Volume 22: 141–151
Publication date: 3 October 2019
dx.doi.org/10.7751/telopea13434

TELOPEA
Journal of Plant Systematics

The Royal
BOTANIC GARDENS
& Domain Trust

plantnet.rbgsyd.nsw.gov.au/Telopea • escholarship.usyd.edu.au/journals/index.php/TEL • ISSN 0312-9764 (Print) • ISSN 2200-4025 (Online)

Discovery of the Northern Hemisphere hybrid *Potamogeton ×salicifolius* in the Pilbara region of Western Australia

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Abstract

The interspecific hybrid *Potamogeton ×salicifolius* (= *P. lucens* × *P. perfoliatus*), so far known only from several countries of Europe and a few countries of Asia, was discovered in Western Australia. Morphology of the Australian specimens fits the range of variation observed in Eurasian specimens. DNA sequencing confirmed the morphological identification as a hybrid *P. lucens* × *P. perfoliatus*, and identified *P. lucens* as the maternal parent. This identity is surprising because neither of the parental species are known to occur in Western Australia and *P. lucens* currently does not even occur in the continent. Three possible explanations for the occurrence of *P. ×salicifolius* in Western Australia are discussed: its relictual occurrence from a time when both the parental species occurred there, long-distance dispersal of a hybrid seed from Eurasia and the remnant of local cultivation or intentional planting.

Introduction

Adaptations of aquatic plants to their water environments often result in considerable morphological reduction, extensive phenotypic plasticity, and parallel evolution of traits (Sculthorpe 1967; Barrett *et al.* 1993; Kaplan 2002). The taxonomic complexity of aquatic plants is often further increased by interspecific hybridization (Cook 1970; Les and Philbrick 1993; Moody and Les 2007). This makes their taxonomy difficult because species often cannot be easily sorted based on morphology (Kaplan and Symoens 2005; Kaplan 2008; Moody and Les 2010; Kaplan and Marhold 2012; Kabátová *et al.* 2014; Prančl *et al.* 2014, 2018).

The cosmopolitan genus *Potamogeton* L. (Potamogetonaceae) has the highest species diversity among all aquatic plants (Wiegleb and Kaplan 1998). It includes about 72 species and at least 100 hybrids (Kaplan 2010; Kaplan and Fehrer, unpublished data), with the centre of diversity in temperate regions of the Northern Hemisphere. The occurrence of hybrids in this genus was first suggested in the late 19th century (see the reviews in Wiegleb *et al.* 2008 and Kaplan *et al.* 2009). Until recent times, detection of hybrids was mostly based on morphological intermediacy, or more specifically, the deviation from the variation ranges of the parental species. Stem anatomy was also used to provide additional characters for comparison with potential parental species (Raunkiær 1903; Fischer 1907, Hagström 1916; Kaplan 2001, 2005a, b; Zalewska-Gałosz *et al.* 2010). However, only the application of isozyme electrophoresis (Hollingsworth *et al.* 1995; Fant *et al.* 2001a, b; Kaplan *et al.* 2002; Kaplan and Wolff 2004) and DNA-based techniques (Kaplan and Fehrer 2004, 2006, 2009; Ito *et al.* 2007; Du *et al.* 2009; Zalewska-Gałosz *et al.* 2009) provided unequivocal evidence for the existence of many of the hybrids. In addition, molecular analyses have recently provided insight into the previously unknown parentage of several hybrids (Kaplan and Fehrer 2011; Zalewska-Gałosz *et al.* 2018), contributed to the discovery of several entirely new hybrid combinations (Kaplan *et al.* 2009, 2018; Zalewska-Gałosz *et al.* 2010; Bobrov *et al.* 2013) and even confirmed the existence of a natural triple hybrid (Kaplan and Fehrer 2007).

Potamogeton hybrids are patchily distributed. Almost all records come from relatively few regions of the Northern Hemisphere (see Kaplan 2010), and only recently have *Potamogeton* hybrids been identified for the Southern Hemisphere, in Australia (Kaplan *et al.* 2011) and Argentina (Ito *et al.* 2016).

The diversity of *Potamogeton* in Australia started to be thoroughly investigated by European botanists in the late 1870's and all the currently recognized species that are confined to Australia were discovered and described during the following four decades (Bentham and Mueller 1878; Bennett 1887, 1892a, b, 1902, 1910; Hagström 1916). The most recent revision of *Potamogeton* for the Flora of Australia recognized 11 species (Papassotiriou *et al.* 2011). However, the recent detection of a new hybrid, *Potamogeton* ×*jacobsii* Z.Kaplan *et al.* (Kaplan *et al.* 2011), indicated that Australian diversity remains incompletely documented.

In 1981, C. B. Hellquist collected a pondweed specimen (*Hellquist 15099*, NASC) in the Fortescue River, Western Australia, which morphologically did not fit any Australian species. With its elliptical submerged leaves that are semi-amplexicaul at the base, it was most similar to *Potamogeton* ×*salicifolius* Wolfg., an interspecific hybrid between *P. lucens* L. and *P. perfoliatus* L. However, while *P. perfoliatus* is known from Australia, *P. lucens* is not, and the identity of the Fortescue River specimen as *P.* ×*salicifolius*, only known from Eurasia, would be very surprising. We therefore visited the site where this plant was first observed and re-collected it for elucidation of its relationships using DNA sequencing.

The aim of this study was to (1) determine whether the Fortescue River pondweed is a so far unknown species with homogenized nuclear DNA sequences or a hybrid with sequence additivity, and if the latter (2) whether or not the identity of *P. lucens* × *P. perfoliatus*, as inferred from morphology, is supported by sequence additivity patterns, and (3) determine which of these parental species has provided the maternal chloroplast DNA.

Materials and methods

Plant material

The putative hybrid (*Jobson 2554 and Baleeiro*; *Jobson 3302*) was collected from Deep Reach Pool (~2.3 km long, 0.1 km wide; 21°37'06.3"S, 117°06'45.8"E; Fig. 1), located along the Fortescue River about 5 km SE of Millstream Station (Millstream-Chichester National Park), Western Australia, in October 2014 and March 2017 (Fig. 1A). This seasonal waterway, located in an arid catchment of the Pilbara region, consists of semi-permanent and permanent pools maintained by groundwater inflows and rainfall (Pinder and Leung 2009). Plants were submerged with leaves and mature inflorescences held just below the surface, and shoots affixed at about 2–3 m depth along both banks (Fig. 1B). The population was observed at seven non-systematically selected sites around the entire waterbody. Samples of the putative parental species used for genetic comparison come from Australia, Europe, Asia and North America (Table 1). Both putative parental species are tetraploid (Kaplan *et al.* 2013). Voucher specimens from the putative hybrid are preserved in both the National Herbarium of New South Wales (NSW) and the herbarium of the Institute of Botany, Průhonice (PRA), and vouchers for all samples of the parental species are preserved in PRA. A morphological assessment of the putative hybrid was carried out, based on fresh material and herbarium vouchers. All characters traditionally used in *Potamogeton* taxonomy were scored.

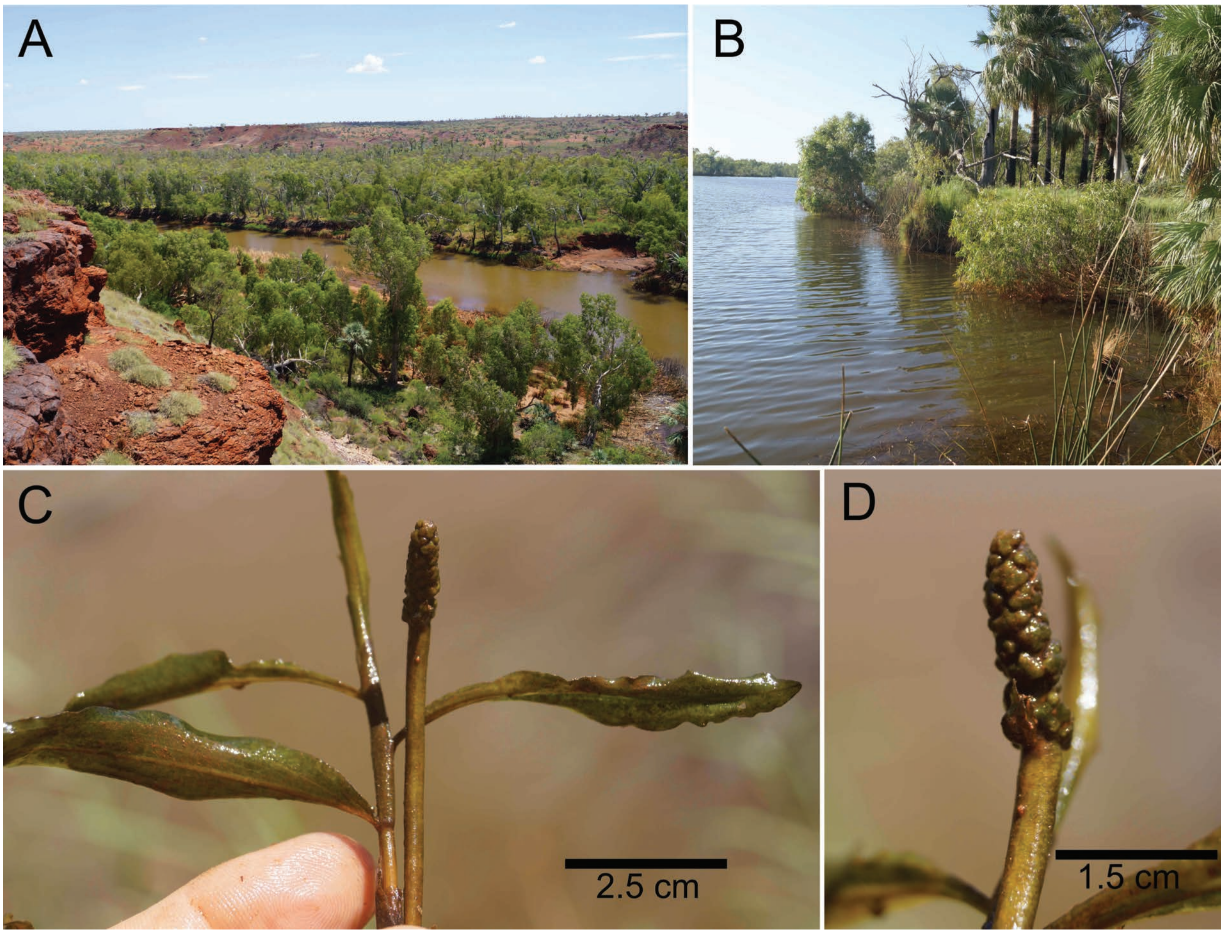

Fig. 1. A. Arid Pilbara landscape showing the Deep Reach Pool of the Fortescue River, WA; B. Habitat along edge of pool; C. *Potamogeton ×salicifolius*, main stem and inflorescence; D. Spike. Material used in C and D = *Jobson 3302*. All images by R.W. Jobson.

Table 1. Accession origins and GenBank accession numbers. 'Sample' indicates the reference numbers of the isolates used in our analyses or are adopted from GenBank.

Species	Sample	Origin	ITS	*rpl*20-5'*rps*12
P. lucens	317	Czech Republic: distr. Pardubice, Hrobice, Baroch fispond, coll. *Z. Kaplan 96/627* (PRA)	EF174584	EF174595
	858	Netherlands: prov. Limburg, Arcen, coll. *P. Denny*, cult. and coll. *Z. Kaplan 858* (PRA)	EF174583	EF174594
	1551	Japan: Kyushu, Fukuoka Prefecture, Kitakyusyu, coll. *N. Tanaka*, cult. and coll. *Z. Kaplan 1551* (PRA)	HQ263508	HQ263452
	1762	Japan: Honshu, Chiba Prefecture, Tokyo, Tega River, coll. *N. Tanaka*, cult. and coll. *Z. Kaplan 1762* (PRA)	HQ263509	HQ263453
	2109	Finland: South Häme, Hollola, Lake Vesijärvi, coll. *Z. Kaplan and P. Uotila 09/318* (PRA)	HQ263510	HQ263454
	2146	Russia: Siberia, prov. Irkutsk, distr. Ziminskiy, Ignay, Zima River, coll. *Z. Kaplan and V. Chepinoga 09/372* (PRA)	HQ263511	HQ263455
	2163	Germany: Mecklenburg-Vorpommern, Feldberg, Lake Schmaler Luzin, coll. *Z. Kaplan 09/334* (PRA)	HQ263512	HQ263456
	TNS:Yu Ito:YI00049	Japan	n.a.	AB871496
P. lucens × P. perfoliatus	3273/3421	Australia: Western Australia, Millstream Station, Fortescue River, coll. *R. W. Jobson 2554 and P. Baleeiro* (NSW, PRA) and *R. W. Jobson 3302* (NSW, PRA)	MK418991, MK418992	MK432608

Species	Sample	Origin	ITS	***rpl*20-5’*rps*12**
P. perfoliatus	NSW807556	Australia	AB937782	n.a.
	979	Switzerland: canton Sankt Gallen, Rorschach, Altenrhein, Lake Constance, coll. *Z. Kaplan 98/125* (PRA)	AY529527	DQ468862
	985	Austria: Vorarlberg, Bregenz, Fußach, Lake Constance, coll. *Z. Kaplan 98/131* (PRA)	HQ263520	HQ263462
	1002	Sweden: prov. Skåne, Sjöbo, Björka, Björkaån stream, coll. *Z. Kaplan 98/338* (PRA)	AY529526	DQ468863
	1470	Germany: Bavaria, Ebing, Main River, coll. *L. Meierott*, cult. and coll. *Z. Kaplan 1470* (PRA)	AY529525	EF174597
	1626	USA: Vermont, Orleans Co., West Glover, Lake Parker, coll. *Z. Kaplan and C. B. Hellquist 05/360* (PRA)	EU596953	EU596944
	1817	Bosnia and Herzegovina: Poklečani, Blidinje Lake, coll. *J. Pokorný*, cult. and coll. *Z. Kaplan 1817* (PRA)	HQ263521	HQ263463
	1861	USA: New York, Clinton Co., Ausable, Lake Champlain, coll. *C. B. Hellquist 16968* (PRA)	HQ263522	HQ263464
	1893	Czech Republic: distr. Sokolov, Dasnice, Ohře River, coll. *Z. Kaplan 07/350* (PRA)	HQ263523	HQ263465

Molecular procedures and data analyses

DNA isolations, PCR amplifications, purification and sequencing of the nuclear ribosomal ITS region and the *rpl*20-5’*rps*12 chloroplast intergenic spacer were all performed as described in Kaplan and Fehrer (2004, 2006). Both molecular markers have been used previously for molecular hybrid identification and are able to unequivocally distinguish all species closely related to the putative parents, *P. perfoliatus* and *P. lucens*. In addition to direct sequencing, ITS of one hybrid sample was cloned as described in Fehrer *et al.* (2009); five clones were sequenced with the forward primer. Sequences were submitted to GenBank; accession numbers are included in Table 1. ITS and *rpl*20-5’*rps*12 sequences of the hybrid samples were aligned with sequences of the putative parents in Bioedit (Hall 1999). Variable positions (intra- and interspecific differences and variation among clones in the hybrid) are summarized in Tables 2 and 3.

Table 2. Diagnostic characters of *P. lucens* and *P. perfoliatus* – ITS region. Variable positions in the alignment are listed. For the hybrid, five cloned sequences are included and forward as well as reverse direct sequence reads of two hybrid samples until indel positions (446, 661-662) from either side. Polymorphic sites (double peaks) are represented by IUPAC ambiguity codes (y: C or T, w: A or T, s: G or C, k: T or G, r: A or G, m: A or C). ‘n.a.’- not available. Lower case letters indicate the unequal proportion of bases derived from the parents; c/- and ca/-- are optional indels creating sequence shifts.

Species / sample	**ITS1**												**ITS2**									
	56	58	81	84	95	113	120	130	182	202	261	273	447	453	457	463	466	661-662	665	673	684	706
P. lucens 317	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 858	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 1551	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 1762	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 2109	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 2146	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens 2163	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens × *P. perfoliatus* 3273 clone1	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens × *P. perfoliatus* 3273 clone2	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	G
P. lucens × *P. perfoliatus* 3273 clone3	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	T
P. lucens × *P. perfoliatus* 3273 clone4	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	T
P. lucens × *P. perfoliatus* 3273 clone5	A	C	A	G	G	A	C	T	C	G	C	G	G	A	T	C	C	CA	C	C	A	T
P. lucens × *P. perfoliatus* 3273 direct reads	A	y	w	s	k	w	y	y	y	k	y	r	k	m	w	y	c/-	ca/--	y	y	w	K
P. lucens × *P. perfoliatus* 3421 direct reads	A	y	w	s	k	w	y	y	y	k	y	r	k	m	w	y	c/-	ca/--	y	y	w	K

Species / sample	**ITS1**												**ITS2**									
	56	58	81	84	95	113	120	130	182	202	261	273	447	453	457	463	466	661-662	665	673	684	706
P. perfoliatus NSW807556	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	n.a.	n.a.	n.a.	n.a.	n.a.
P. perfoliatus 979	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 985	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1002	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1470	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1817	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1893	A	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1626	T	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G
P. perfoliatus 1861	T	T	T	C	T	T	T	C	T	T	T	A	T	C	A	T	-	--	T	T	T	G

Table 3. Diagnostic indels and substitutions of *P. lucens* and *P. perfoliatus* – chloroplast DNA (*rpl*20-5'*rps*12).

Species / sample	**Position in alignment**							
	97	**255**	**429-436**	**516-522**	**551**	**554**	**616**	**751-761**
P. lucens 317	C	G	TTCACAAT	TTCAAGA	A	C	C	TCATTGATACT
P. lucens 858	C	G	TTCACAAT	TTCAAGA	A	C	C	TCATTGATACT
P. lucens 2109	C	G	TTCACAAT	TTCAAGA	A	C	C	TCATTGATACT
P. lucens 2146	C	G	TTCACAAT	TTCAAGA	A	C	C	TCATTGATACT
P. lucens 2163	C	G	TTCACAAT	TTCAAGA	A	C	C	TCATTGATACT
P. lucens 1551	C	G	TTCACAAT	-------	A	C	C	TCATTGATACT
P. lucens 1762	C	G	TTCACAAT	-------	A	C	C	TCATTGATACT
P. lucens TNS:Yu Ito:YI00049	C	G	TTCACAAT	-------	A	C	C	TCATTGATACT
P. lucens × *P. perfoliatus* 3273	C	G	TTCACAAT	-------	A	C	C	TCATTGATACT
P. perfoliatus 979	G	T	--------	-------	G	C	C	-----------
P. perfoliatus 985	G	T	--------	-------	G	C	C	-----------
P. perfoliatus 1002	G	T	--------	-------	G	C	T	-----------
P. perfoliatus 1470	G	T	--------	-------	G	C	C	-----------
P. perfoliatus 1626	G	T	--------	-------	G	T	C	-----------
P. perfoliatus 1817	G	T	--------	-------	G	C	C	-----------
P. perfoliatus 1861	G	T	--------	-------	G	C	C	-----------
P. perfoliatus 1893	G	T	--------	-------	G	C	C	-----------

Results

Morphological assessment

The *Potamogeton* from the Fortescue River shows the following morphology (Figs 1 & 2). Stems are terete, unbranched or sparingly branched, with internodes 4–46 mm long. Submerged leaves are sessile, oblong to elliptical, sometimes slightly dorsally recurved, 33–92 mm long, 10–18 mm wide, 2.4–6.1 times longer than wide, translucent, yellowish green, sometimes with a pink tinge, 7–15-veined, with a prominent midrib, sometimes slightly undulate at margins, very minutely and sparsely denticulate on young leaves, with denticles fugacious and absent on older leaves, acute to rounded and apiculate at the apex, semi-amplexicaul at the base. Floating leaves are absent. Stipules are axillary, convolute, 7–22 mm long. Inflorescences are terminal, with peduncle 30–70 mm long and spike 7–17 mm long. Flowers are numerous, with 4 abortive carpels (Fig. 1C, D). Fruits have not been observed and are presumably not produced, as in the great majority of *Potamogeton* hybrids, which are consistently sterile. This morphology fits the descriptions of *P. ×salicifolius* given in the literature (Preston 1995; Zalewska-Gałosz 2003; Kaplan 2007).

Fig. 2. Herbarium specimen of vegetative shoots of *Potamogeton ×salicifolius* from the Deep Reach Pool cultivated in a garden. Image by Z. Kaplan.

Molecular identification

ITS sequences of the putative hybrid samples were almost entirely homogenized. Surprisingly, the dominant sequence corresponded to *P. lucens* (Table 2); also, all five cloned sequences were identical with that species except for a single polymorphism (position 706) not present in either putative parent. Careful inspection of forward and reverse direct sequencing reads showed 18 small additional peaks and two shifts corresponding to diagnostic positions differing between *P. lucens* and *P. perfoliatus*. This variation was additive for the two species, indicating they are indeed parents of the hybrid. According to relative peak heights observed in direct sequencing, the *P. perfoliatus* ITS variant constituted 2–5% of the total amount and would therefore be detectable among clones only if a very large number of them were sequenced. Eurasian ITS sequences representing *P. lucens* were identical, while those of *P. perfoliatus* differed between European / Australian and North American accessions, with the latter showing a single substitution at a non-additive site (position 56; Table 2). To verify the unexpected findings obtained from our initial sample (*Jobson 2554 and Baleeiro*), a second hybrid sample (*Jobson 3302*; Table 1), collected three years later, showed matching patterns in terms of relative ratios of parental ITS variants and the same unique shared polymorphic site indicating they may belong to the same vegetative clone (Table 2).

Chloroplast DNA, known to be inherited maternally in *Potamogeton* (Kaplan and Fehrer 2006), showed sequence identity between the hybrid and Japanese accessions of *P. lucens*, which differed from other samples of that species by a single indel mutation (Table 3). All other *Potamogeton* species can be excluded as potential parents as they differ morphologically, genetically and phylogenetically (Lindqvist *et al.* 2006; Kaplan *et al.* 2013).

Distribution in Australia

Examination of herbarium collections of CANB, MEL, NSW and PERTH did not reveal other occurrences of *P. ×salicifolius* in Australia. However, two earlier specimens from the Fortescue River were discovered in PERTH: one was collected in 1974 (*G.W.Carr & A.C.Beauglehole 5073*, PERTH 3866297) and the other in 1975 (*M.Evans s.n.*, PERTH 3866300). Both specimens are sterile and were identified as *P. tepperi* A.Benn. by their collectors.

Discussion

DNA sequences have confirmed the morphological identification of the *Potamogeton* samples from the Fortescue River as a hybrid *P. lucens* × *P. perfoliatus*. This hybrid is known from Eurasia under the binomial *P. ×salicifolius*, where it has been recorded in several European countries (Preston 1995, Zalewska-Gałosz 2003; Kaplan and Zalewska-Gałosz 2004; Kaplan 2007; Bobrov and Sinjushin 2008; Wiegleb *et al.* 2008; Lastrucci *et al.* 2010), in Turkey (Aykurt *et al.* submitted) and southern Siberia (Kashina 2000). In none of these regions is it common, instead *P. ×salicifolius* is confined to a single or a few sites. It usually co-occurs with its parental species, or at least it is found in areas where both species are recorded. However, in this case, neither of the parental species is known to occur in Western Australia. *Potamogeton perfoliatus* has a large range, it is widespread mainly in the Northern Hemisphere (Wiegleb and Kaplan 1998) and is also found in eastern and south-eastern Australia (Papassotiriou *et al.* 2011). *Potamogeton lucens* is distributed in Eurasia, southwards to northern and eastern Africa, Luzon and Guam, but not Australia (Stone 1971, Wiegleb and Kaplan 1998, Wiegleb 2002, Papassotiriou *et al.* 2011). With the absence of both parental species in Western Australia, the question of origin is raised for the presence of *P. ×salicifolius* found in the Fortescue River. We provide below three possible explanations:

(1) Relict from a past occurrence of the parental species in Western Australia: Previous studies conducted in Europe showed that sterile *Potamogeton* hybrids can persist vegetatively in the absence of the parental species for very long periods, up to centuries or even millennia, presumably as remnants after one or both parents disappeared (e.g. Preston *et al.* 1998; Kaplan and Fehrer 2004, 2009, 2011, 2013; Kaplan *et al.* 2009; Kaplan and Uotila 2011). Recently, Kaplan *et al.* (2018) identified a sterile clone of *P. pulcher* Tuck. × *P. oakesianus* J.W.Robbins persisting for a long time in Florida, USA, although *P. oakesianus* currently does not occur in Florida, and its nearest sites are as distant as in Virginia, approximately 1,100 km away. Almost complete homogenization of ITS sequences towards one of the parents in the absence of meiotic recombination suggests in both cases that the vegetative clones do not represent recently formed hybrids. Under this hypothesis, *P. lucens* and *P. perfoliatus* both occurred in Western Australia in the past and gave rise to their hybrid, which has persisted there after the extinction of the parents.

(2) Long-distance dispersal of a hybrid seed: The parental species need not be present in Western Australia, but the hybridization event may have occurred in the area of sympatry of the parental species in Asia, perhaps in Japan according to the particular chloroplast haplotype of *P. lucens*, with the hybrid seed transported to Australia via birds, in their guts or embedded in their feather matrix. Both continents are indeed connected

by bird migration routes (e.g., Alerstam 1990, Berthold 2001, Newton 2008), which are known as a means of transport of plant propagules (e.g., Figuerola and Green 2002, Santamaría *et al.* 2002). However, this opens up the question of why it was only the hybrid seed that was transported but not a seed of the parental species, considering the fact that in the regions where *P.* ×*salicifolius* was recorded, it is always rarer than the parental species. Although hybrid seed is likely more common in the habitat than established adult individuals, the number of populations of either of the parental species (thus not affected by hybridization), would be higher than the number of mixed or sympatric populations containing both parent species. Consequently, seeds of the parental species would much more likely be transported by birds than the hybrid seeds. An explanation may be that even if seeds of *P. lucens* were transported to Australia they may not have found suitable ecological conditions to persist, in contrast to the hybrid seed that may have been fitter due to heterosis resulting from mixing the genetic contributions of its parents (Rieseberg and Carney 1998). A similar situation involves *P.* ×*lanceolatifolius* (Tiselius) C.D.Preston, a sterile hybrid of *P. gramineus* L. × *P. nodosus* Poir., which persists in Sweden despite *P. nodosus* (apparently adapted to warmer climates) never being recorded from Scandinavia (Kaplan and Fehrer 2011).

(3) Remnant of local cultivation or intentional planting: The plants of *P.* ×*salicifolius* in the Fortescue River may have either been planted there or escaped from cultivation. The major spring feeding the Deep Reach Pool is fed from the dolomite-rich Millsteam aquifer (Pinder and Leung 2009). Not far from the Deep Reach Pool (*c.* 3 km), is an associated small spring-fed pondage formerly used as a water supply for the household and vegetable garden for the former Millstream Station Homestead which was a working sheep/cattle station between 1865 and 1975. The pondage contains various introduced aquatic plants that may have been sourced directly or indirectly from foreign material. Although the creek leading from the pondage runs directly into Deep Reach Pool, the shallow waterbody seemed an unsuitable habitat for *P.* ×*salicifolius*, with a search uncovering no plants (R.W.Jobson pers. obs.). However, *Potamogeton* are generally very rarely grown in garden pools, and cultivation of sterile hybrids is probably restricted to a few specialized gardens worldwide. It is therefore highly unlikely that this rather rare hybrid of Eurasian (probably Japanese) origin was grown in Western Australia. In any case, examination of herbarium collections of CANB, MEL and NSW did not reveal other occurrences of *P.* ×*salicifolius* in Australia, either in cultivation or in the wild.

Conclusions

We discovered the hybrid *Potamogeton lucens* × *P. perfoliatus* in Western Australia. So far, this sterile hybrid has been known only from Eurasia under the binomial *P.* ×*salicifolius*. Its identification is unequivocal based on both morphology and the DNA sequence data. In Western Australia it occurs in the absence of its parental species. There are several potential explanations for how it got there, including its relict occurrence from the time when both the parental species occurred in Western Australia, long-distance dispersal of a hybrid seed and the remnant of local cultivation or intentional planting. While there is circumstantial evidence for each of these possibilities, the actual origin of the vegetative hybrid clone remains unknown.

Acknowledgements

We are grateful to C. B. Hellquist (North Adams, Massachusetts, USA) for drawing our attention to a taxonomically unclear specimen from the Fortescue River and Veronika Bambasová for performing the molecular labwork. The research was supported by project no. 17-06825S from the Czech Science Foundation and by long-term research development project no. RVO 67985939 from the Czech Academy of Sciences. We thank the WA Government for providing permits for our scientific collections (CE004826, CE005524).

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Manuscript received 5 May 2019, accepted 7 August 2019