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Potamogeton ×jacobsii (Potamogetonaceae) from New South Wales, Australia – the first *Potamogeton* hybrid from the Southern Hemisphere

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Abstract

Although more than 80 hybrids are documented for *Potamogeton* in the Northern Hemisphere, the hybrid diversity in the Southern Hemisphere is largely unknown. A taxonomically uncertain *Potamogeton* plant discovered in New South Wales, Australia, was subjected to detailed morphological investigation and molecular analyses to discover its exact identity. Both approaches resulted in the identification of the *Potamogeton* specimen as *P. crispus* × *P. ochreatus*, which is a previously unrecorded hybrid and is here described as **Potamogeton × jacobsii** nothosp. nova. Although *P. crispus* is known to hybridise with many other species, *P. ×jacobsii* is the first known hybrid involving *P. ochreatus*.

Introduction

The occurrence of hybridisation in pondweeds (*Potamogeton* and *Stuckenia*, Potamogetonaceae) has been known for more than a century (see the reviews in Wiegleb et al. 2008 and Kaplan et al. 2009). Besides traditional morphological comparisons, stem anatomy (e.g., Raunkiær 1896, 1903; Fischer 1904, 1905, 1907; Hagström 1916; Ogden 1943; Symoens et al. 1979; Wiegleb 1990a, 1990b; Kaplan 2001, 2005a, 2005b; Kaplan & Symoens 2004, 2005; Zalewska-Gałosz et al. 2010), isozyme electrophoresis (e.g., Hollingsworth et al. 1995, 1996; Preston et al. 1998; Fant et al. 2001a, 2001b; Iida & Kadono 2002; Kaplan et al. 2002; Fant & Preston 2004; Kaplan & Wolff 2004; Kaplan 2007) and DNA-based techniques (King et al. 2001; Fant et al. 2003; Kaplan & Fehrer 2004, 2006, 2007, 2009; Ito et al. 2007; Wang et al. 2007; Du et al. 2009; Kaplan et al. 2009; Zalewska-Gałosz et al. 2009, 2010) have all contributed to our understanding of diversity, morphological variation and distribution in Potamogetonaceae hybrids.

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Table 1. Samples used in the molecular analyses.

Taxon	Ref. no.	Origin and collection records	ITS GenBank no.	*rpl*20-5′-*rps*12 GenBank no.
P. crispus	1463	Czech Republic, Bohemia, Bohuslavice, coll. *Z. Kaplan 03/121* (PRA)	GU814242	GU814253
	1464	Czech Republic, Bohemia, Velká Jesenice, coll. *Z. Kaplan 03/122* (PRA)	GU814243	GU814254
	1472	Germany, Bavaria, Ebing, coll. *L. Meierott*, cult. *Z. Kaplan 1472* (PRA)	AY529523	GU814255
	1473	Czech Republic, Bohemia, Poděbrady, coll. *Z. Kaplan 03/142* (PRA)	AY529524	GU814256
	2022	India, Jammu and Kashmir State, Manasbal Lake, coll. *A. H. Ganie*, cult. *Z. Kaplan 2022* (PRA)	GU814244	GU814257
	2215	Australia, West Australia, Joondalup Lake, coll. *M. Moody WP271* (PERTH)	GU814245	HM852142
P. ×jacobsii	2034	Australia, New South Wales, Narellan, coll. *C. B. Hellquist 17201 & G. Sainty* (PRA)	GU814246–GU814249	GU814258
P. ochreatus	1071	New Zealand, North Island, Lake MacLaren, coll. *D. Hofstra*, cult. *Z. Kaplan 1071* (PRA)	GU814250	GU814259
	1072	New Zealand, North Island, Lake Rotoaira, coll. *D. Hofstra*, cult. *Z. Kaplan 1072* (PRA)	GU814251	GU814260
	2214	Australia, West Australia, Gwellup Lake, coll. *M. Moody WP270* (PERTH)	GU814252	HM852141

Table 2. Comparison of the most important diagnostic characters of *Potamogeton crispus*, *P. ochreatus* and their hybrid, *P. ×jacobsii*.

Character	*P. crispus*	*P. ×jacobsii*	*P. ochreatus*
Shape of leaves	linear-oblong	linear to linear-oblong	linear
Length of leaves (mm)	25–95	28–70	35–90
Width of leaves (mm)	(4–)6–12	4–9	1.7–5.2
Length:width ratio of leaves	5–9(–13)	6–14	13–28
Shape of leaf apex	acute or subobtuse to rounded	rounded to truncate or slightly retuse, not mucronate	obtuse or rounded to truncate, often also shortly mucronate at the top
Shape of leaf margin	serrulate and often strongly undulate in adult plants	entire (or rarely only very minutely denticulate) and plane	entire and plane
Presence and number of sclerenchymatous strands in leaves in addition to vascular veins	always absent	0–12, present only in some leaves, very indistinct	(4–)10–34, always present, apparent under the microscope
Shape of stem in cross-section	compressed and shallowly grooved	slightly compressed and shallowly grooved	terete

Although much attention has been paid to the identification of Potamogetonaceae hybrids in taxonomic research, our knowledge is confined to relatively few regions. In their recent worldwide revision of *Potamogeton* including *Stuckenia*, Wiegleb and Kaplan (1998) listed 50 confirmed hybrids, all from the Northern Hemisphere. The number of identified *Potamogeton* hybrids has increased to more than 80 (Z. Kaplan, unpubl.). A higher frequency and diversity of pondweed hybrids is associated with regions with specific ecological characteristics associated with postglacial dynamics (Kaplan 2007, Kaplan et al. 2009). These centres of hybridisation in *Potamogeton* and *Stuckenia* are mainly in the northern half of Europe, Japan, and eastern North America (Hagström 1916, Ogden 1943, Preston 1995, Wiegleb & Kaplan 1998, Kaplan et al. 2009). *Potamogeton* hybrids have been, however, occasionally recorded from other regions and it can be assumed that they may also occur in areas where no targeted searches have been performed yet.

In this paper, we focus on the identity of a recently discovered *Potamogeton* plant in New South Wales (*Hellquist 17201 & Sainty*), which showed morphology that was not consistent with the normal range of variation of Australian species, and did not fit the characteristics of any recognised taxon worldwide (Wiegleb & Kaplan 1998). We employed a detailed investigation of morphology combined with molecular analyses to reveal the exact origins and identity of the *Potamogeton* plant.

Material and Methods

Plant material

The new plant, hereafter referred to as *P.* ×*jacobsii*, was collected during fieldwork in New South Wales, Australia. The site was a water-way running through a housing complex. The plant was growing in shallow water, not associated with any other *Potamogeton* taxon. Additional material used for comparison came from Australia, Europe, Asia and North America (*P. crispus* L.) and Australia and New Zealand (*P. ochreatus* Raoul). Plants of both *P. crispus* and *P. ochreatus* were cultivated in the Experimental Garden at the Institute of Botany, Průhonice, Czech Republic, and tested for phenotypic plasticity in order to better understand the origin of morphological variation (see also Kaplan 2002). In addition, herbarium specimens from around the world were investigated (for the complete list of herbaria see Kaplan 2008 and Kaplan 2010). Specimens included in the molecular analyses are summarised in Table 1. Most voucher specimens are held in the herbarium of the Institute of Botany, Průhonice (PRA) but vouchers for two samples collected by Michael Moody are lodged in the Western Australian Herbarium, Perth (PERTH).

Morphological evaluation

Morphological observations were made on recently collected herbarium material. Details of leaf morphology were studied using a stereomicroscope at a magnification of 20–75×. Abundant morphological data compiled for our previous studies were also considered in order to cover the morphological variation of the respective species as completely as possible. Only a brief description, mainly consisting of diagnostic characters, is given here for *P.* ×*jacobsii*. The most important diagnostic characters are shown in microphotographs and summarised in Table 2 to facilitate comparison. Rare extremes were excluded from the quantitative data.

Identification of hybrids

Potamogeton and *Stuckenia* hybrids are generally intermediate between the parental species (e.g., Hagström 1916, Preston 1995, Wiegleb & Kaplan 1998). Although character expression in hybrids is generally unpredictable (Rieseberg & Ellstrand 1993, Kaplan et al. 2009), several previous molecular studies (Hollingsworth et al. 1995; Kaplan et al. 2002; Fant & Preston 2004; Kaplan & Fehrer 2004, 2006; Kaplan & Wolff 2004; Kaplan 2007) have demonstrated that for most *Potamogeton* and *Stuckenia* hybrids, either the parental species themselves or at least their respective species group can be reliably identified morphologically, as long as adequate expert inspection of a large set of key features is adopted. However, molecular proof of identification of *Potamogeton* hybrids is always advisable (Kaplan & Fehrer 2007, 2009; Kaplan et al. 2009), particularly if a previously unknown hybrid combination is suspected.

Our previous studies have revealed a very low level of intra-individual polymorphism in the multicopy nuclear ribosomal ITS region for *Potamogeton* species as well as relatively little intraspecific variation while hybrids, the majority of which are sterile, maintained the ribotypes of their respective parents (Kaplan & Fehrer 2007, 2009; Kaplan et al. 2009). These features provide an opportunity for the use of ITS sequencing as a very helpful tool in the detection of hybrids, which show additive patterns of their parents, i.e., superimposed peaks and insertions/deletions (indels) at diagnostic positions (Kaplan & Fehrer 2007, Kaplan et al. 2009). We therefore performed direct ITS sequencing, complemented by cloning of the hybrid accession, in order to identify both parents. The maternal parent of the hybrid was revealed by sequencing of the *rpl*20-5'*rps*12 intergenic spacer region from chloroplast DNA.

Molecular analyses

DNA from the majority of samples was isolated from CTAB-preserved leaves (Štorchová et al. 2000) and used for both ITS and *rpl*20-5'*rps*12 sequencing. Isolated DNA of samples 2214 and 2215 was obtained from Michael Moody (Crawley, Australia). PCR amplifications and direct sequencing of the ITS and *rpl*20-5'*rps*12 regions were done as described in Kaplan & Fehrer (2004, 2006). To investigate the possible hybrid origins of

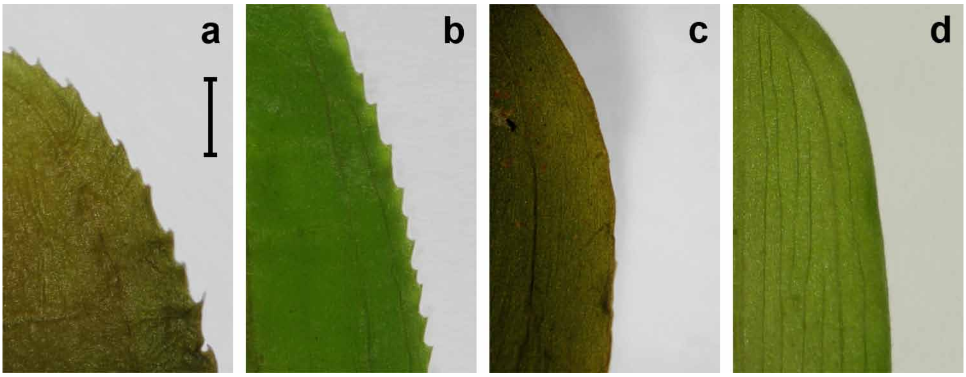

Fig. 1. Shape of leaf margin in *Potamogeton*. **a,** *P. crispus* (*Kaplan 08-384*); **b,** *P. crispus* (*Kaplan 08-646*); **c,** *P.* ×*jacobsii* (*Hellquist 17201 & Sainty*); **d,** *P. ochreatus* (*Kaplan 1071*). Scale bar identical for all figures: 1 mm.

P. ×*jacobsii*, the ITS region was also cloned using a pooled sample consisting of three separate PCR reactions to ensure representative amplification of the parental copies (Fehrer et al. 2009). Four clones were sequenced with the forward PCR primer and compared with each other and with direct sequencing to identify *Taq* DNA polymerase errors. GenBank accession numbers of all sequences are included in Table 1.

Results

Morphological evaluation

The new *Potamogeton* plant (*P.* ×*jacobsii*) resembles *P. crispus* in its general appearance, branching pattern and basic superficial features such as shape and colour of leaves, however, it differs in other characters of the leaves. Leaves of *P. crispus* have several characteristic features that are unlikely to be confused with those of any other species. In particular, they are serrulate (Figs 1a & 1b), with teeth that are visible to the naked eye, and often strongly undulate along the margins. In contrast, the leaves of *P.* ×*jacobsii* are basically entire (Fig. 1c) and plane, as in *P. ochreatus* (Fig. 1d). The venation of *P. crispus* leaves usually consists of two veins on each side of the midrib,

Fig. 2. Arrangement of vascular veins in *Potamogeton* leaves. **a,** *P. crispus* (*Kaplan 07-41*); **b,** *P.* ×*jacobsii* (*Hellquist 17201 & Sainty*). Scale bar identical for both figures: 3 mm.

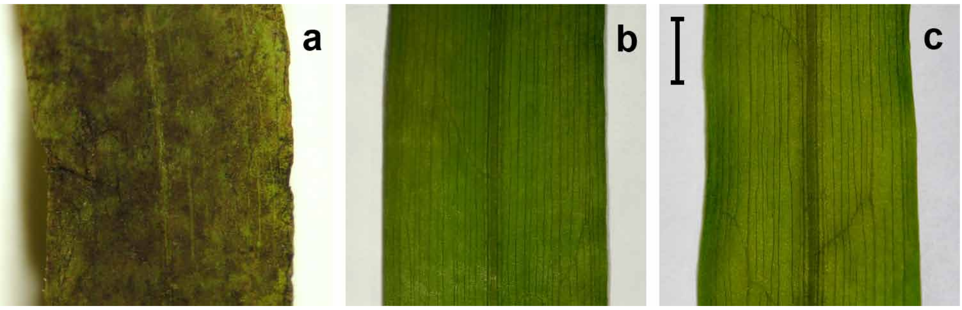

Fig. 3. Occurrence of sclerenchymatous strands in leaves of *Potamogeton*. **a,** *P.* ×*jacobsii* (*Hellquist 17201 & Sainty*); **b & c,** *P. ochreatus* (*Kaplan 1071*). Scale bar identical for all figures: 1 mm.

with the outer pair of veins being faint and running close along the margins and the inner pair being more prominent and running longitudinally at approximately ½–⅔ of the distance between the midrib and the margins (Fig. 2a). This venation pattern can be observed also in *P.* ×*jacobsii* (Fig. 2b), but some leaves contain also faint and irregular sclerenchymatous strands in addition to the vascular veins (Fig. 3a), which is clearly a character inherited from *P. ochreatus*, the only Australian species showing this structure (Fig. 3b). The leaves of *P.* ×*jacobsii* are also similar to those of *P. crispus* in size, resembling narrow-leaved forms of this species (Figs 4a & 4b), but in the hybrid they are often truncate or slightly retuse at the apex (Figs 4c & 4d), and thus more similar to that of *P. ochreatus* (Figs 4e & 4f). Hybrids of *P. crispus* often differ from their parents in the shape of the stem in cross-section (e.g., Hagström 1916, Preston 1995, Kaplan & Fehrer 2004, Alix & Scribailo 2006). The stem of *P. crispus* is compressed and shallowly grooved whereas that of *P. ochreatus* is terete. As far as it can be observed in the pressed specimen, the stem of the hybrid is also compressed, at least in some sections, but

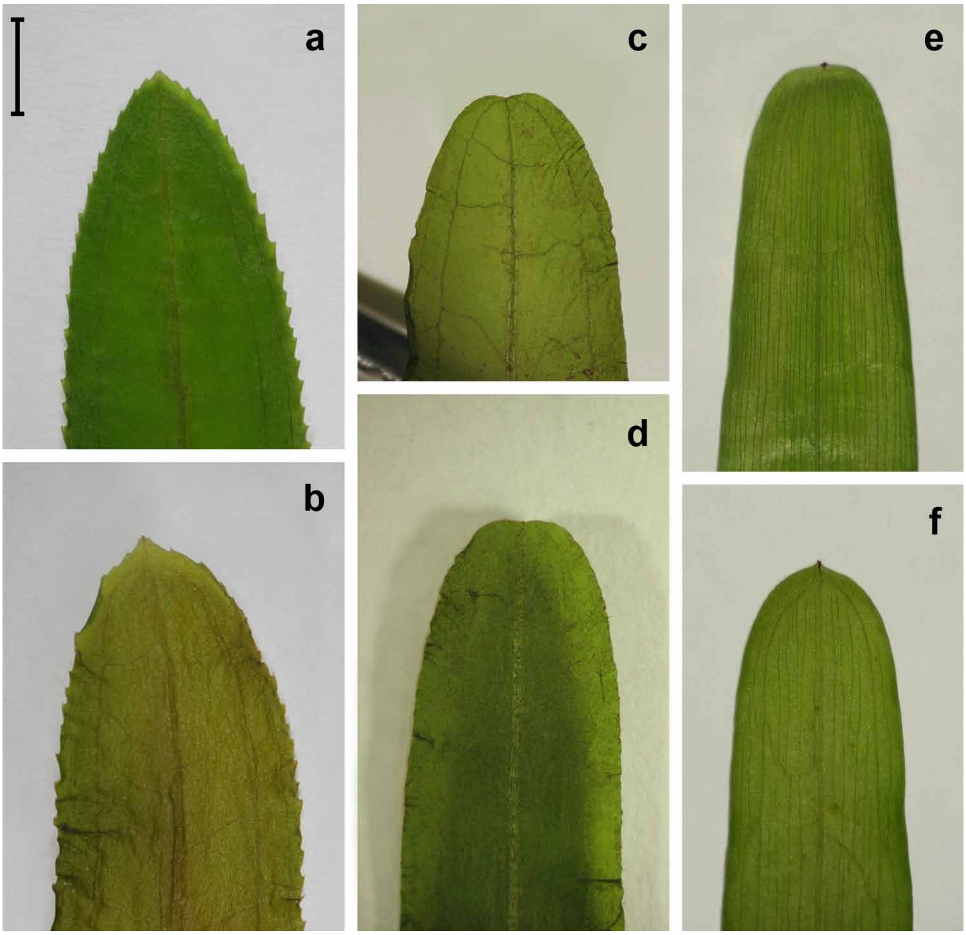

Fig. 4. Shape of leaf apex: **a,** *P. crispus* (*Kaplan 08-646*), **b,** *P. crispus* (*Kaplan 08-384*), **c & d,** *P.* ×*jacobsii* (*Hellquist 17201 & Sainty*), **e & f,** *P. ochreatus* (*Kaplan 1071*). Scale bar identical for all figures: 2 mm.

clearly less markedly than is usual in *P. crispus*. These morphological observations clearly indicated that *P. ×jacobsii* shows a combination of characters of *P. crispus* and *P. ochreatus*, most of them being intermediate between these species. The most important diagnostic characters of all three taxa are summarised in Table 2.

Both *P. crispus* and *P. ochreatus* are widespread throughout Australia but more common in temperate areas. All other species or species groups of *Potamogeton* documented from Australia can be easily excluded on morphological grounds as none have the characters that could explain the morphology shown by the putative hybrid. This is particularly true for all broad-leaved and mostly heterophyllous species with elliptical to ovate leaves, which differ by a suite of characters. These species constitute about two thirds of Australian *Potamogeton* species diversity. The only remaining species with linear submerged leaves, *Potamogeton octandrus* Poir., is also unlikely to be involved in the parentage of the investigated hybrid, particularly because the very narrow submerged leaves of the latter species lack the sclerenchymatous strands observed in *P. ×jacobsii*.

Molecular evaluation

Direct ITS sequencing of *P. ×jacobsii* was mostly unreadable due to a large number of superimposed peaks and indel mutations, which is consistent with a hybrid origin. However, the readable 5'-end of the sequence contained 13 substitutions and one diagnostic indel suggesting a contribution of both *P. crispus* and *P. ochreatus*, when compared with our database of *Potamogeton* ITS sequences (Fehrer & Kaplan, unpubl.). Sequencing of four clones of *P. ×jacobsii* revealed that one (clone 11) was identical to *P. ochreatus* and three (clones 3, 6 & 15) were identical or nearly identical to Australian (and Indian) *P. crispus*, which slightly differed from four European accessions of *P. crispus* (Table 3). The ITS sequences of *P. crispus* and *P. ochreatus* (730 bp in alignment) differ by 32 nucleotide substitutions, one 2 bp- and four 1 bp-indels. Molecular data comparisons with other species also allowed us to exclude other putative parents: *P. crispus* is not only morphologically, but also genetically very distinct from all other *Potamogeton* species according to molecular phylogenies based on chloroplast or nuclear markers (Iida et al. 2004, Lindqvist et al. 2006, Zhang et al. 2008, Fehrer & Kaplan, unpubl.). The species most closely related to *P. crispus* are *P. maackianus* A.Benn. and *P. robbinsii* Oakes (Lindqvist et al. 2006), which occur in eastern Asia and North America, respectively (Wiegleb & Kaplan 1998) but not in the Southern Hemisphere. Their ITS sequences differ from *P. crispus* by more than 20 nucleotide substitutions and four indels (Fehrer & Kaplan, unpubl.). Both species can also be excluded on the basis of their morphology. *Potamogeton oxyphyllus* Miq. is genetically most similar to *P. ochreatus* according to our ITS sequences (Fehrer & Kaplan, unpubl.) but no phylogeny including both species has been published to date. Both species differ by four nucleotide substitutions and one indel. *Potamogeton oxyphyllus* can also be excluded on morphological grounds and because it occurs in eastern and southeastern Asia, but not in Australia (Wiegleb & Kaplan 1998).

The chloroplast *rpl*20-5'*rps*12 sequences of *P. crispus* and *P. ochreatus* (totalling 794 bp in alignment), did not show any intraspecific variation and differed by two nucleotide substitutions and one diagnostic 11 bp-indel (Table 4). While the overall variation is expectedly lower than that of the nuclear ITS region, these differences are sufficient to clearly distinguish between *P. crispus* and *P. ochreatus*; the *Potamogeton* hybrid sample had the *P. crispus* haplotype.

Table 3. Nucleotide sequence variation and ITS ribotypes of *Potamogeton crispus*, *P. ochreatus* and their hybrid, *P. ×jacobsii*.

Taxon	Position in alignment[1]																																										
	55	70	75	86	87	130	198	202	222	235	247	249	250	251	264	270	274	403	448	453	459	461	464	465	469	477	513	514	523	525	528	531	567	594	595	602	606	613	655	658	659	662	663
P. ochreatus	T	A	T	A	A	C	A	G	G	T	G	A	C	T	A	T	A	C	C	C	C	A	T	C	T	G	C	C	–	T	C	C	T	C	T	T	G	C	A	C	C	–	–
P. ×jacobsii clone 11	T	A	T	A	A	C	A	G	G	T	G	A	C	T	A	T	A	C	C	C	C	A	T	C	T	G	C	C	–	T	C	C	T	C	T	T	G	C	A	C	C	–	–
P. crispus (Europe)	C	T	A	A	T	T	A	A	C	A	T	T	T	A	G	–	G	T	A	T	T	T	C	T	T	A	T	T	T	T	T	G	–	T	C	C	A	T	C	A	–	C	A
P. crispus (India)	C	T	A	A	T	T	T	A	C	A	T	T	T	A	G	–	K	T	A	T	T	T	C	T	T	A	T	T	T	K	T	G	–	T	C	T	A	T	C	A	–	C	A
P. crispus (Australia)	C	T	A	A	T	T	T	A	C	A	T	T	T	A	G	–	G	T	A	T	T	T	C	T	T	A	T	T	T	T	T	G	–	T	C	T	A	T	C	A	–	C	A
P. ×jacobsii clone 3	C	T	A	G	T	T	T	A	C	A	T	T	T	A	G	–	G	T	A	T	T	T	C	T	T	A	T	T	T	T	T	G	–	T	C	T	A	T	C	A	–	C	A
P. ×jacobsii clone 6	C	T	A	G	T	T	T	A	C	A	T	T	T	A	G	–	G	T	A	T	T	T	C	T	A	A	T	T	T	T	T	G	–	T	C	T	A	T	C	A	–	C	A
P. ×jacobsii clone 15	C	T	A	A	T	T	T	A	C	A	T	T	T	A	G	–	G	T	A	T	T	T	C	T	T	A	T	T	T	T	T	G	–	T	C	T	A	T	C	A	–	C	A

[1] position 86: intra-individual polymorphism in the hybrid sample (also apparent in direct sequencing); positions 198 and 602: intraspecific polymorphisms in *P. crispus* differing between Australian/Indian and European samples; positions 274 and 525: intra-individual polymorphisms in Indian *P. crispus* (K = G or T); position 469: polymerase error in *P. ×jacobsii* clone 6. The alignment starts with the first base after the forward primer sequence. Only positions that differ among the sequences are shown.

Table 4. Nucleotide sequence variation and haplotypes of *Potamogeton crispus*, *P. ochreatus* and their hybrid, P. ×jacobsii in the *rpl*20-5′*rps*12 intergenic spacer region.

Taxon	**Position in alignment**		
(no. of sequences)	**67**	**249–259**	**525**
P. crispus (6)	T	–	C
P. ×jacobsii (1)	T	–	C
P. ochreatus (3)	G	AAAAAATAAAT	T

Discussion

The detailed morphological and molecular analyses led to the conclusion that *P. ×jacobsii* is a hybrid between *P. crispus* and *P. ochreatus* with the former as the maternal parent. Because our previous studies (Kaplan 2002, Kaplan & Fehrer 2004, Kaplan et al. 2009) demonstrated that some extreme phenotypes of true species may mimic hybrids, and vice versa, the definitive and conclusive evidence came from molecular analyses. Because this hybrid combination has never been recorded before (see e.g., Graebner 1907, Hagström 1916, Wiegleb & Kaplan 1998), it is here formally described and a new name is provided:

Potamogeton ×jacobsii Z.Kaplan, Fehrer & Hellq., **nothosp. nova**

(= *P. crispus* L. × *P. ochreatus* Raoul)

Diagnosis. Planta hybrida inter parentes intermedia; primo aspectu *P. crispum* revocans sed differt foliis angustioribus, margine integerrimis, apice truncatis vel retusis, et lineis sclerenchymatosis nerviformibus inter nervos foliorum primarios interjectis; a *P. ochreato* foliis latioribus, ordinatione nervis primariis diversa, lineis sclerenchymatosis multo paucioribus praecipue distinguitur.

Type. Australia: New South Wales: South Coast: Mt. Annan Cascades, Narellan, along shallow edge of lower pond in a series of man-made ponds along stream in housing development, 34°03.125'S, 150°45.237'E, elevation 10 m, 17 February 2009, *C.B. Hellquist 17201 & G. Sainty* (holotype: PRA).

Description. Rhizome slender, creeping. Stem simple to sparingly branched, slender, slightly compressed and shallowly grooved. All leaves submerged, sessile, linear to linear-oblong, 28–70 mm long, 4–9 mm wide, 6–14 times as long as wide, bright green to dark green; base broadly cuneate; margin entire (rarely very minutely denticulate); apex rounded to truncate or slightly retuse (never mucronate); venation of 5 longitudinal vascular veins and either lacking sclerenchymatous strands or with up to 12 additional sclerenchymatous strands (only apparent in some leaves), with a narrow band of lacunae bordering midrib. Stipules axillary, convolute, short, decaying early. Generative organs not available.

Etymology. Named for the late Dr. Surrey W. L. Jacobs, an expert in Australian aquatic plants.

Final Note

A combination of morphological and molecular approaches has recently led to several new discoveries concerning the diversity of *Potamogeton* hybrids. Character additivity of the ITS region has contributed to the discovery and/or exact identification of several entirely new hybrid combinations (Kaplan et al. 2009, Zalewska-Gałosz et al. 2010), detection of the occurrence of a long-overlooked hybrid (Zalewska-Gałosz et al. 2009) and even confirmed the existence of a triple hybrid in *Potamogeton* (Kaplan & Fehrer 2007). It can be expected that more hybrids and other unrecorded components of *Potamogeton* and *Stuckenia* diversity will be discovered in the Southern Hemisphere. Australian field botanists interested in cooperation are encouraged to contact us. We offer our help in both morphological and molecular checking of the identity of unclear *Potamogeton* samples.

Acknowledgments

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