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A new species of *Vallisneria* L. for Western Australia and recircumscription of *V. triptera* S.W.L.Jacobs & K.A.Frank

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Abstract

Vallisneria kristinae R.W.Jobson & Dunning (Hydrocharitaceae) is described as a new species endemic to North Kimberley, Western Australia. It was previously included within the Northern Territory species *V. triptera* S.W.L.Jacobs & K.A.Frank. Here it is distinguished from that species based on its overall smaller size and weakly-winged fruit capsules. We also find strong genetic differentiation with accessions sampled from across the entire distribution of both taxa. We present a phylogeny based on nuclear ITS and plastid *trnK 5′* intron data that also includes all Australian species of *Vallisneria*. Along with a redescription of *V. triptera s. str.*, diagnostic features are illustrated, a taxonomic key containing all Australian species is provided, and distribution, habitat, and conservation status are discussed.

Introduction

Worldwide there are 17 recognised species of *Vallisneria* P.Micheli ex L. (Shen 2000, 2001; Les et al. 2008; Martin & Mort 2023), with Australia having the greatest diversity with eight species (Jacobs & McColl 2011, 2023). A major vegetative character distinguishing groups within the genus involves growth form, with three facultative annual species sharing a caulescent vegetative growth form, while the remainder are annuals or perennials sharing a basally rosulate vegetative growth form (Les et al. 2008; Jacobs & McColl 2011). Following the molecular phylogenetic results of Les et al. (2008), *Maidenia rubra* Rendle was included under *Vallisneria*, with close phylogenetic affinity shown to *V. triptera*. All three of the cauline species *V. rubra* (Rendle) Les & S.W.L.Jacobs, *V. triptera* S.W.L.Jacobs & K.A.Frank and *V. caulescens* Bailey & F.Muell. are northern Australian endemics, with *V. triptera* restricted to a few creeks near Jabiru, NT (the vicinity of the type collection) and disjunct populations in northern Kimberley region of Western Australia (Jacobs & McColl 2011). *Vallisneria triptera* and *V. rubra* differ from *V. caulescens* in having female flowers being tripartite instead of bipartite (McConchie 1983; Jacobs & Frank 1987; McConchie & Kadereit 1987). The fruit of *V. triptera* differs from *V. caulescens* in being tripartite vs. bipartite, while that of *V. rubra* is cylindrical (rarely tripartite) and not winged (Jacobs & McColl 2011).

Les et al. (2008) found all three caulescent species to be monophyletic but with weak support (BS = 32; BB = 0.18) using combined nuclear ribosomal (nrDNA) ITS and plastid gene regions *rbcL* and *trnK 5′* intron. Martin and Mort (2023) added 18 accessions to this dataset and found the African/Eurasian species *V. spiralis* L. and *V. denseserrulata*

Richard W. Jobson & Luke T. Dunning (2024) A new species of *Vallisneria* L. for Western Australia and recircumscription of *V. triptera* S.W.L.Jacobs & K.A.Frank. *Telopea* 27: 27–38. doi:10.7751/telopea17993

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(Makino) Makino sister to a supported clade (BS = 99; BB = 1.0) containing two accessions of *V. caulescens* which in turn was sister to all other species, including *V. rubra* and *V. triptera* that were supported (BS = 86; BB = 1.0) as sister species. From that study Martin & Mort (2023) described the new rosulate species *V. jacobsii* A.P.Martin & D.N.Wilson which was supported (BS = 80; BB = 1.0) as sister species to three accessions of *V. australis* S.W.L.Jacobs & Les, and distinguished morphologically from that species based on leaf colour and the former species' apparent distichous growth form at the base of rosettes.

In the current study, we increased the molecular sampling of Martin & Mort (2023), particularly a geographically-dispersed sampling of the three caulescent species, including the first sequenced samples of *V. triptera* populations from the northern Kimberley. From an analysis of molecular data and an examination of morphology we here differentiate the NT populations of *V. triptera* from those of the northern Kimberley region, describing the latter as a new species *V. kristinae* R.W.Jobson & Dunning. Comparisons with closely related *Vallisneria triptera* accessions from across the Northern Territory distribution are provided. Diagnostic features are illustrated, and distribution, habitat, and conservation status are discussed. We also include a modified key to the *Vallisneria* species of Australia (expanding Jacobs and McColl 2011).

Methods and materials

Taxon sampling

Relevant dried and alcohol-preserved material representing all related species, held at the National Herbarium of New South Wales (NSW), Queensland Herbarium (BRI), Australian National Herbarium (CANB), Northern Territory Herbarium (DNA), and Western Australia Herbarium (PERTH), were examined. The description of *V. triptera* used in this text is a modification of that included in Jacobs & McColl (2011) with new measurements of fruit and leaves obtained from the holotype and additional Jabiru specimens held at NSW, excluding previously included data from Kimberley specimens. Two samples of *Nechamandra alternifolia* (Roxb. ex Wight) Thwaites were selected as outgroups for *Vallisneria* based on Les et al. (2008): nuDNA ITS (GenBank: AY870381, EF143023) and plastid *trnK* 5′ intron marker (GenBank: AY335967, EF142957). GenBank numbers of all previously published ingroup accessions can be found in the tables of Les et al. (2008) and Martin and Mort (2023). The final dataset contained 93 ingroup accessions of which 26 were previously unpublished (Table 1). DNA isolation was performed as for Jobson et al. (2017). Location details of newly published ingroup accessions relevant to this study were used to create a distribution map (Fig. 1).

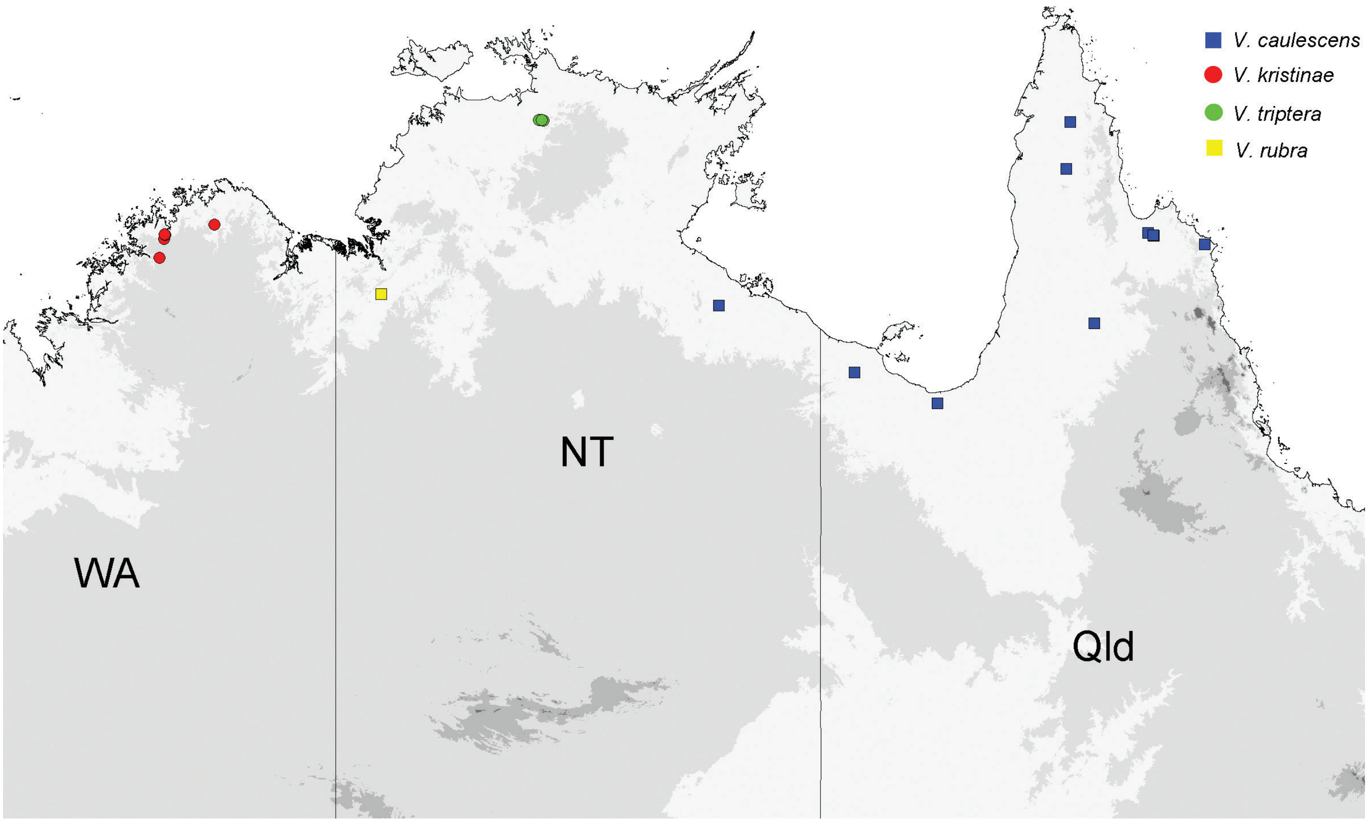

Fig. 1. Distribution map showing accessions of species with cauline leaf arrangement used in the molecular phylogeny of *Vallisneria*. *V. kristinae* (red circle), *V. triptera* (green circles), *V. rubra* (yellow square), *V. caulescens* (blue squares). Accession details shown in Table 1.

Table 1. Previously unpublished accessions used in the nrDNA ITS and cpDNA *trnK 5′* matrices. Secondary collectors are not included. Locality abbreviations: NT: Northern Territory; Qld: Queensland; WA: Western Australia. GenBank accession numbers for sequences are shown. N.A. indicates sequencing failed or was not carried out.

Lab #	Taxon	Collector	Coll. Date	Location	ITS	matK
V22	*V. australis* S.W.L.Jacobs & Les	Keighery, G.J. 5905 (CANB)	23/02/1983	Margaret River Bridge, Margaret River, WA	OR936254	N.A.
V05	*V. caulescens* F.M.Bailey & F.Muell.	Hellquist, B. 8204 (NSW)	3/08/1997	Hann River Crossing, Cook, Qld	OR936240	N.A.
V06	*V. caulescens* F.M.Bailey & F.Muell.	Jacobs, S.W.L. 8232 (NSW)	10/08/1997	WNW of Rokeby Ranger Station, Cook, Qld	OR936241	N.A.
V08	*V. caulescens* F.M.Bailey & F.Muell.	Jacobs, S.W.L. 1360 (NSW)	30/04/1974	Forked Lagoon, "Wernadinga", Burke, Qld	OR936242	N.A.
V09	*V. caulescens* F.M.Bailey & F.Muell.	Jacobs, S.W.L. 9934 (NSW)	17/06/2008	NW of Chillagoe, Cook, Qld	OR936243	N.A.
V10	*V. caulescens* F.M.Bailey & F.Muell.	Jacobs, S.W.L. 5368 (NSW)	29/07/1987	N of Starcke River, Cook, Qld	OR936244	N.A.
V11	*V. caulescens* F.M.Bailey & F.Muell.	Forster, P.I. 21088 (MEL)	24/05/1997	Hells Gate on Cliffdale Creek, Burke, Qld	OR936245	N.A.
V17	*V. caulescens* F.M.Bailey & F.Muell.	Craven, L. 4757 (CANB)	14/07/1977	Day Lagoon, McArthur River, NT	OR936250	N.A.
V01	*V. caulescens* F.M.Bailey & F.Muell.	Jobson, R.W. 4193 (NSW)	29/06/2022	Batavia Downs, Cook, Qld	OR936236	OR940931
V04	*V. caulescens* F.M.Bailey & F.Muell.	Nelder, J. 3806b (DNA)	8/04/1992	N of Lakefield Homestead, Cook, Qld	OR936239	N.A.
V19	*V. caulescens* F.M.Bailey & F.Muell.	Paijmans, K. 2870 (CANB)	12/08/1978	ESE of 'Breeza Plains' homestead, NT	OR936251	N.A.
V14	*V. kristinae* R.W.Jobson & Dunning	Fryxell, P.A. 4067 (CANB)	11/05/1983	Mitchell Plateau, NW Kimberley, WA	OR936247	OR940935
V15	*V. kristinae* R.W.Jobson & Dunning	Fryxell, P.A. 4139 (CANB)	15/05/1983	Carson River Station, North Kimberley, WA	OR936248	OR940936
V32	*V. kristinae* R.W.Jobson & Dunning	Jobson, R.W. 4360 (NSW)	24/05/2023	Airport Swamp, Mitchell Plateau, WA	OR936260	OR940942
V02	*V. kristinae* R.W.Jobson & Dunning	Barrett, R.L. 4664 (NSW)	24/04/2008	Doongan Station, North Kimberley, WA	OR936237	OR940932
V03	*V. kristinae* R.W.Jobson & Dunning	Jacobs, S.W.L. 8049 (NSW)	20/05/1996	Airport Swamp, Mitchell Plateau, WA	OR936238	OR940933
V12	*V. kristinae* R.W.Jobson & Dunning	Kenneally, K.F. 7144 (CANB)	9/02/1979	Mitchell Plateau, NW Kimberley, WA	OR936246	OR940934
V20	*V. nana* R.Br.	Sands, M.J.S. 5090 (CANB)	13/07/1988	King Leopold Ranges, West Kimberley, WA	OR936252	N.A.
V21	*V. nana* R.Br.	Trudgen, M.E. 10102 (CANB)	10/07/1991	SW of Pannawonica, WA	OR936253	N.A.
V23	*V. nana* R.Br.	George, A. 13568 (CANB)	8/08/1975	Drysdale River National Park, WA	OR936255	N.A.
V33	*V. rubra* (Rendle) Les & S.W.L.Jacobs	Jobson, R.W. 4413 (NSW)	24/05/2023	W of Timber Creek, NT	OR936261	OR940943
V16	*V. triptera* S.W.L.Jacobs & K.Frank	Barker, W. 442 (CANB)	6/05/1983	E of South Alligator River, NT	OR936249	OR940937
V28	*V. triptera* S.W.L.Jacobs & K.Frank	Jacobs, S.W.L. 7971 (NSW)	5/05/1996	W of Jabiru, on the Arnhem Highway, NT	OR936256	N.A.
V29	*V. triptera* S.W.L.Jacobs & K.Frank	Barker, W. 442 (NSW)	18/05/2023	E of South Alligator River, NT	OR936257	OR940939
V30	*V. triptera* S.W.L.Jacobs & K.Frank	Jobson, R.W. 4331 (NSW)	18/05/2023	Jabiru, NT	OR936258	OR940940
V31	*V. triptera* S.W.L.Jacobs & K.Frank	Jobson, R.W. 4335 (NSW)	20/05/2023	Jabiru, NT	OR936259	OR940941

Amplification and sequencing

Amplifications were performed using the nrDNA ITS gene marker and the plastid *trnK* 5′ intron as for Branwhite et al. (2023). The *trnK* 5′ intron was amplified with primers described in Les et al. (2008) using parameters described in Shaw et al. (2005). The ITS region was amplified for all samples using the forward primer ITS5A (Stanford et al. 2000) and the universal reverse primer ITS4 (White et al. 1990). Polymerase chain reaction (PCR) conditions for ITS were performed as described in White et al. (1990).

Phylogenetic analyses

Phylogenetic analyses were performed on (1) the two individual datasets, and (2) the concatenated matrix comprising both markers. The most suitable nucleotide substitution model for each of the three markers was assessed using the Akaike information criterion (AIC) implemented in jModelTest (ver.2.1.7, see https://en.bio-soft.net/tree/MODELTEST.html, accessed 20 November 2023; Guindon & Gascuel 2003; Posada 2008). The best fit model was GTR+G+I and with a burn-in involving the first 25% of the samples, we estimated Bayesian posterior probability using MrBayes (ver. 3.2.6; Ronquist 2012), with five independent runs of 30 million generations, using four chains with sampling of trees every 1000 generations. Substitution rates and state frequencies were set to the Dirichlet distribution, with all other priors unlinked with a flat multinomial distribution. Stationarity was assessed by examining plots of the –lnL across generations in Tracer (ver. 1.6, Rambaut et al.; https://beast.community/tracer, accessed 20 November 2023). The effective sample size (ESS) was set to >1000, and these remaining trees were used to construct a 50% majority rule consensus tree, visualised using FigTree (ver. 1.4.0; http://tree.bio.ed.ac.uk/software/figtree/).

Results

Sequences and alignment

The ITS matrix was 772 bp long, of which 269 characters (35%) were parsimony informative, and the *trnK 5*′ intron matrix was 1209 bp long of which 122 characters (11%) were parsimony informative. Both datasets included samples from all ingroup taxa; ITS (*n* = 93), *trnK 5*′ intron (*n* = 68) (unpublished accessions shown in Table 1; published accessions listed in Les et al. 2008 and Martin & Mort 2023). The concatenated matrix contained 93 ingroup and two outgroup taxa and was 1981 bp long with 334 parsimony informative characters (17%).

Phylogenetic relationships

Phylogenetic trees were maximally topologically congruent for the individually analysed ITS and *trnK 5*′ intron datasets, although the latter provided no resolution between maximally supported clades, forming a polytomy including a monophyletic *V. rubra* and two maximally supported clades of *V. triptera* (data not shown). We therefore concatenated and analysed both datasets as a single matrix (Fig. 2). The 50% consensus tree showed strongly supported major clades (posterior probability (PP) = 1.0). The phylogeny shows the well supported African/Eurasian clade of *V. spiralis* + *V. denseserrulata* is sister to a maximally supported clade (PP = 1.0) containing all other included species. The maximally supported clade (PP = 1.0) containing accessions of *V. caulescens* is sister to an unsupported clade containing two subclades; an unsupported clade of the caulescent growth form species *V. rubra* + *V. triptera*, and a fully supported clade of rosulate growth form taxa from Australian/Asian/North America (Fig. 2). The *V. rubra* clade is supported as monophyletic, while the weakly supported *V. triptera* clade (PP = 0.90) is divided into two clades representing distributions in either NT (location of type material for *V. triptera*) or the northern Kimberley region of WA (Fig. 2). Based on the strongly supported genetic division between WA and NT specimens of *V. triptera*, coupled with morphology (see below), the two clades are recognised as two species, *V. triptera* s.s. (NT) and the new species *V. kristinae* (WA). Removal of the mostly smaller morphological measurements of *V. kristinae* from the *V. triptera* protologue (Jacobs and Frank 1997), warranted a redescription of *V. triptera*.

Taxonomy

Vallisneria triptera S.W.L.Jacobs & K.A.Frank, *Telopea* 7: 114 (1997).

Type: Northern Territory: c. 5 km W. of Jabiru, Arnhem Highway, 12°39.19′S, 132°48.97′E, 5 May 1996, *S.W.L. Jacobs 7970* (holo (♀) [prepared as two parts]: NSW 430934 (sheet); NSW 706555 (spirit); iso: DNA, MEL, BRI, Z, B, K).

A male specimen was collected from the same locality: *S.W.L. Jacobs 7971* (B, BRI, DNA, NSW, Z).

Illustration. Jacobs & McColl (2011: fig. 6).

Submerged, dioecious, stoloniferous annual. *Roots* fibrous. *Leaves* cauline, alternate to sub-opposite near stem apex, 2–9 cm long, 5–12 mm wide, green to red-green; margins finely toothed at least in the upper half; apex acute; 5–7 major longitudinal veins. *Male plant* with 1 to several inflorescences in each leaf axil; numerous flowers (95–190) enclosed in a spathe; spathe ovate, 2.5–4.5 mm long, 2–3 mm diam. *Male flowers* ovoid, c. 0.5 mm long, 0.5 mm wide, uniseriate; *perianth segments* 3; anthers 2, bisporangiate; filaments appearing fused medially (like forming a ‘Y’), hairs present on the base of the androecium. *Female plant* with 1 to several simple inflorescences in each leaf axils of the upper 1/3 of the shoot, coiled peduncle ranging from 10–30 mm long; spathe thin, translucent, 4.6–5 mm long, enclosing 1 sessile flower; apex obtuse. *Female flowers* 1.2–1.7 mm long, c. 2.4 mm wide; sepals 3, elliptical, 1.6–1.9 mm long, 0.7–0.8 mm wide, apex obtuse; petals 3, inconspicuous, minute; staminodes 3, inconspicuous; stigmas 3, bifid, deeply divided or cleft, shorter than sepals, 15–25% as wide as sepals, covered with minute filiform papillae. *Fruit* ovate in outline, trigonous in transection, 3-winged at margins, wings 2–4 mm at widest point, 17–30 mm long, 5–8 mm diam, smooth, green to red-brown. *Seeds* numerous, narrow-ovoid to ellipsoid, 0.7–0.8 mm long, 0.25–0.3 mm diam, with the surface reticulate with oblong to hexagonal cells with ribbed side walls. **Figure 3.**

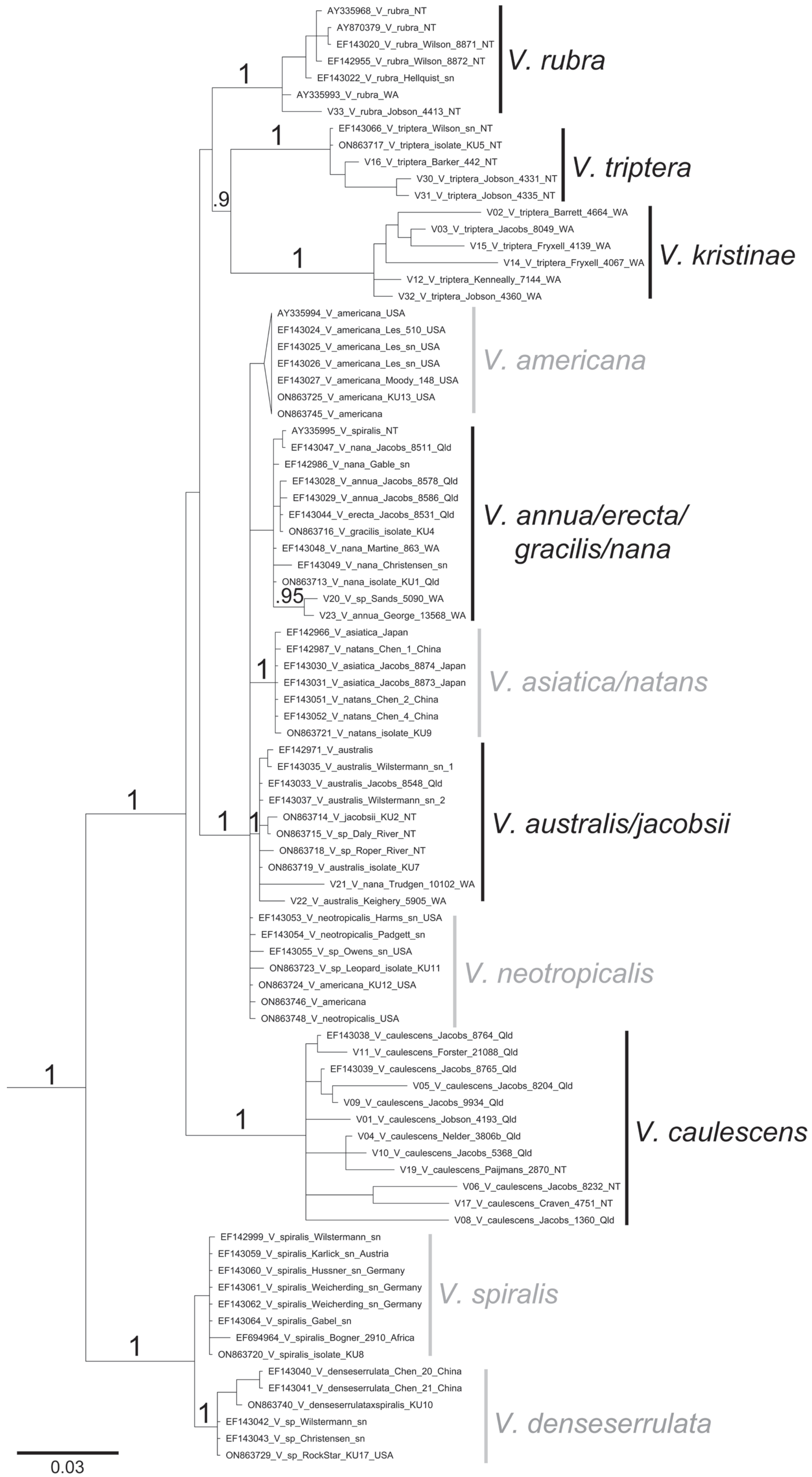

Fig. 2. 50% majority-rule Bayesian inference consensus tree for concatenated ITS/*5'trnK* data. Posterior probability (PP) support values are shown above important branches. PP = 0.95–1.00: strong support; 0.84–0.94: weak support (not shown); unsupported (not shown). Bars show clades representing taxa of Australian (black) and exotic (grey) *Vallisneria*.

Fig. 3. Holotype of *Vallisneria triptera* S.W.L.Jacobs & K.A.Frank (NSW 430934).

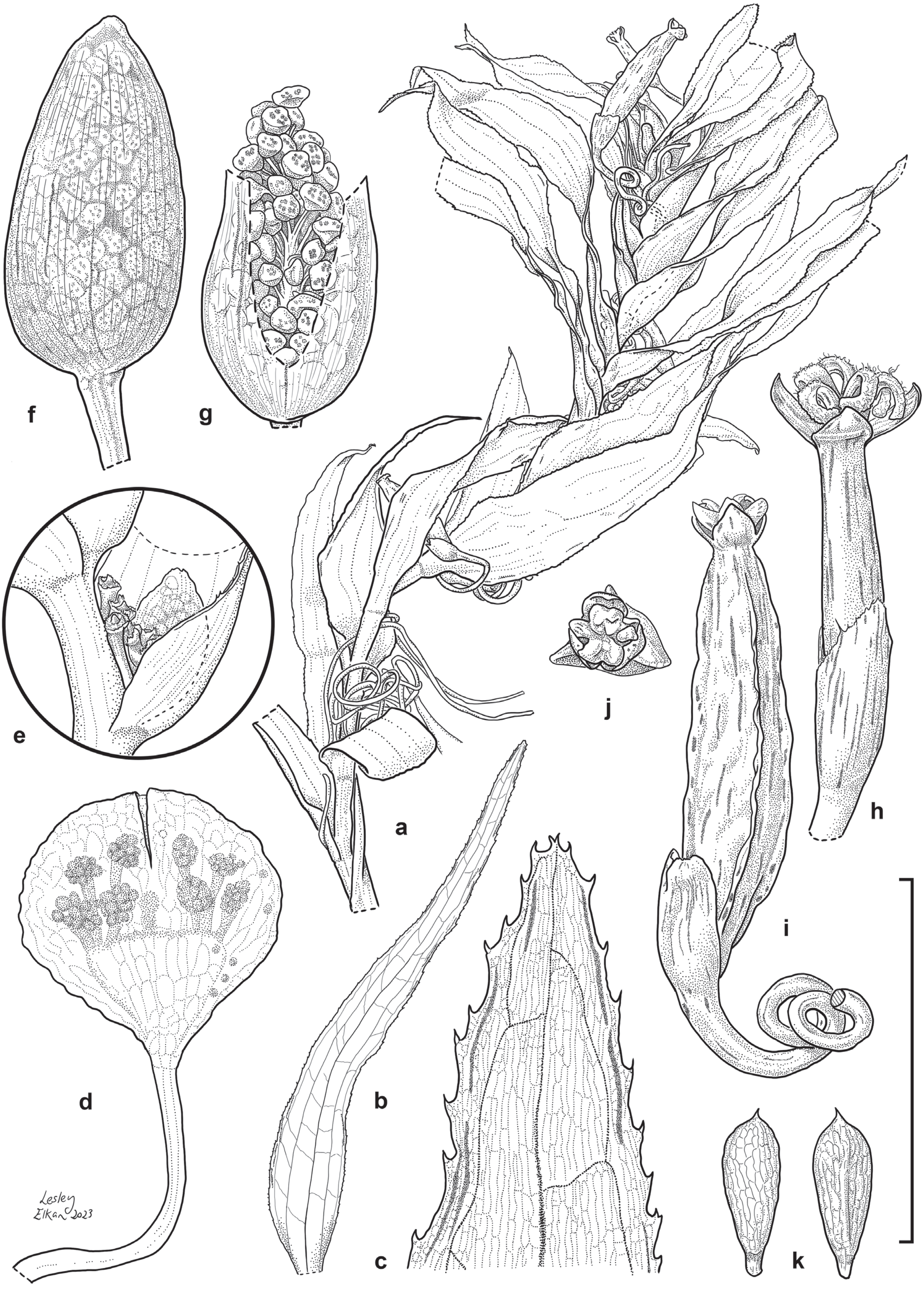

Fig. 4. *Vallisneria kristinae*. a, habit (female plant); b, leaf; c, leaf apex; d, male flower; e, leaf axil showing male spathe *in situ*; f, male spathe; g, male spathe partially excised showing male flowers; h, female flower side view; i, fruit lateral view; j, fruit dorsal view; k, seed frontal and rear view. Scale bar: a & b = 30 mm; c = 2.5 mm; d = 0.4 mm; e, i & j = 10 mm; f & g = 3.3 mm; h = 5 mm; k = 1.5 mm. Material used: *R.W. Jobson 4360 & L. Dunning* (NSW 963090 - spirit). Illustration by Lesley Elkan.

Additional specimens examined. Northern Territory, Darwin & Gulf: Arnhem Highway, 31 km E of South Alligator River, 6 May 1983, *R.M. Barker 442* (NSW 387144); Gulungul Ck, N of Arnhem Hwy, 13 Mar 1986, *I.D. Cowie 307* (DNA); between Mt Brockman and Jabiru, 21 Feb 1973, *L.A. Craven 2353* (CANB); Nanambu Ck, 27 Mar 1981, *L.A. Craven 6575* (CANB); Mt Brockman, 21 Feb 1973, *C.R. Dunlop 3325* (DNA); Baralil Creek, Kakadu National Park, 15 May 1987, *S.W.L. Jacobs 4994* ♂, *4995* ♀, *4996* ♂, *5003* ♂, *5004* ♀, *G. Sainty & K.L. Wilson* (NSW).

Distribution, Ecology & Phenology. Occurs in the vicinity of Jabiru in the Northern Territory (Fig. 1). It inhabits freshwater ephemeral creeks, waterholes, swamps or billabongs. Flowers from February to May and sets fruit from April to July.

Notes. When observed in the field, *V. triptera* is unlikely to be confused with any other species due to its broadly winged, trigonous mature fruit (Fig. 3). This is particularly the case when compared to the thinly winged trigonous fruit of *V. kristinae* (Fig. 4), the bipartite fruit of *V. caulescens*, and the cylindrical fruit of *V. rubra* (Jacobs & McColl 2011, 2023).

Conservation status. The current study proposes to geographically delimit *V. triptera* to a few sites in the vicinity of Jabiru, NT (Fig. 1). Based on previous surveys and collections, *V. triptera* is likely very localised, and very rare at the known sites. Several of the sites are within crown land at Jabiru, while others are within the boundary of Kakadu National Park. It is currently considered Near Threatened in the Northern Territory, under the *Territory Parks and Wildlife Conservation Act* (Woinarski & Wunderlich 2014) and we recommend a reassessment of this conservation status.

Vallisneria kristinae R.W.Jobson & Dunning, ***sp. nov.***

Type: Western Australia: Scull Creek camp and trap site, Doongan Station, 15°12′35.8″S, 125°43′39.2″E, 24 Apr 2008, *R.L. Barrett RLB 4664* (holo (♀): NSW929006; iso (♀): PERTH 08103259).

Diagnosis. Similar to *V. triptera* with trigonous, winged fruit, but differs in having fruit only weakly winged, and lanceolate in outline, 10–15 mm long; leaves up to 50 mm long; and male flowers 0.2 mm long.

Submerged, dioecious, stoloniferous annual. *Roots* fibrous. *Leaves* cauline, alternate to subopposite near stem apex, 20–50 mm long, 5–9 mm wide, red-green, entire margin finely toothed; apex obtuse or acute, with 3–5 major longitudinal veins. *Male plant* with 1–4 inflorescences in each leaf axil; numerous flowers (120–180) enclosed in each spathe; spathe ovate, 2.8–3.0 mm long, 1.4–1.7 mm diam. *Male flowers* ovoid, c. 0.2 mm long, c. 0.25 mm wide; *perianth segments* 3 (only observed in fused state); anthers 2, bisporangiate, filaments not clearly observed. *Female plant* with 1–5 inflorescences per leaf axil of the upper 1/3 of the shoot, coiled peduncle ranging from 10–100 mm long; spathe enclosing ¾ of ovary, 1 sessile flower, 3.1–3.6 mm long, obtuse, thin, translucent. *Female flowers* 1.1–1.3 mm long, c. 1.4 mm wide; *sepals* 3, ovate, c. 0.9 mm long, c. 0.4 mm wide, apex acute; *petals* 3, inconspicuous, minute; staminodes 3, inconspicuous; stigmas 3, bifid, deeply divided, ± length of sepals, upper surface minutely papillose. *Fruit* lanceolate in outline, trigonous in transection, 3-winged at margins, 10.0–15.2 mm long, 1.9–2.2 mm diam, wings 0.08–0.12 mm at widest point, surface slightly undulate, green with red flecks. *Seeds* numerous, ovoid, 0.45–0.60 mm long, 0.20–0.21 mm diam., with reticulate surface. **Figure 4**.

Etymology. The specific epithet *kristinae* honours Mrs Kristina McColl (nee Frank; curator at the National Herbarium of New South Wales, Botanic Gardens of Sydney) whose work on *Vallisneria* was integral in defining *V. triptera* and establishing a taxonomic treatment of the genus in Australia.

Additional specimens examined. Western Australia: Gardner: Kalumburu Community, 3 Apr 2000, *K.F. Kenneally 9820* (PERTH); Airfield Swamp, c. 5 km N of Mining Campsite, Mitchell Plateau, 21 Apr 1982, *K.F. Kenneally 8043* (CANB); Airfield Swamp, c. 5 km N of Mining Campsite, Mitchell Plateau, 20 Apr 1996, *S.W.L. Jacobs 8044* (NSW 406176); Mitchell R. above falls, 19 May 1996, *S.W.L. Jacobs 8041* (NSW); Carson River Station, 14 km S of Carson River Homestead, 15 May 1983, *P.A. Fryxell 4139 & L.A. Craven* (CANB); near Mining Campsite, Mitchell Plateau, 11 May 1983, *P.A. Fryxell 4067 & L.A. Craven* (CANB); Airfield Swamp, Mitchell Plateau, 20 May 2023, *R.W. Jobson 4360 & L.T. Dunning* ((a) female plants; (b) male plants; NSW 963090); c. 24 km WSW of old Theda Homestead on the Kalumburu Road, 16 Apr 2015, *R.W. Jobson 2672 & W. Cherry* (NSW 934780).

Distribution, Ecology & Phenology. Restricted to the Kimberley region of Western Australia (Fig. 1). Inhabits shallow freshwater ephemeral creeks and swamps (Fig 6). Flowers from February to May with mature fruit observed between April and July.

Conservation status. Although there are 14 collections from across the North Kimberley region, most are from the same vicinity at Airfield Swamp, suggesting it is likely to be regionally and locally rare. Eight of the collected specimens represent several sites that are conserved within the Mitchell River National Park at Mitchell Plateau (Airfield Swamp), while all other accessions were collected from non-protected leasehold land from near Kalumburu (*Kenneally 9820*), south-west to just north of Prince Regent National Park (*R.L.Barrett 4664*). Further field survey of this species both locally and across its distribution is required for better assessment of conservation status. We therefore recommend that this species be listed as Data Deficient in WA under either Priority code P2 or P3.

Discussion

The Bayesian analysis of the combined ITS + *trnK 5′* intron shows that samples of each of the three species *V. rubra*, *V. kristinae* and *V. triptera* each form a maximally supported monophyletic clade (PP = 1.0; Fig. 2). The distribution of *V. rubra* overlaps that of both of the latter species providing the possibility one of these species is derived through hybridization with *V. rubra*. When analysed individually the maternally inherited plastid *trnK 5′* dataset provided no resolution between maximally supported clades within a polytomy representing a monophyletic *V. rubra*, *V. kristinae*, and *V. triptera* respectively (data not shown). Under a scenario of hybridization between *V. rubra* and either *V. kristinae* or *V. triptera*, we would expect a closer relationship with its theoretical maternal parent. The individually analysed ITS phylogeny shows the same phylogenetic topology as that of the combined tree and provides the information that led to the ancestral relationships between the three taxa (Fig. 2). Under the likely scenario in which the nuDNA ITS is biparentally inherited, and the plastid *trnK 5′* is maternally inherited we would expect to see any hybridization drive discordance in the topology of, or within, the three clades. However, apart from the lack of resolution in the plastid tree, we observed no discordance between the two tree topologies at an intraspecific level, discounting the possibility of recent hybrid origins (data not shown). Regardless, a single observation of potential hybridization between *V. rubra* and *V. triptera* was recorded by L.A. Craven in 1983 (*Craven 6576*, NSW 691255) where these species co-occur in a small creek near Jabiru, Northern Territory (Les et al. 2008; Martin & Mort 2023). This potential hybrid specimen (*Craven* 6576) has not been included in previous molecular phylogenetic studies, and although we were able to amplify and sequence it for the paternally inherited nuclear ITS (sequence available upon request), we failed to amplify the maternally inherited *trnK 5′*. Although the ITS sequence was found to be nested within the *V. rubra* clade, we also observed several polymorphisms in the ITS chromatogram linking it to *V. triptera* (data not shown). For this reason, we did not include *Craven 6576* in the current phylogeny, and we intend on presenting a more thorough analysis in future work. There have been no observations of such co-occurrence or potential hybridization occurring in Western Australian populations of *V. rubra* and *V. kristinae*.

All three species, *V. rubra*, *V. kristinae* and *V. triptera*, have a cauline vegetative growth form, anchored to the substrate via fibrous roots. The overall plant habit of *V. kristinae* and *V. triptera* is generally more robust (length of leaves ranging from 20–90 mm) than *V. rubra*, (leaves 20–30 mm long). Both *V. kristinae* and *V. triptera* differ from *V. rubra* in the shape of their fruit being trigonous 3-winged vs. non-winged and cylindrical (Fig. 5; McConchie 1983; Jacobs & Frank 1997; Jacobs & McColl 2011).

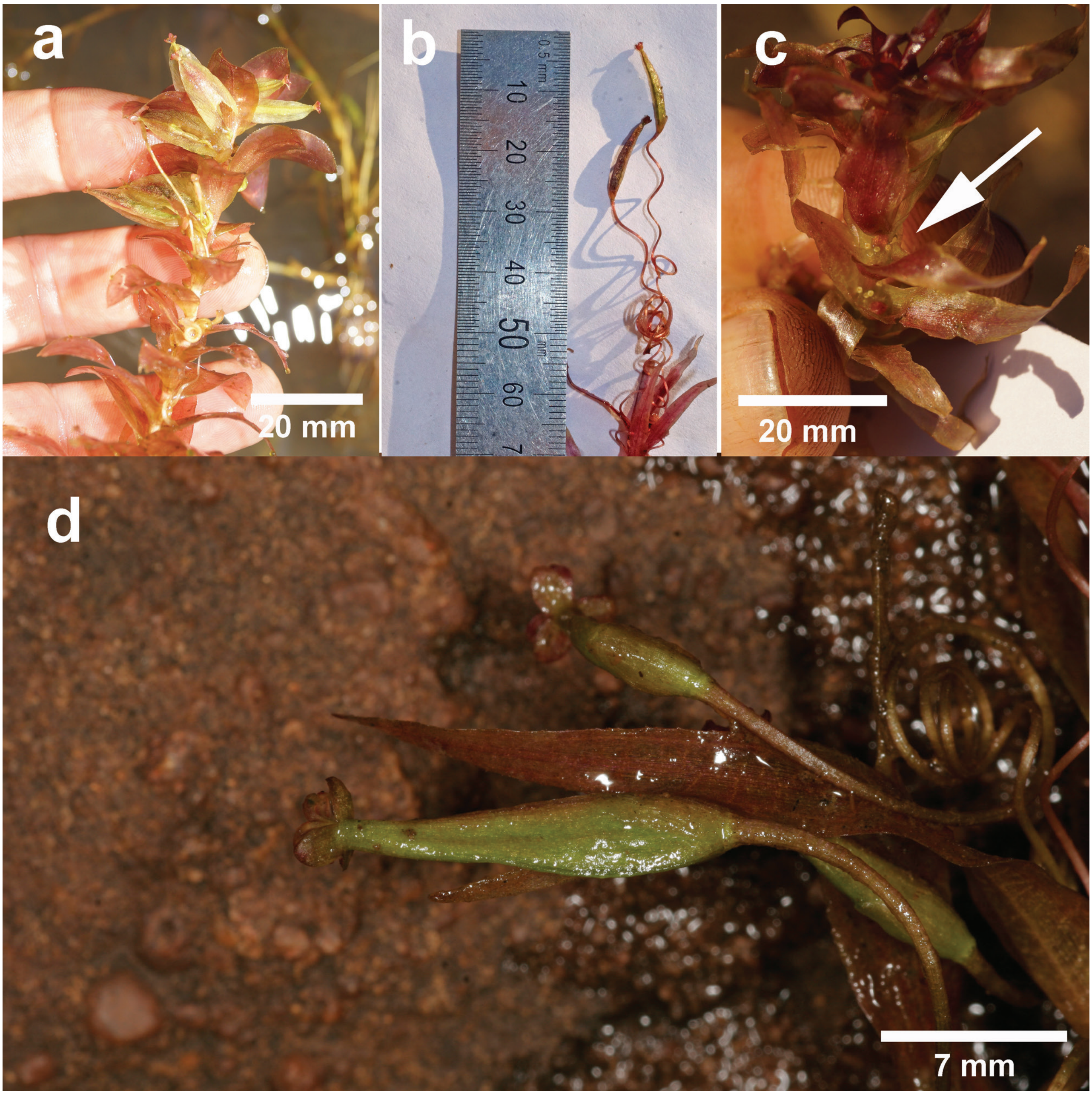

Fig. 5. Mature fruit of *Vallisneria triptera* a, *V. kristinae* b. Male plant of *V. kristinae* with spathe of flowers in leaf axil (arrow) c. mature fruit of *V. kristinae* at Theda Station d. Images: a = R. Jobson (*R.W. Jobson 4336 & L. Dunning*); b–c = R. Jobson (*R.W. Jobson 4360 & L. Dunning*); d = R. Barrett (*R.L.Barrett RLB 4664*). Scale bars shown.

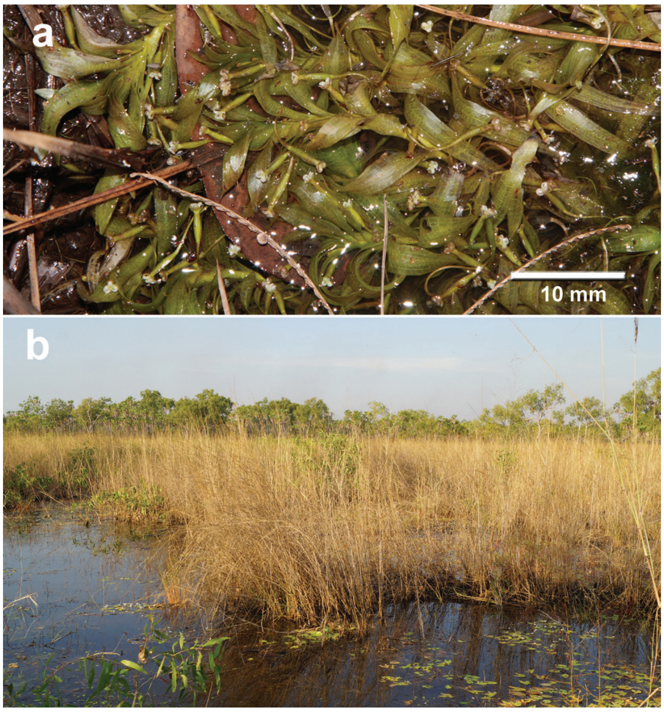

Fig. 6. *Vallisneria kristinae* habit at type site (a) and habitat on Mitchell Plateau (b). Images: a = R. Barrett (*R.L. Barrett RLB 4664*); b = R. Jobson (*R.W. Jobson 4360 & L. Dunning*). Scale bars shown.

Vegetative and sexual organs of *V. kristinae* are generally smaller than those of *V. triptera* (Figs 5 & 7). Female flowers of *V. kristinae* are c. 1.4 mm wide with ovate sepals (c. 0.9 mm long, c. 0.4 mm wide) vs. flowers of *V. triptera* c. 2.4 mm wide with elliptical sepals (1.6–1.9 mm long, 0.7–0.8 mm wide). Male flowers of *V. kristinae* are also smaller (0.2 mm long, c. 0.25 mm wide) than those of *V. triptera* (<0.5 mm long, 0.5 mm wide; Fig. 7).

Vallisneria kristinae is similar to *V. triptera* with both possessing trigonous 3-winged fruit. However, the most salient difference between the two species is that *V. kristinae* has the fruit weakly winged, lanceolate in outline and small (10–15 mm long, 1.9–2.2 mm diam.) while in *V. triptera* they are broadly winged, ovate in outline, and larger (17–30 mm long, 5–8 mm diam). Seeds are also smaller in *V. kristinae* (0.45–0.60 mm long, 0.20–0.21 mm diam.) vs. those of *V. triptera* (0.7–0.8 mm long, 0.25–0.3 mm diam.) (Fig. 7).

Although the original description of *V. triptera* (Jacobs & Frank 1997) accounts for the overall small vegetative and sexual organs of the Kimberley plants (i.e., *V. kristinae*), it does not include the weakly winged mature fruit and small seeds. This is likely due to the observed rarity of mature fruit on specimens collected in WA with the few observable fruit probably assumed by the authors to be large, immature flowers.

Our *Vallisneria* phylogeny based on the combined sequence data (Fig. 2) supports the results of Martin and Mort (2023). The phylogeny shows the newly described species *V. jacobsii* nested within a clade containing accessions of *V. australis*. In this study we have not been able to examine morphological characters for inclusion of *V. jacobsii* in our key. Our main concern involved the taxonomic description of Martin and Mort (2023) that states the basal leaf arrangement 'appears distichous' as opposed to singularly rosulate in *V. australis*. Based on the degree of doubt regarding the presence of this key character, the populations of *V. jacobsii* requires further examination.

Key to species of *Vallisneria* in Australia (modified from Jacobs and McColl 2011)

1a Leaves all cauline; facultative annuals **2**

1b Leaves basal, tufted; annuals or perennials.......... **5**

2a Fruit not winged, leaf blade nearly filiform (0.1–0.2 mm wide) .... ***V. rubra***

2b Fruit winged, bi- or tripartite in transverse section, leaf blade flat (4.5–15 mm wide) **3**

3a Fruit 2-winged, flattened, usually >40 mm long; female flowers with 2 perianth segments .... ***V. caulescens***

3b Fruit 3-winged, trigonous; female flowers with 3 perianth segments **4**

4a Mature fruit broadly winged, 7.0–8.0 mm wide, 17–30 mm long .... ***V. triptera***

4b Mature fruit slightly winged, 3.0–3.5 mm wide, 10–15 mm long .... ***V. kristinae***

5a Plants facultatively annual (commonly in ephemeral waterbodies); leaf-tips long-acute; female tufts commonly producing numerous (>12) flowers at maturity in first year while still quite small.......... ***V. annua***

5b Plants normally perennial; leaf tips acute or obtuse; female tufts not flowering until plant is several years old and then only producing few flowers (<12) at any one time **6**

6a Stranded plants with erect, stiff, acute leaves; plants in deeper water with obtuse leaves (Daintree R., N Qld) .... ***V. erecta***

6b All leaves lax, and either all acute or all obtuse.......... **7**

7a Leaves obtuse; filaments in male flower fused for about 75% of their length; leaves often >10 mm wide .... ***V. australis***

7b Leaves acute, with or without a fine apical mucro; filaments in male flower free to fused for about 75% of their length; leaves often <1 cm wide **8**

8a Leaves usually 3–10 mm wide, mostly obtuse, not with a fine apical mucro; various habitats of the Australian Monsoon Tropics and sub tropics.......... ***V. nana***

8b Leaves <3 mm wide, with a fine apical mucro; plants of fast-flowing shallow water of the Australian Wet Tropics biome, often in shaded habitats .... ***V. gracilis***

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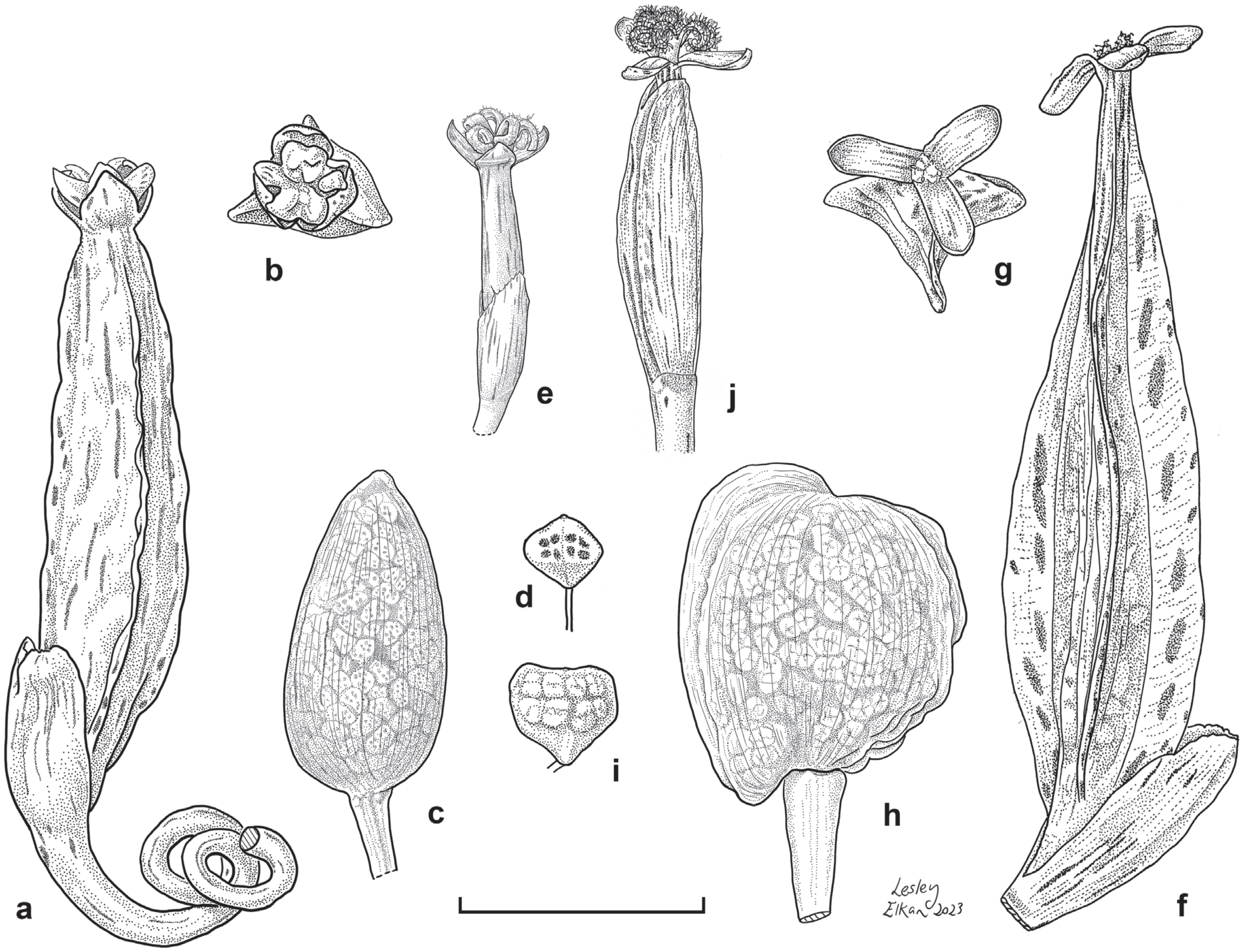

Fig. 7. Comparison of *Vallisneria kristinae* (a–e), *V. triptera* (f–i), a & f, fruit in situ; b & g, fruit dorsal view; c & h, male spathe; d & i, male flower lateral view; e & j, female flower lateral view. Scale bar: a, b, e, f, g, j = 5 mm; c & h = 2.5 mm; d & i = 1.0 mm. Material used: a–e = *R.W. Jobson 4360 & L. Dunning* (NSW 963090 - spirit); f, g, j = *S.W.L. Jacobs 4994* (spirit 3233; NSW 387142); h, i = *R.W. Jobson 4335* (NSW 963079).

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