

## *Pteris epaleata*, a new fern species from Australia and New Zealand segregated from *P. comans* (Pteridaceae)

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### Introduction

*Pteris* L. is a large genus of 250 species in Pteridaceae subfamily Pteridoideae that occurs on all continents except Antarctica (Zhang *et al.* 2015; PPG 1 2016). Three subgenera and 15 sections that represent major lineages have been proposed (Zhang and Zhang 2018). The *Pteris comans* G.Forst. subclade is an easily identifiable lineage within subgenus *Campteria*, section *Tripedipteris* (Zhang and Zhang 2018). This group comprises seven species with anastomosing veins from Australia and the Pacific. These are: *Pteris comans*, putatively widespread through the Pacific and south-east Australia, *P. litoralis* Rech., from the tropical Pacific, *P. macilenta* A.Rich., from New Zealand, *P. microptera* Mett. ex Kuhn, from Lord Howe Island, *P. saxatilis* (Carse) Carse, from New Zealand, *P. tahuataensis* Lorence & K.R.Wood, from the Marquesas Islands, and *P. vieillardii* Mett., from New Caledonia. The recently segregated *P. carsei* Braggins & Brownsey, from New Zealand, also belongs here (Brownsey *et al.* 2020). *Pteris laevis* Mett. from New Caledonia and *P. zahlbruckneriana* Endl. from Norfolk Island, which were not sampled by Zhang and Zhang (2018), also have anastomosing veins and are morphologically similar

### Abstract

*Pteris comans* G.Forst. has been broadly circumscribed to encompass a variable assemblage of ferns throughout the Pacific and eastern Australia with anastomosing veins and large, dissected fronds. Populations from the tropical Pacific differ from populations in Australia and New Zealand in frond dissection and indumentum. DNA sequences for the chloroplast loci *rbcl* and *trnL-trnF* were generated to assess their relationships. Australian and New Zealand populations were shown to be more closely related to other New Zealand species than to tropical Pacific *P. comans* and are here described as a new species, *P. epaleata* D.J.Ohlsen.

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to some of the previously listed species. It is likely that these species also belong to the *P. comans* subclade.

Within the *P. comans* subclade, Zhang and Zhang (2018) recovered two well-supported lineages, one comprising *P. litoralis*, *P. tahuataensis* and *P. microptera* and the other comprising Australian *P. comans*, *P. carsei* (included at the time under *P. comans*), *P. macilenta* and *P. saxatilis*. *Pteris vieillardii* was sister to these two clades (Zhang and Zhang 2018). *Pteris carsei* (then included in a broader *P. comans*) was shown to be more closely related to *P. macilenta* and *P. saxatilis* than it was to Australian *P. comans*, supporting previous suggestions that the *P. comans* group required taxonomic revision (Green 1994). This revision was undertaken in part by Brownsey *et al.* (2020), who described *P. carsei*, which was a step toward restoring monophyly of species within the *P. comans* subclade. Brownsey *et al.* (2020) also detailed how *P. carsei* and other recognised species in the *P. comans* subclade can be distinguished morphologically, by a combination of frond size, dissection and indument. This showed that narrowly defined species delimitations in the *P. comans* subclade, although followed with hesitation in the past (Green 1994), could be both monophyletic and morphologically recognisable.

*Pteris comans* still remains a rather broadly circumscribed species even with the exclusion of *P. carsei*. In its current circumscription it is also far more widespread than the other species of the *P. comans* subclade, being recognised in subtropical and temperate south-east Australia, as well as throughout the tropical Pacific in the Solomon Islands (Chen *et al.* 2017), Vanuatu (Nakamura 2008) where the type was collected (Nicolson and Fosberg 2003; Brownsey *et al.* 2020), New Caledonia (Brownlie 1969), Fiji (Brownlie 1977), Samoa (Christensen 1943), Tonga (Yuncker 1959), Cook Islands (Sykes 2016) and French Polynesia (Brown and Brown 1931; Lorence *et al.* 2011). There are two clear morphological forms that currently reside within *P. comans*. Populations from Australia have more dissected fronds, being 3–4-pinnate-pinnatifid with pinnatifid segments near the apex, whereas populations in the tropical Pacific have fronds that are at most 3-pinnate-pinnatisect at the base and have segments towards the apex that remain more or less entire. Retaining this level of morphological heterogeneity

within the one species is inconsistent with the trend of adopting narrower species concepts for the rest of the *P. comans* subclade.

Brownsey *et al.* (2020) suggested that mainland Australian and Tasmanian *P. comans* may be better treated as a separate species with *P. comans s.str.* only occurring in the tropical Pacific, but did not conduct any formal revision on the Australian plants. In the current study, morphological and chloroplast DNA variation is assessed across the distribution of plants formerly assigned to *P. comans*, including populations from across Australia and also from the tropical Pacific, as well as two collections from Fiordland, New Zealand, that are morphologically similar to Australian plants. This was done to aid taxonomic judgement on whether *Pteris comans* should be retained as a single variable and widespread species or whether some populations would be better treated as a separate species.

## Materials and methods

### DNA Isolation and amplification

Fifteen new DNA sequences belonging to the *P. comans* subclade were generated in this study (Table 1). The methods either followed Shepherd *et al.* (2019) or were as described here. DNA was extracted from 20 mg of silica gel-dried young leaf tissue. Leaf tissue was ground using a mortar and pestle with the aid of acid-washed grinding sand (Ajax Finechem, Aus). DNA was isolated from ground samples using a DNeasy Plant Mini Kit (QIAGEN, Germany), following the manufacturer's instructions. DNA was eluted in 100 µL of the supplied elution buffer.

Samples were sequenced for the chloroplast regions *rbcl* and *trnL-trnF*. These regions were chosen because several species of the *P. comans* subclade have been previously sequenced for *rbcl* (Chao *et al.* 2014; Zhang *et al.* 2015; Zhang and Zhang 2018) allowing easy comparison, and *trnL-trnF* is a region routinely employed in studies of fern systematics because it contains non-coding regions that have demonstrated high levels of genetic variation in fern species (e.g. Su *et al.* 2005; Shepherd *et al.* 2007; Wang *et al.* 2011). A herbarium specimen from Fiordland, New Zealand (Johnson *s.n.*, CHR 253997) suspected of being more closely related to Australian populations than other New Zealand

populations, some herbarium samples of *P. carsei* and two silica-gel dried samples of *P. comans* specimens from the Solomon Islands were sequenced for *trnL-trnF* but efforts to amplify *rbcl* were unsuccessful.

Chloroplast DNA markers were amplified by Polymerase Chain Reaction (PCR), performed on a MyCycler thermal cycler (Bio-Rad, USA). Reaction mixtures comprised 5 µL of 5x MyTaq Reaction Buffer containing 5 mM of each dNTP and 15 mM MgCl<sub>2</sub> (Bioline, Australia), 0.125 µL (0.625 units) MyTaq DNA Polymerase (Bioline, Australia), 10 pmol of each primer, 2.0 µL of extracted DNA, and distilled water added to make a total volume of 25 µL. The *trnL* intron, *trnL* 3'-exon and *trnL-trnF* intergenic spacer were amplified and sequenced using the primers F (5'-ATT TGA ACT GGT GAC ACG AG-3') (Taberlet *et al.* 1991) and Fern1 (5'-GGC AGC CCC CAR ATT CAG GGR AAC C-3') (Trewick *et al.* 2002). The *rbcl* gene was amplified using the primers aF (5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3') and cR (5'-GCA GCA GCT AGT TCC GGG CTC CA-3') (Hasebe *et al.* 1994).

PCR thermocycling conditions involved an initial denaturation step of 95°C for 1 minute, followed by 33 cycles of 95°C for 1 minute, 55°C for 1 minute, and 65°C for 4 minutes, and a final extension at 65°C for 5 minutes. PCR products were quantified by electrophoresis against Hyperladder I and EasyLadder I (Bioline, Australia) and purified using illustra ExoSTAR 1-step enzymatic purification (GE Healthcare Life Sciences, UK). Purified PCR products were then sent to the Australian Genome Research Facility (AGRF), Melbourne Branch, where sequencing reactions, and capillary separation, using the 96-capillary analyser AB 3730xl sequencing platform, were performed.

### Sequence editing, alignment and analysis

Sequences were edited using Sequencher v. 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA) and were aligned manually in Se-Al Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). Maximum Parsimony analysis was performed in PAUP\* v. 4.0 β10 (Swofford 2002). Gap characters in the alignment were treated as a fifth

**Table 1. Details of samples used in phylogenetic analyses. Newly generated sequences are given in bold. A '-' is given for samples for which *rbcl* was not generated.**

Species	Specimen Details	<i>rbcl</i> GenBank Number	<i>trnL-trnF</i> GenBank Number
<i>Pteris carsei</i>	New Zealand, Waitakere Ranges, L.R. Perrie 3406 & L.D. Shepherd (WELT P020796)	EF469954	<b>MT180118</b>
	New Zealand, Great Barrier Island, M.E. Young s.n. (AK 298327)	GU136796	<b>MT180117</b>
	New Zealand, Kermadec Islands, Raoul Is., E. Cieraad s.n. (WELT P020739)	-	<b>MT180120</b>
<i>P. comans</i>	New Zealand, Whangarei, L.R. Perrie 4661 & L.D. Shepherd (WELT P029533)	-	<b>MT180119</b>
	Solomon Islands, Kolombangara Is., C.-W. Chen <i>et al.</i> Wade2727 (TAIF 445137)	-	<b>MT180113</b>
<i>P. epaleata</i>	Solomon Islands, Nggatokae Is., C.-W. Chen <i>et al.</i> Wade3238 (TAIF 452521)	-	<b>MT180112</b>
	Australia, Victoria, Main Creek, D.J. Ohlsen 913 & V. Stajsic (MEL 2469967A–2469973)	<b>MT180110</b>	<b>MT180115</b>
	Australia, Queensland, Toolona Circuit Track, L.R. Perrie & D.J. Ohlsen BB122 (MELUP 118484)	<b>MT180109</b>	<b>MT180114</b>
<i>P. macilenta</i>	New Zealand, Fiordland, Resolution Island, P.N. Johnson s.n. (CHR 253997)	-	<b>MT181523</b>
	New Zealand, Pohangina Valley, L.R. Perrie 3770 (WELT P021006)	GU136797	<b>MT180121</b>
<i>P. saxatilis</i>	New Zealand, Aranga Beach, L.R. Perrie 3662 <i>et al.</i> (WELT P022567)	GU136798	<b>MT180122</b>
<i>P. tahuataensis</i>	French Polynesia, Marquesas Islands, K. Wood 10250 (PTBG)	MF972821	MF972861
<i>P. vieillardii</i>	New Caledonia, Plateau de Dogny, L.R. Perrie 7696 <i>et al.</i> (WELT P028681)	<b>MT180111</b>	<b>MT180116</b>

character state. For indels of multiple-bases, characters were excluded from analysis so that indels were each represented only by a single gap character. Samples for which *rbcl* could not be generated had their *rbcl* section of the alignment scored as missing data. A heuristic tree search was used, with delayed character-state optimisation (DELTRAN) and starting trees were obtained by a closest addition sequence, followed by tree bisection-reconnection (TBR) branch swapping. Bootstrap support for nodes was determined using 1000 'full heuristic' replicates. *Pteris vieillardii* was chosen as an outgroup because this species is sister to the remaining species of the *P. comans* subclade (Zhang and Zhang 2018).

### Morphological Observations

All herbarium material belonging to the *P. comans* subclade was inspected in the home herbaria of the authors (MEL, MELU, TAIF and WELT) along with all material in AK and CHR. This covers around 30% of all Australian *Pteris comans* specimens in herbaria and was expected to be representative of the morphological diversity harboured within Australian *P. comans*. All online images of types of species in the *P. comans* subclade, including *P. comans*, were also viewed. Morphology that is variable among the *P. comans* subclade, such as frond size, dissection, colour, and indumentum, was a focus for assessing any possible major morphological forms that could be residing within *P. comans s.lat.*, and a new description was written based on herbarium specimens seen, augmented by ranges reported in existing descriptions (e.g. Kramer and McCarthy 1998).

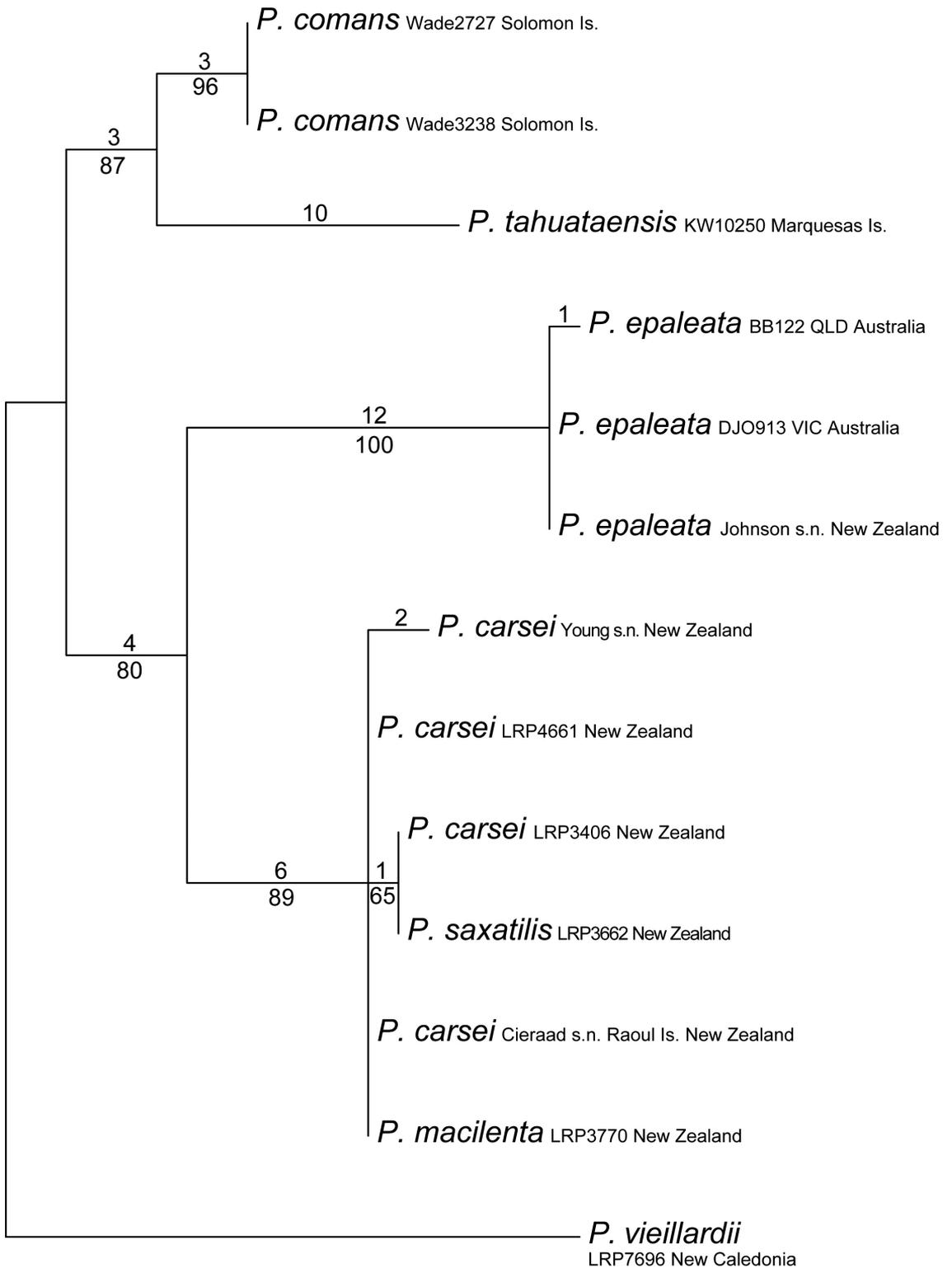
### Results and Discussion

Both Australian populations of the new species had identical *rbcl* sequences but differed in their *trnL-trnF* sequences by one base substitution and one nucleotide in a mononucleotide repeat. The *trnL-trnF* sequence from the collection from Fiordland, New Zealand (*Johnson s.n.*, CHR 253997) was identical to *trnL-trnF* of the Victorian population except for a difference of one nucleotide in a mononucleotide repeat. This is a similar amount of genetic variation to what is harboured in *P. carsei*, which had two base substitution differences between the two

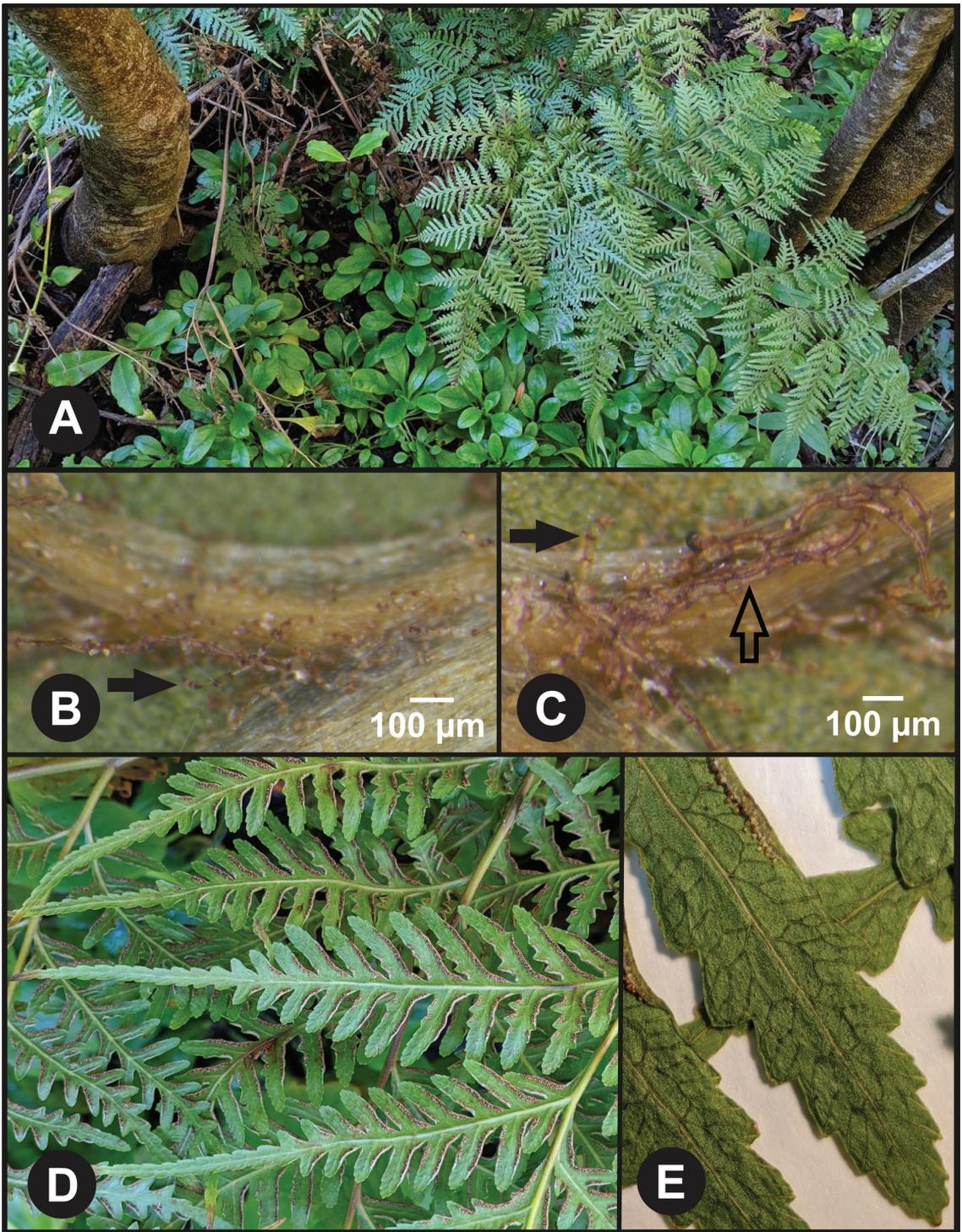
*rbcl* sequences, and one base substitution difference and slight differences in length of mononucleotide repeats among the four *trnL-trnF* sequences.

Twenty-six most parsimonious trees that were 63 steps long were generated, one of which is shown in Figure 1. Clades with strong support (bootstrap of at least 80%) were also recovered in all trees not shown and so the following discussion of the results of the presented tree apply to all 26 most parsimonious trees. Australian populations and the Fiordland sample of *P. comans* were resolved as sister to a clade of *P. carsei*, *P. macilenta* and *P. saxatilis*, the same relationship found by Zhang and Zhang (2018) using a single Australian sample of *P. comans* and a different set of DNA regions. The *P. comans* sample from the Solomon Islands was sister to *P. tahuataensis* (Figure 1), making *P. comans* in its current circumscription polyphyletic. It is also likely that *P. comans* from the Solomon Islands is closely related to *P. litoralis* and *P. microptera* given that these species were in a well-supported lineage with *P. tahuataensis* based on a different set of chloroplast regions (Zhang and Zhang 2018). *Pteris carsei* did not form a clade separate from the other New Zealand species due to a lack of genetic variation between samples. It is likely that these species share a recent ancestry and that *P. macilenta* is an allopolyploid derived from *P. carsei* and *P. saxatilis* (Braggins 1975; Brownsey and Smith-Dodsworth 2000). All three species are morphologically distinct in New Zealand (Brownsey and Smith-Dodsworth 2000) and the lack of resolution between these species in the tree is not taken here to reflect a need for taxonomic re-evaluation of these species.

All mainland Australian and Tasmanian specimens of *P. comans* examined and two collections from Fiordland in New Zealand (Resolution Island, *Johnson s.n.*, CHR 253997; Coal River, *Johnson s.n.*, CHR 320041) shared a similar set of morphological features, including having highly dissected fronds that were at least 3-pinnate throughout the lower half of the frond and 3-pinnate-pinnatifid at the base (Figure 2A), dull green to yellow-green fronds (Figure 2A), hairy pinnae, and costae that may occasionally have linear scales 2–3(–4) cells wide (Figure 2B, 2C). This combination of features distinguishes this group of populations from other members of the *P. comans* subclade, including *P. comans* from the tropical Pacific (see Recognition section below



**Figure 1.** One of 26 most parsimonious trees (length = 63, CI = 0.968, RI = 0.965). Branch lengths are given above branches and bootstrap support is given below branches.



**Figure 2.** Morphology of *Pteris epaleata* based on the living plant of D.J. Ohlsen & V. Stajsic DJO913.

**A.** Frond from above. **B.** Abaxial lamina and costules showing typical indument and with the arrow pointing to a uniseriate hair. **C.** Abaxial lamina and costule with the open arrow pointing to an example of a rare linear hair-like scale. The filled-in black arrow points to a hair for comparison. **D.** Abaxial surface of secondary pinnae. **E.** Tertiary pinna showing the anastomosing venation.

for more details). No specimens from mainland Australia or Tasmania examined possessed the diagnostic features of closely related species in the *P. comans* subclade such as *P. carsei*.

The morphological observations and genetic results support recognition of all mainland Australian and Tasmanian specimens examined and the Fiordland specimens as a single species. They are all unified by a set of morphological features, there are no distinctive morphological variants that can be easily discerned among these specimens, and geographically-distant samples have been shown to share very similar *rbcl* and *trnL-trnF* sequences. The results of this study also support the recognition of these populations as a separate species from *P. comans* in the Solomon Islands, given that the *P. comans* samples are polyphyletic and populations from the tropical Pacific can be easily distinguished morphologically from the populations in mainland Australia, Tasmania and Fiordland. The Solomon Islands specimens are morphologically similar to the type from nearby Vanuatu, making it very probable that our Solomon Islands samples are representative of *P. comans s.str.* The two species also appear to be geographically disjunct as the *P. comans* subclade has not been recorded from the intervening wet tropics region of north-east Queensland. Consequently, the following taxonomy section describes the populations in mainland Australia, Tasmania and Fiordland as the new species, *P. epaleata* D.J.Ohlsen, with *P. comans* now being restricted to the tropical Pacific.

## Taxonomy

*Pteris epaleata* D.J.Ohlsen sp. nov.

## Etymology

*Epaleata* is Latin (feminine, nominative case) for without scales, which refers to the absence of well-developed triangular scales on the lamina, a feature that helps to distinguish this species from the closest related species.

## Holotype

Australia, Victoria, Mornington Peninsula National Park, Main Creek south of Boneo Road, 30 August 2019, *D.J. Ohlsen & V. Stajsis* *DJO913* (MEL 2469967A–2469973A). Isotypes: CANB, NSW, WELT.

## Description

Rhizomes erect, scaly. Rhizome scales narrowly ovate, 7–8 mm long, c. 2 mm wide, pale brown, concolorous, lacking marginal projections. Fronds (Figure 2A) 77–174(–250) cm long. Stipes 40–103(–140) cm long, 6–11 mm diameter near the base, purplish-brown, red-brown or chestnut-brown proximally, yellow-brown or chestnut-brown distally, occasionally with scattered scales proximally. Rachises yellow-brown or chestnut-brown, adaxially sulcate, glabrous. Laminae 3–4-pinnate-pinnatifid at base, broadly ovate, 33–75(–110) cm long, 30–100 cm wide, dark green and dull adaxially, paler abaxially, herbaceous, bearing multicellular hairs (Figure 2B, 2C) and occasionally hair-like linear scales 2–3(–4) cells broad (Figure 2C) on the costae; veins anastomosing. Primary pinnae in 5–11 pairs below pinnatifid apex, overlapping; the longest at or near the base, 12–58 cm long, 12–28 cm wide, elliptic or narrowly- to broadly-ovate, straight; pinna apices acute to acuminate, bases long-stalked. Longest secondary pinnae (Figure 2D) 10–27 cm long, 5–15 cm wide, usually narrowly ovate, sometimes ovate, subopposite at base becoming alternate toward apex of primary pinnae, longest at base to midway along primary pinnae and then becoming gradually smaller toward apex, apices acute to acuminate, bases adnate or stalked. Longest tertiary segments (Figure 2E) 15–90 mm long, 5–30 mm wide, elliptic, ovate or oblong; apices acute to obtuse, margins crenate, serrate or divided to more than halfway, bases adnate or shortly stalked. Quaternary segments to 22 mm long, 2–9 mm wide, oblong to triangular, apices acute to obtuse, margins crenate, serrate or divided to more than halfway, bases adnate, occasionally with further divisions to 4 mm long, 1–3 mm wide; ultimate segments 1–5.5 mm wide. Sori elongated along margins of the ultimate segments, with paraphyses among sporangia; indusia 0.35–0.55 mm wide. Spores 25–35 µm in polar diameter, 35–47.5 µm in equatorial diameter.

**Specimens examined:** **QUEENSLAND:** Binna Burra, *N.A. Wakefield* 425, 13 Nov. 1942 (MEL); Lamington National Park, Toolona Circuit Track, *L.R. Perrie & D.J. Ohlsen* *BB122*, 29 Aug. 2010 (MELU; BRI). **NEW SOUTH WALES:** Deervale, *N.A. Wakefield* 144, 5 Jan. 1941 (MEL); Mount Dromedary, *N.A. Wakefield* 143, 12 Jan. 1941 (MEL); Belmore Falls, *E.F. Constable* 6251, 14 Oct. 1965 (MEL); Dawsons Springs, Mount Kaputar

National Park, *R.G. Coveny 8707*, 17 Nov. 1976 (MEL); Brindle Creek, *R.G. Coveny 9949*, 4 Dec. 1977 (MEL). **VICTORIA:** Cape Otway, *C. Walter s.n.*, 1 Dec. 1873 (MEL); Joanna River, *C. Walter s.n.*, 1 Mar. 1874 (MEL); Ferntree Gully, *P.R.H. St John s.n.*, 20 Sep. 1906 (MEL); Apollo Bay, *S.G.M. Fawcett s.n.*, 31 May 1955 (MELU); Clematis Gully, Dandenong Ranges, *O. Singleton s.n.*, 18 Apr. 1943 (MEL); Hardy's Gully, Kallista, *R. Melville 3838*, 11 May 1953 (MEL); Reserve opposite Tarra National Park, *G. Isaac s.n.*, 2 Nov. 1975 (MEL); Sherbrook Forest, *P.K. Gullan & A.M. Opie 44*, 23 Feb. 1977 (MEL); Nepean State Park, along a tributary of Main Creek, c. 0.75 miles from its junction with Lightwood Creek, *W.R. Archer s.n.*, 13 May 1979 (MEL); Tributary of Main Creek, *B.D. Duncan 79143*, 28 Aug. 1979 (MEL); Tributary of Main Creek, *B.D. Duncan 79144*, 28 Aug. 1979 (MEL); Willies Gully, tributary of Main Creek, *B.D. Duncan s.n.*, 28 Aug. 1979 (MEL); Near Sherbrook-Kallista Road, *B.D. Duncan 80056*, 21 Mar. 1980 (MEL); Near the junction of Main Creek and small tributary, *L.K.M. Elmore s.n.*, 1 Sep. 1980 (MEL); Tributary of Main Creek, *W. Archer BDD 81063*, 15 Feb. 1981 (MEL); Northern heathlands, Wilsons Promontory, *E.A. Chesterfield 2061*, 19 Nov. 1987 (MEL); Highfield State Park, tributary flowing into Main Creek, N of Boneo Road, S of Lightwood Creek, *J. Westaway 601*, 23 Jan. 1989 (MEL); Minor Gully draining into Main Creek south of Boneo River [Road], *J. Westaway 606*, 24 Jan. 1989 (MEL); Wongungurra River upstream from Blowfly Creek junction, *G. Johnson 263*, 3 Dec. 1996 (MEL); Warnambool-Cobden Rd, *L. Weedon s.n.*, 8 Dec. 2011 (MEL); 15 metres above west Bank of Main Creek, south of Boneo Road, *D.J. Ohlsen BB245*, 9 Sep. 2012 (MELU); N-S tributary of Main Creek, c. 110 metres ESE from Bushrangers Bay Walking Track, *V. Stajsic 9059*, 14 Mar. 2019 (MEL); Main Creek south of Boneo Road, *D.J. Ohlsen & V. Stajsic DJO913*, 30 August 2019 (MEL). **TASMANIA:** Between Circular Head and the Arthur River, *F. Mueller s.n.*, 1 Feb. 1875 (MEL); Golden Valley, *R.A. Black s.n.*, 6 Apr. 1915 (MEL); Cumberland Creek, King Island, *P. Barnett s.n.*, 12 June 1967 (MEL); Little Beach Creek, *M. Garrett s.n.*, 1 July 1984 (MEL); Blackwater Road, Spur 5, *G. Kantvilas 90*, 15 Sep. 1984 (MEL). **NEW ZEALAND:** Resolution Island, Fiordland, *P.N. Johnson s.n.*, Dec. 1974 (CHR); Coal River, Fiordland, *P.N. Johnson s.n.*, 8 Nov. 1977 (CHR)

## Chromosome Number

$2n=58$  (S.K. Roy and B.G. Briggs in Tindale and Roy 2002, as *Pteris comans*).

## Distribution

Australia and New Zealand. In Australia it is sporadically distributed from south-east Queensland south to Tasmania. In Queensland it is known from Lamington

National Park near the border with New South Wales. In New South Wales it occurs on and east of the Great Dividing Range but with an inland occurrence at Mount Kaputar. In Victoria it occurs south of the Great Dividing Range and extends west to around Warnambool. It is widespread throughout Tasmania. In New Zealand it is only known from two collections from Fiordland National Park, where it may be a relatively recent arrival by long-distance dispersal similar to *Sticherus tener* (R.Br.) Ching (Brownsey et al. 2013).

## Habitat

*Pteris epaleata* is a terrestrial fern found in subtropical to cool-temperate rainforest and wet-sclerophyll forests usually along creeks or muddy drainage lines, but sometimes also at the base of cliffs or on outcrops in areas of water seepage. It grows from sea-level to around 1100 metres on the east edge of the New England Plateau in New South Wales.

## Recognition

*Pteris epaleata* resembles several species of the *Pteris comans* subclade *sensu* Zhang and Zhang (2018). *Pteris epaleata* differs from all of these species by indumentum and frond division. The indumentum on the abaxial side of the rachis, costae and costules in *Pteris epaleata* comprises uniseriate hairs or sometimes also very occasional linear scales that are 2–3(–4) cells broad at the base (Figure 2B, 2C). In morphologically similar species, such as *P. carsei*, *P. microptera* and *P. zahlbruckneriana*, the rachis and costae have uniseriate hairs and some triangular scales that are several cells wide at the base or in the case of *P. comans s.str.* are glabrous. *Pteris epaleata* can be further distinguished from the other species that have been included in *P. comans* by its more divided fronds that are at least 3-pinnate throughout the lower half of the frond and 3-pinnate-pinnatifid at the base, with the segments adnate to the rachis near the apex being pinnatifid (Figure 2A). In this respect it resembles *P. tremula* R.Br., which has free venation and is not part of the *P. comans* subclade (Zhang and Zhang 2018). The other species that have been included in *P. comans* are 2-pinnate-pinnatifid or less divided or are more divided only in the basal primary pinnae, and the segments adnate to the rachis near the apex have at most small teeth near their apices rather than being incised along

the entire margin of the segment. *Pteris epaleata* can be further distinguished from *P. carsei* and from *P. macilenta* and *P. saxatilis* by its dull adaxial frond surface compared with the lustrous adaxial frond surface in *P. carsei*. *Pteris carsei* also usually has broader ultimate segments (4–28 mm wide) than *P. epaleata* (1–5.5 mm wide) and *P. carsei* is confined to coastal areas, whereas *P. epaleata* can extend further inland, including into mountainous areas.

### Hybridisation

A collection from Mount Elephant in eastern Tasmania (Garrett *s.n.* HO 326490) has been claimed by the collector on the sheet to be a hybrid between *P. epaleata* and *P. tremula*, which are both much more abundant than the putative hybrid at that site.

### Conservation Status

In the Victorian part of its range, *P. epaleata* has been classified as rare on the basis of relatively few known populations (Gullan *et al.* 1990). Globally, it is probably best considered not threatened under IUCN Red List Threatened Species criteria. In New Zealand, because there has been no targeted surveying, we suggest a provisional assessment under Townsend *et al.* (2008) as Data Deficient with the qualifier Secure Overseas.

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### References

- Braggins, J.E. (1975). Studies on the New Zealand, and some related, species of *Pteris* L. PhD Dissertation. University of Auckland: Auckland.
- Brownlie, G. (1969). 'Fasc. 3, Ptéridophytes' in A. Aubréville (ed.) *Flore de la Nouvelle Calédonie et Dependances*. Museum National d'Histoire Naturelle: Paris.
- Brownlie, G. (1977). The pteridophyte flora of Fiji. *Nova Hedwigia Beiheft* 55: 1–397.
- Brownsey, P. J., and Smith-Dodsworth, J.C. (2000). *New Zealand Ferns and Allied Plants*. 2nd edition. David Bateman Ltd.: Auckland, New Zealand.
- Brownsey, P.J., Braggins, J. and Perrie L.R. (2020). *Pteris carsei*, a new endemic fern species from New Zealand previously treated as *P. comans*. *New Zealand Journal of Botany* **58**, 214–222.
- Brownsey, P.J., Ewans, R., Rance, B., Walls, S. and Perrie, L.R. (2013). A review of the fern genus *Sticherus* (Gleicheniaceae) in New Zealand with confirmation of two new species records. *New Zealand Journal of Botany* **51**, 104–115.
- Chao, Y.-S., Rouhan, G., Amoroso, V.B. and Chiou, W.-L. (2014). Molecular phylogeny and biogeography of the fern genus *Pteris* (Pteridaceae). *Annals of Botany* **114**, 109–124.
- Chen, C.-W., Perrie, L., Glenney, D. and Chiou, W.-L. (2017). *Sol amazing: lycophytes and ferns of the Solomon Islands*. National Museum of Natural Science: Taichung.
- Christensen, C. (1943). A revision of the Pteridophyta of Samoa. *Bernice P. Bishop Museum Bulletin* **177**, 1–138.
- Green, P.S. (1994). 'Pteridaceae' in A.J.G. Wilson (ed.) *Flora of Australia* **49**: Oceanic Islands 1, pp. 567–570. Australian Government Publishing Service: Canberra.
- Gullan, P.K., Cheal, D.C. and Walsh, N.G. (1990). *Rare or threatened plants in Victoria*. Department of Conservation and Environment: East Melbourne, Victoria.
- Hasebe, M., Omori, T., Nakazawa, M., Sano, T., Kato, M., and Iwatsuki, K. (1994). *rbcl* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 5730–5734.
- Kramer, K.U. and McCarthy, P.M. (1998). 'Pteris' in P.M. McCarthy (ed.) *Flora of Australia* **48**: Ferns, Gymnosperms and Allied Groups, pp. 242–248. ABRIS: Canberra; and CSIRO: Melbourne.
- Lorence, D., Wagner, D., Wood, K. and Smith, A. (2011). New pteridophyte species and combinations from the Marquesas Islands, French Polynesia. *Phytokeys* **4**, 5–51.
- Nakamura, M. (ed.) (2008). *Illustrated flora of ferns and fern allies of South Pacific Islands*. Tokai University Press: Japan.
- Nicolson, D.H. and Fosberg, F.R. (2003). The Forsters and the Botany of the Second Cook Expedition (1772–1775). *Regnum Vegetabile* **139**, 1–760.
- PPG 1. (2016). A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* **54**, 563–603.
- Rambaut, A. (2002). 'Se-AL: sequence alignment editor'. Available at: <http://tree.bio.ed.ac.uk/> [Verified 2019]

- Shepherd, L.D., Perrie, L.R. and Brownsey, P.J. (2007). Fire and Ice: volcanic and glacial impacts on the phylogeography of the New Zealand forest fern *Asplenium hookerianum*. *Molecular Ecology* **16**, 4536–4549.
- Shepherd, L.D., Brownsey, P.J., Stowe, C., Newell, C. and Perrie, L.R. (2019) Genetic and morphological identification of a recurrent *Dicksonia* tree fern hybrid in New Zealand. *PLoS One* **14**(5), e0216903.
- Su, Y.-J., Wang, T., Zheng, B., Jiang, Y., Chen, G.-P., Ouyang, P.-Y. and Sun, Y.-F. (2005). Genetic differentiation of relictual populations of *Alsophila spinulosa* in southern China inferred from cpDNA *trnL-F* noncoding sequences. *Molecular Phylogenetics and Evolution* **34**, 323–333.
- Sykes, W.R. (2016). *Flora of the Cook Islands*. National Tropical Botanical Garden: Kaua'i, Hawai'i.
- Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**, 1105–1109.
- Tindale, M.D. and Roy, S.K. (2002). A cytotoxic survey of the Pteridophyta of Australia. *Australian Systematic Botany* **15**, 839–937.
- Townsend, A., de Lange, P., Duffy, C., Miskelly, C., Molloy, J. and Norton, D. (2008). *New Zealand threat classification system manual*. Department of Conservation, Wellington.
- Trewick, S.A., Morgan-Richards, M., Russell, S.J., Henderson, S., Rumsey, F.J., Pinter, I., Barrett, J.A., Gibby, M. and Vogel, J.C. (2002). Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Molecular Ecology* **11**, 2003–2012.
- Wang, L., Wu, Z.-Q., Bystriakova, N., Ansell, S.W., Xiang, Q.-P., Heinrichs, J., Schneider, H. and Zhang, X.-C. (2011). Phylogeography of the Sino-Himalayan Fern *Lepisorus clathratus* on “The Roof of the World”. *PLoS ONE* **6**: e25896. doi:10.1371/journal.pone.0025896.
- Yuncker, T.G. (1959). Plants of Tonga. *Bernice P. Bishop Museum Bulletin* **220**, 1–283.
- Zhang, L., Rothfels, C.J., Ebihara, A., Schuettelpelz, E., Le Pêcheon, T., Kamau, P., He, H., Zhou, X.-M., Prado, J., Field, A., Yatskievych, G., Gao, X.-F. and Zhang, L.-B. (2015). A global plastid phylogeny of the brake fern genus *Pteris* (Pteridaceae) and related genera in the Pteridoideae. *Cladistics* **31**, 406–423.
- Zhang, L. and Zhang, L.-B. (2018). Phylogeny and systematics of the brake fern genus *Pteris* (Pteridaceae) based on molecular (plastid and nuclear) and morphological evidence. *Molecular Phylogenetics and Evolution* **118**, 265–285.