent; fruit surface with dense and well recognizable papillae . . . . . *T. cowieana* (Fig. 10)
- 8 Fruit surface without papillae. . . . . 9
- 9 Seeds smooth, translucent, cells of exotesta not recognizable from the surface . . . . . *T. lanterna* (Fig. 9)
- 9. Mature seeds clearly sculptured, honeycombed when dry, with individual exotesta cells well recognizable from the surface . . . . . 10
- 10 Reproductive units typically with four (rarely three or up to seven, very rarely two) acute or apiculate bracts. Reproductive units typically on distinct, often long peduncles. (Less frequently, reproductive units almost sessile, but such plants were not collected from Western Australia, where the next species occurs.) . . . . . *T. submersa* (Fig. 3C–D, I)
- 10 Reproductive units with two (very rarely four) acuminate bracts. Reproductive units virtually sessile (only very rarely with long peduncles) . . . . . *T. bibracteata* (Figs 2C, 3F–G)

**ACKNOWLEDGEMENTS**

We are grateful to the curators of several Herbaria (B, BRI, CHR, DNA, E, HO, MEL, NSW, NT, PERTH) for sending loans of specimens to Kew, David Simpson and Paul Wilkin for providing equipment and benchspace in the Kew Herbarium and Ludwig Martins (B) for permission to dissect a reproductive unit on the type specimen of *Hydatella leptogyne*. We also thank Richard Bateman, Alexander C. Timonin and Neil Gibson for discussion, Rolf Rutishauser and two anonymous referees for helpful suggestions, Vadim Mokievsky for help in drawing maps and Greg Keighery for discussion and making many valuable collections in south western Australia. DDS and MVR acknowledge support from the Bentham-Moxon trust and RFBR (grant No. 06-04-48113), and the work of DDS was supported by President of Russia grant No. MD-1056.2007.4.

## LITERATURE CITED

- Barrett, S.C.H.** 2002. The evolution of plant sexual diversity. *Nature Reviews, Genetics* 3: 274–283.
- Baum, D.A. & Hileman, L.C.** 2006. A developmental genetic model for the origin of the flower. Pp. 3–27 in: Ainsworth, C. (ed.), *Flowering and its Manipulation*. Blackwell, Sheffield.
- Bentham, G.** 1878. *Flora of Australia*, vol. 7. Lovell Reeve & Co., London.
- Buzgo, M., Soltis, P.S. & Soltis, D.S.** 2004. Floral developmental morphology of *Amborella trichopoda* (Amborellaceae). *Int. J. Pl. Sci.* 165: 925–947.
- Cheeseman, T.F.** 1907. Notice on the occurrence of *Hydatella*, a new genus to the New Zealand flora. *Trans. New Zealand Inst.* 39: 433–434.
- Cooke, D.A.** 1981. New species of *Schoenus* (Cyperaceae) and *Trithuria* (Hydatellaceae). *Muelleria* 4: 299–303.
- Cooke, D.A.** 1983. Two Western Australian Hydatellaceae. *Muelleria* 5: 123–125.
- Cooke, D.A.** 1987. Hydatellaceae. Pp. 1–5 in: George, A.S. (ed.), *Flora of Australia*, vol. 45. Australian Government Publishing Service, Canberra.
- Diels, L. & Pritzel, E.** 1904–1905. Fragmenta Phytographiae Australiae occidentalis. Beiträge zur Kenntnis der Pflanzen Westaustraliens, ihrer Verbreitung und ihrer Lebens-Verhältnisse. *Bot. Jahrb.* 35: 55–662. [*Hydatella*, pp. 93–94, 1904]
- Edgar, E.** 1966. The male flowers of *Hydatella inconspicua* (Cheesem.) Cheesem. (Centrolepidaceae). *New Zealand J. Bot.* 4: 153–158.
- Endress, P.K.** 2004. Structure and relationships of basal relictual angiosperms. *Austral. Syst. Bot.* 17: 343–366.
- Frohlich, M.W. & Parker, D.S.** 2000. The mostly male theory of flower evolutionary origins: from genes to fossils. *Syst. Bot.* 25: 155–170.
- Gaikwad, S.P. & Yadav, S.R.** 2003. Further morphotaxonomical contribution to the understanding of family Hydatellaceae. *J. Swamy Bot. Club* 20: 1–10.
- Grant, S.R.** 1999. Genetics of gender dimorphism in higher plants. Pp. 247–273 in: Geber, M.A., Dawson, T.E. & Delph, L.F. (eds.), *Gender and Sexual Dimorphism in Flowering Plants*. Springer, Berlin.
- Grob, V., Moline, P., Pfeifer, E., Novelo, A.R. & Rutishauser, R.** 2006. Developmental morphology of branching flowers in *Nymphaea prolifera*. *J. Pl. Res.* 119: 561–570.
- Hamann, U.** 1976. Hydatellaceae—a new family of Monocotyledoneae. *New Zealand J. Bot.* 14: 193–196.
- Hamann, U.** 1998. Hydatellaceae. Pp. 231–234 in: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants. IV. Flowering Plants – Monocotyledons – Alismatanae and Commelinanae*. Springer, Berlin.
- Hamann, U., Kaplan, K. & Rübtsamen, T.** 1979. Über die Samenschalenstruktur der Hydatellaceae (Monocotyledoneae) und die systematische Stellung von *Hydatella filamentosa*. *Bot. Jahrb. Syst.* 100: 555–563.
- Hooker, J.D.** 1858. *Flora of Tasmania*, vol. 2. Lovell Reeve & Co., London.
- Hutchinson, J.** 1959. *The Families of Flowering Plants*, 2nd ed. Clarendon Press, Oxford.
- Mabberley, D.J.** 1987. *The Plant-book. A Portable Dictionary of the Higher Plants*. Cambridge Univ. Press, Cambridge.
- Meagher, T.S.** 2007. Linking the evolution of gender variation to floral development. *Ann. Bot.* 100: 165–176.
- Paczkowska, G. & Chapman, A.R.** 2000. *The Western Australian Flora, a Descriptive Catalogue*. Wildflower Society of Western Australia, Western Australian Herbarium, CALM, and Botanic Gardens and Parks Authority, Nedlands.
- Pledge, D.H.** 1974. Some observations on *Hydatella inconspicua* (Cheesem.) Cheesem. (Centrolepidaceae). *New Zealand J. Bot.* 12: 559–561.
- Qiu, Y.L., Li, L., Hendry, T.A., Li, R., Taylor, D.W., Issa, M.J., Ronen, A.J., Vekaria, M.L. & White A.M.** 2006. Reconstructing the basal angiosperm phylogeny: evaluating information content of mitochondrial genes. *Taxon* 55: 837–856.
- Renner, S.S. & Won, H.** 2001. Repeated evolution of dioecy from monoecy in Siparunaceae (Laurales). *Syst. Biol.* 50: 700–712.
- Rudall, P.J., Sokoloff, D.D., Remizowa, M.V., Conran, J.G., Davis, J.I., Macfarlane, T.D. & Stevenson, D.W.** 2007. Morphology of Hydatellaceae, an anomalous aquatic family recently recognized as an early-divergent angiosperm lineage. *Amer. J. Bot.* 94: 1073–1092.
- Saarela, J.M., Rai, H.S., Doyle, J.A., Endress, P.K., Mathews, S., Marchant, A.D., Briggs, B.G. & Graham, S.W.** 2007. Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. *Nature* 446: 312–315.
- Soltis, D.E., Gitzendanner, M.A. & Soltis P.S.** 2007. A 567-taxon data set for angiosperms: the challenges posed by Bayesian analyses of large data sets. *Int. J. Pl. Sci.* 168: 137–157.
- Soltis, D.E., Soltis, P.S., Endress, P.K. & Chase, M.W.** 2005. *Phylogeny and Evolution of Angiosperms*. Sinauer Associates, Sunderland.
- Theißen, G. & Becker, A.** 2004. Gymnosperm orthologues of class B floral homeotic genes and their impact on understanding flower origin. *Crit. Rev. Pl. Sci.* 23: 129–148.
- Thien, L.B., Sage, T.L., Jaffre, T., Bernhard, P., Pontieri, V., Weston, P.H., Malloch, D., Azuma, H., Graham, S.W., McPherson, M.A., Rai, H.S., Sage, R.F. & Dupre, J.L.** 2003. The population structure and pollination biology of *Amborella trichopoda* (Amborellaceae). *Ann. Missouri Bot. Gard.* 90: 466–490.
- Wells, R.D.S., Clayton, J.S. & de Winton, M.D.** 1998. Submerged vegetation of Lakes Te Anau, Manapouri, Monowai, Hauroko, and Poteriteri, Fiordland, New Zealand. *New Zealand J. Mar. Freshwater Res.* 32: 621–638.
- Yadav, S.R. & Janarthanam, M.K.** 1994. Hydatellaceae: a new family to Indian flora with a new species. *Rheedea* 4: 17–20.
- Yadav, S.R. & Janarthanam, M.K.** 1995. *Trithuria konkanensis* (Hydatellaceae), eine neue Art aus Indien. *Aqua-Planta* 3: 91–97.



**Appendix. List of herbarium specimens examined (except new taxa). Species assignments are according to the updated taxonomy.**

*Trithuria australis*. **Australia: Western Australia:** *Annels & Hearn 5347*, 500 m north of junction of Wingebellup and Southfield Roads, 4.3 km south of Tonebridge, 11 Oct. 1994 (CHR, NSW, NY, PERTH); *Diels 4983*, Hammersley River, Oct. 1901 (B, PERTH); *Diels 5698*, Hutt River (B); *Fitzgerald s.n.*, Midland Junction, Sep. 1901 (NSW); *Fitzgerald s.n.*, Midland Junction, Oct. 1903 (B, K); *Hearn s.n.*, 4 km S of Tone Bridge settlement along Wingebellup Road, c. 50 km E of Manjimup, 16 Dec. 2003 (K, PERTH); *Hearn s.n.*, Pindicup Nature Reserve, 16 Dec. 2003 (CANB, PERTH); *Hearn s.n.*, Pindicup Nature Reserve, 16 Dec. 2003 (K, PERTH); *Keighery G.J. & Gibson 2584*, Cobertup Nature Reserve, 22 Oct. 1997 (PERTH); *Keighery G.J. & al. 7251*, D'Entrecasteaux National Park, 4 Dec. 2002 (PERTH); *Macfarlane & Annels 2283*, 4.3 km S of Tonebridge townsite, 27 Oct. 1994 (CANB, K, MEL, PERTH); *Macfarlane & Hearn 3357*, 4 km S of Tone Bridge settlement along Wingebellup Road, c. 50 km E of Manjimup, 15 Dec. 1999 (NSW, PERTH); *Macfarlane & Hearn 3361*, Kulunilup Nature Reserve, c. 56 km ESE of Manjimup, 15 Dec. 1999 (PERTH); *Sandiford 929*, Road Reserve, N side of South Coast Highway, E of Hay River, 7 Nov. 2003 (PERTH). — *Trithuria bibracteata*. **Australia: Western Australia:** *Annels 5349*, Mordallup Road just W of Lake Muir Road, 27 Oct. 1994 (PERTH); *Annels & Hearn 4626*, Boronia Road, 3.1 km E of Middle Road, N of Walpole, 13 Oct. 1994 (CANB, PERTH); *Annels & Hearn 5350*, Perup Ecology Centre, Oct. 1994 (K, PERTH); *Anonymous 2*, Midland Junction, Sep. 1901 (K); *Archer 311051*, 2 km SW of Mt Merivale, 3 Nov. 2005 (PERTH); *Clifford s.n.*, Cannington, 10 km S of Perth, 29 Oct. 1982 (BRI); *Fitzgerald s.n.*, Midland Junction, Sep. 1901 (NSW, PERTH); *Fitzgerald s.n.*, Midland Junction, Oct. 1903 (B, K, PERTH); *Gunness & al. 13/37*, ca 19 km NNE of the Goomalling, 25 Oct. 1999 (PERTH); *Keighery B.J. 2007*, eastern side of Pearce Airforce base, 20 Sept. 1994 (PERTH); *Keighery B.J. & Gibson 682*, water reserve 1459 on Yoongarillup Road, off the Vasse Highway 12 km SE of Busselton, 14 Oct. 1992 (PERTH); *Keighery B.J. & Gibson 801*, Fish Road Reserve, 10 km SSE Busselton, 9 Nov. 1992 (PERTH); *Keighery G.J. 4125*, 31 km N on Mimegarra Road, start 18 km E Lancelin (c. 50 km ENE Lancelin), 17 Oct. 1981 (PERTH); *Keighery G.J. 4202*, 13 km N Midland, 27 Oct. 1982 (PERTH); *Keighery G.J. 4217*, Boundary Rd., Orange Grove, 10 Oct. 1981 (PERTH); *Keighery G.J. 4280*, 20 km S of Bridgetown on Manjimup road, 20 Nov. 1981 (PERTH); *Keighery G.J. 6719*, 1 km S of Tambellup to Cranbrook, 20 Oct. 1983 (PERTH); *Keighery G.J. 10496*, Duranilling, 4 Nov. 1988 (PERTH); *Keighery G.J. 10904*, Brixton St., Beckenham, 15 km E of Perth, 21 Oct. 1989 (PERTH); *Keighery G.J. & Gibson 2798*, E boundary of Yarnup Nature Reserve, 25 Oct. 1997 (PERTH); *Kelly 90/50*, N end of Block bounded by Brixton St., Wanaping Road and park Road, Kenwick, 31 Oct. 1990 (PERTH); *Kelly 90/62*, Ellen Brook Nature Reserve, Upper Swan, 2 Nov. 1990 (PERTH); *Macfarlane 3479*, Perup Reserve, c. 45 km ENE of Manjimup, 11 Oct. 2000 (PERTH); *Macfarlane 3903*, 15.1 km N of Manjimup, 24 Oct. 2006 (PERTH); *Macfarlane & Hearn s.n.*, Wingebellup Road, c. 1.5 km W of Yarnup Road, 15 Dec. 1999 (PERTH); *Macfarlane & Hearn 3366*, Yarnup Nature Reserve, c. 68 km SE of Manjimup, 15 Dec. 1999 (PERTH); *Newbey 9627*, Murray Rock, 30 Sept. 1982 (PERTH); *Royce 4327*, Harvey, 15 Sept. 1953 (PERTH); *Royce 2265*, Boyanup, 15 Oct. 1947 (PERTH); *Royce 6148*, Upper Swan, 11 Nov. 1959 (PERTH); *Staer s.n.*, Midland Junction, Oct. 1905 (E). — *Trithuria filamentosa*. **Australia: Tasmania:** *Brown 340*, Hardwood River Valley, 24 Jan. 1978 (HO); *Buchanan 1194b*, Twisted tarn, 1 Apr. 1983 (HO); *Buchanan 12328*, Mersey, betw. Lake Ina and Lake Norman, 1 Mar. 1992 (HO); *Buchanan 13009*, Wedge, Lake Rhona, 30 Jan. 1993 (HO); *Buchanan 8092 and 8094*, near Giblin River, 7 km from mouth 23 Jan. 1986 (HO); *Buchanan 9938*, Lake Ewart, 4 Feb. 1987 (HO); *Burns 438*, Lake Dove, Cradle Mt., 4 Feb. 1961 (HO); *Curtis s.n.*, Lake Augusta, 4 Apr. 1971 (HO); *Curtis s.n.*, Lake Dobson, 12 Mar. 1951, 18 Jan. 1959, 23 Jan. 1968 and 20 Jan. 1969 (HO, K); *de Malahide & Curtis s.n.*, Lake Dobson, 15 Feb. 1972 (K); *Gibbs 6674*, Lake St. Clair, Jan. 1915 (BM, K); *Gibbs 6809*, Hartz mts., Mar. 1915 (BM); *Gibbs 6809*, Lake Hartz, 1914–1915 (K); *Jarman s.n.*, Mt. Anne, 12 Apr. 1985 (HO); *Jarman s.n.*, Olga River, 19 Jan. 1978 (HO); *Jarman s.n.*, Giblin River Valley, Jan. 1979 (HO); *Jarman s.n.*, S of Frenchmans Cap, 15 Dec. 1978 (HO); *Malahide s.n.*, Lake Dove, 7 Feb. 1966 (K); *Moscal 13940 and 13942b*, Wilson Bight, 13 Jan. 1987 (HO, K); *Moscal 1426*, Mersey, Lake Ball, 19 Jan. 1983 (HO); *Moscal 1517*, Mersey, Mount Oakleigh, 28 Jan. 1983 (HO); *Moscal 1522*, Mersey, Lake Rosa, 28 Jan. 1983 (HO); *Moscal 2008*, Lake Will, 28 Jan. 1983 (HO); *Moscal 20292*, 5 km NNE of Waddamana, 1 Dec. 1990 (HO); *Moscal 2052*, Lake Holmes, 27 Feb. 1983 (HO); *Moscal 6881*, Meander, Second Bar Lake, 11 Mar. 1984 (HO); *Moscal 7003*, Devils Den., 13 Mar. 1985 (HO); *Rodway 907a*, Mt. Field, Dec. 1896 (HO, MEL); *Rodway s.n.*, 29 Sep. 1898 (MEL); *Rodway s.n.*, Broad River, Dec. 1896 (MEL, NSW); *Rodway s.n.*, Mt. Field, Dec. 1906 and Feb. 1919 (HO); *Tyler s.n.*, Lake Rhona, Mar. 1976 (HO); *Wells & al. VM31*, Lake St. Clair, 29 Jan. 1997 (HO); *Wells & al. VM9*, Lake Dobson, 27 Jan. 1997 (HO). — *Trithuria inconspicua*. **New Zealand: North Is.:** *Anonymous s.n.*, Shag Lake, 18 Nov. 1987 (CHR); *Butcher s.n.*, Lake Waiparera, 3 Jan. 1966 (HO, 3 specimens, K, NSW, 2 specimens); *Carse 1796*, Lake Ngatu, Jan. 1913 (K); *Carse 2131*, Lake Ngatu, Jan. 1902 (K); *Carse s.n.*, Lake Ngatu, 20 Dec. 1914 (CHR); *Cooper s.n.*, Lake Waiparera, 5 Feb. 1966 (K); *de Lange 1146*, Lake Rotokawau, 18 Nov. 1991 (CHR); *de Lange 2504*, Lake Kaiwi, 16 Mar. 1996 (CHR); *Matthews s.n.*, Lake Ngatu, 10 Feb. 1902 (B); *Pledge s.n.*, Lake Taharoa, 12 and 22 Jan. 1971 (CHR, E); *Powell s.n.*, Russel Lake, Waiharara [Lake Waiparera], 30 Jan. 1950 (CHR); *Tanner s.n.*, Lake Waikere, 21 Jan. 1981 (CHR); **South Is.:** *Clayton 1145*, Lake Te Anau, 10 Mar. 1993 (CHR); *Clayton s.n.*, Lake Poteriteri, 13 Mar. 1993 (CHR); *Clayton & Johnson 1138*, Lake Hauroko, 8 Mar. 1993 (CHR); *Wells s.n.*, Lake Manapouri, 11 Mar. 1993 (CHR). — *Trithuria lanterna*. **Australia: Queensland:** *Bean 16480*, North Kennedy, Bruce Highway, 13 km W of Bowen, 3 May 2000 (BRI, DNA); *McKee 9231*, Gorge Creek, Mareeba, 20 Apr. 1962 (BRI, NSW); **Northern Territory:** *Dunlop 9345 & Leach*, Bickerton Island, 29 Apr. 1993 (DNA); *Egan 4781 & Knox*, Kakadu NP, 7 km NNE of Jabiru, 25 Apr. 1995 (DNA); *Egan 4816 & Knox*, Kakadu NP, 10 km NNE of Jabiru, 26 Apr. 1995 (DNA); *Egan 5290 & Michell*, Howard River, Gunn Point, 11 Apr. 1996 (DNA); *Graven & Wightman 8331*, Mt Gilruth, 28 Mar. 1984 (DNA, MEL); *Harwood 370*, Mary River N.P., 20 May 1998 (DNA); *Johnson 4555 and 4597*, Arnhem Land, 19 km E of Jabiru, 18 and 19 Apr. 1989 (BRI, DNA); *Latz 3699*, Lake Deane, 26 May 1973 (NT); *Latz 3816*, Munmalary Stn., 15 Apr. 1973 (NT); *Michell & Deichmann 3400*, Nitmiluk National Park, 6 Apr. 2001 (DNA); *Michell 2698*, Edith river area, 11 May 2000 (DNA); *Specht 413*, Groote Eylandt, Gulf of Carpentaria, 27 May 1948 (BRI, MEL); *Specht 566*, Bickerton Island, Gulf of Carpentaria, 14 Jun. 1948 (BRI, MEL, PERTH); *Symon 7806*, Arnhem Land, 21 Jun. 1972 (DNA). — *Trithuria occidentalis*. **Australia: Western Australia:** • Male plants: *Fitzgerald s.n.*, Midland Junction, Oct. 1903 (B, K, NSW); *Fitzgerald s.n.*, Midland Junction, Sep. 1901 (NSW, 2 specimens in PERTH); *Keighery G.J. 4201*, J.R. & B. Martyn Reserve, Ellen Brook, 13 km N of Midland, 27 Oct. 1982 (PERTH);

## Appendix. Continued.

*s.n.*, Midland Junction, 16 Nov. 1898 (E, PERTH); *Morrison s.n.*, Midland Junction, Swan River, 22 Nov. 1899 (E, BM, K); • Female plants: *Drummond 689*, Swan River (B, BM, K, MEL, PERTH); *Fitzgerald s.n.*, Midland Junction, Oct. 1901 (NSW); *Fitzgerald s.n.*, Midland Junction, Sept. 1901 (K, PERTH); *Fitzgerald s.n.*, Midland Junction, Sept. 1902 (K); *Keighery G.J. 4204*, Ellen Brook, 13 km N Midland, 27 Oct. 1982 (PERTH); *Keighery G.J. 4520*, Warbrook Siding, Upper Swan, 18 Oct. 1978 (PERTH); *Morrison s.n.*, Midland Junction, Lower Swan River, 16 Nov. 1898 (BRI, E, K, PERTH); *Morrison s.n.*, Midland Junction, Swan River, 22 Nov. 1899 (B, BM, K); *Stær s.n.*, Midland Junction, Oct. 1905 (E). — ***Trithuria submersa*. Australia: New South Wales: McBaron 5403 and 5932**, Jindera, 28 Nov. 1950 and 7 Nov. 1952 (NSW); *McBaron 5688*, Henty, 9 Nov. 1951 (NSW); *von Mueller s.n.*, Edward River, Oct. 1875 (NSW); **South Australia: Beaulhole & Krachenbuel 19268**, Yallum, 5 Jan. 1965 (MEL); *Hunt s.n.*, 4 miles W of Lucindale, 22 Nov. 1964 (NSW); *Krachenbuel & Beaulhole 5722*, Hundred of Coles, near road to Lucindale, 12 Oct. 1963 (MEL); *Tate s.n.*, Kangaroo Island (MEL); *Tepper 1247*, Kangaroo Island, 10 Mar. 1884 (MEL); **Tasmania: Curtis s.n.**, Poatina Highway, 22 Jan. 1970 (HO, MEL); *Curtis s.n.*, 10 miles W of Ross, 11 Mar. 1971, 11 Nov. 1971, 11 Apr. 1971 (HO, MEL); *Curtis s.n.*, Epping, 14 Dec. 1955 and 20 Nov. 1968 (HO); *Curtis s.n.*, Epping, NW of Conara junction, Jan. 1955 (MEL); *Gunn s.n.*, Lake River, 2 Dec. 1848 (NSW); *Gunn s.n.*, “Formosa”, 2 Dec. 1848 (HO); *Gunn 2014*, Macquarie River, 6 Nov. 1845 (K); *Moscal 20272*, 5 km NNE of Waddamana, 1 Dec. 1990 (HO); *Rodway 153*, near Campbell Town, 25 Jan. 1931 (HO); *Smith s.n.*, Middle Lagoons, 11 Dec. 1998 (HO); *Stuart s.n.*, South Esk River, 1848 (MEL); *Stuart s.n.*, South Esk, Dec. 1849 (B); *von Mueller s.n.*, Edwards River (HO); **Victoria: Beauglehole & Courtney 69473**, 16 km W of Maryborough P.O., 26 Oct. 1981 (MEL); *Beauglehole & Elmore 71248*, Lang Warrin Flora Reserve, 08 Nov. 1982 (MEL); *Beauglehole 29726*, Mt. Arapiles, 22 Nov. 1968 (MEL); *Beauglehole 29805*, NE Arapiles, 25 Nov. 1968 (MEL); *Beauglehole 29971A*, S of Glenisla Station, 7 Dec. 1968 (MEL); *Beauglehole 43325*, N of Penola - Dergholm Road, 9 Nov. 1964 (MEL); *Beauglehole 61240*, 9 km NNE of Ararat P.O., 2 Nov. 1978 (MEL); *Beauglehole 66275*, 23 km SSE of Kaniva P.O., 8 Nov. 1979 (MEL); *Beauglehole 67218*, 1.5 km NE of Willarura P.O., 9 Dec. 1979 (MEL); *Beauglehole 6742*, 1 mile W of Greenwald School, 7 Dec. 1964 (MEL); *Beauglehole 75483*, Wannon District, Black Waterhole, 30 Nov. 1983 (MEL); *Beauglehole 76028*, Grampians National Park, 14 Jan. 1984 (MEL); *Beauglehole 76145*, Black Swamp Wildlife Reserve, 27 Feb. 1984 (MEL); *Beauglehole 76554*, 24 km W of Casterton, 15 Mar. 1984 (MEL); *Beauglehole 87094*, Morea Wildlife Reserve, 14 Nov. 1986 (MEL); *Clarke 2671*, 8 km NNW of Dunkeld, 29 Oct. 1995 (MEL); *Corrick 4862*, W of Kaniva, 10 Nov. 1974 (MEL); *Corrick 729*, 3 km NW of Minimay, 2 Dec. 1984 (MEL); *Eckert s.n.*, Wimmera, Nov. 1896 (MEL); *Hicks s.n.*, Broughton’s Waterhole, Dec. 1964 and 5 Nov. 1971 (MEL); *Marshall 504*, 5 km SSW of Nathalia P.O., Nov. 1983 (MEL); *Melville & al. 1201*, 18 miles SW of Kaniva, 22 Sep. 1952 (K); *Mulham 1522*, near Barmah S.F., Nov. 1983 (NSW); *Reader s.n.*, Little Desert, Lowan, 3 Nov. 1900 (BM); *Reader s.n.*, Swamp Borung, 04 Nov. 1903 (MEL); *Robinson s.n.*, Melbourn, suburb of Deer Park, 28 Oct. 1991 (MEL); *Short & Corrick 6770*, 33 km S of Nhill, 3 Oct. 1980 (MEL); *Stebbing s.n.*, Ulupna Island Reserve, 1972–1980 (MEL); *Strudwick 0073*, Reef Hills Regional Park, 15 Nov. 1988 (MEL); *Strudwick 604*, 2.9 km W of Goulburn Valley Highway, 7 Nov. 1989 (MEL); *von Mueller 20369*, Mount Emu Creek (BM); *von Mueller s.n.*, Mount Emu Creek, Nov. 1853 (K, MEL); *von Mueller s.n.*, Hopkins River, Nov. 1853 (B); *von Mueller s.n.*, Axe River, Nov. 1854 (B); *Williamson s.n.*, Hawkesdale, Feb. 1904 (MEL); *Williamson s.n.*, Yambuk, Nov. 1927 (MEL); *Willis s.n.*, 11 miles N of Serviceton North, 11 Sep. 1948 (MEL); **Western Australia: Annels 5930**, 100 m W from Northern Road on DeLandgraff Road, 7 Nov. 1997 (PERTH); *Butler s.n.*, Perup River, E of Manjimup, Oct. 1948 (PERTH); *Clifford s.n.*, Cannington Swamp, 28 Oct. 1982 (BRI); *George 15027*, 17 km W of Frankland R., Muir Highway, 2 Nov. 1977 (PERTH); *Hearn s.n.*, Pindicup Nature Reserve, western shore of Pindicup Swamp, 16 Dec. 2003 (K, PERTH); *Keighery B.J. & Gibson 109*, Meelon Nature Reserve, 9 km SE Pinjarra, 15 Oct. 1993 (PERTH); *Keighery B.J. & Gibson 201*, Lake Muckinburra Reserve, E of Gingin, 27 Oct. 1993 (PERTH); *Keighery G.J. 4231*, Waterloo, 3 Nov. 1981 (PERTH); *Keighery G.J. 5667*, 9 km N of Capel to Bunbury, 1 Oct. 1982 (PERTH); *Keighery G.J. 6718*, 1 km S of Tambellup to Cranbrook, 20 Oct. 1983 (PERTH); *Keighery G.J. 10962*, Lake Muir, 55 km E of Manjimup to Mount Barker, 2 Dec. 1988 (PERTH); *Keighery G.J. & Gibson 2396*, Cobertup Nature Reserve, 22 Oct. 1997 (PERTH); *Keighery G.J. & Gibson 2584*, Cobertup Nature Reserve, 22 Oct. 1997 (PERTH); *Keighery G.J. & al. 7251*, D’Entrecasteaux National Park, 4 Dec. 2002 (PERTH); *McCallum Webster 640*, 1 mile from junction of Denmark, Albany Rd., 21 Nov. 1980 (PERTH); *Macfarlane 3902*, 15.1 km N of Manjimup, 24 Oct. 2006 (PERTH); *Macfarlane & Hearn 3358*, 4 km S of Tone Bridge settlement along Wingebellup Road, c. 50 km E of Manjimup, 15 Dec. 1999 (CANB, K, NSW, PERTH); *Macfarlane & Hearn 3362*, Kulunilup Nature Reserve, c. 56 km ESE of Manjimup, 15 Dec. 1999 (K, MEL, PERTH); *Macfarlane & Hearn 3374*, Kodjinup Nature Reserve, c. 60 km SE of Manjimup, 15 Dec. 1999 (PERTH); *Royce 2651*, W of Harvey, 21 Sept. 1948 (PERTH); *Royce 3866*, Elgin, 16 Oct. 1952 (PERTH); *Royce 4356*, Elgin, 17 Sept. 1953 (PERTH); *Sandiford 902*, S of Muir Highway, ITC Perillup Tree farm, Kent River, 21 Oct. 2003 (PERTH); *Stær s.n.*, Midland Junction, Oct. 1905 (E); *Wilson 6262*, 7 km E of Bunbury, 30 Sept. 1967 (PERTH).