Received: 1 February 2023 | Accepted: 5 May 2023

DOI: 10.1002/ajb2.16221

RESEARCH ARTICLE

A new fossil *Acmopyle* with accessory transfusion tissue and potential reproductive buds: Direct evidence for ever-wet rainforests in Eocene Patagonia

Ana Andruchow-Colombo[1,2,3] | **Gabriella Rossetto-Harris**[4] | **Timothy J. Brodribb**[5] | **María A. Gandolfo**[1,2,3] | **Peter Wilf**[4]

[1]Museo Paleontológico Egidio Feruglio, Av. Fontana 140, Trelew, Chubut 9100, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

[3]LH Bailey Hortorium, Plant Biology Section, School of Integrative Plant Science, Cornell University, New York 14853, USA

[4]Department of Geosciences and Earth and Environmental Systems Institute, Pennsylvania State University, University Park, Pennsylvania 16802, USA

[5]School of Biological Sciences, University of Tasmania, Sandy Bay, Tasmania 7001, Australia

Correspondence

Ana Andruchow-Colombo, Museo Paleontológico Egidio Feruglio, Av. Fontana 140, Trelew, Chubut, 9100; and Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

Email: ana.andruchow.colombo@gmail.com

Abstract

Premise: *Acmopyle* (Podocarpaceae) comprises two extant species from Oceania that are physiologically restricted to ever-wet rainforests, a confirmed fossil record based on leaf adpressions and cuticles in Australia since the Paleocene, and a few uncertain reports from New Zealand, Antarctica, and South America. We investigated fossil specimens with *Acmopyle* affinities from the early Eocene Laguna del Hunco site in Patagonia, Argentina.

Methods: We studied 42 adpression leafy-shoot fossils and included them in a total evidence phylogenetic analysis.

Results: *Acmopyle grayae* sp. nov. is based on heterophyllous leafy shoots with three distinct leaf types. Among these, bilaterally flattened leaves uniquely preserve subparallel, linear features that we interpret as accessory transfusion tissue (ATT, an extra-venous water-conducting tissue). Some apical morphologies of *A. grayae* shoots are compatible with the early stages of ovuliferous cone development. Our phylogenetic analysis recovers the new species in a polytomy with the two extant *Acmopyle* species. We report several types of insect-herbivory damage. We also transfer *Acmopyle engelhardti* from the middle Eocene Río Pichileufú flora to *Dacrycarpus engelhardti* comb. nov.

Conclusions: We confirm the biogeographically significant presence of the endangered West Pacific genus *Acmopyle* in Eocene Patagonia. *Acmopyle* is one of the most drought-intolerant genera in Podocarpaceae, possibly due to the high collapse risk of the ATT, and thus the new fossil species provides physiological evidence for the presence of an ever-wet rainforest environment at Laguna del Hunco during the Early Eocene Climatic Optimum.

KEYWORDS

accessory transfusion tissue (ATT), *Acmopyle*, conifers, Early Eocene Climatic Optimum, insect damage, Laguna del Hunco, Podocarpaceae, reproductive buds, seed cone development, total evidence phylogeny

Acmopyle Pilg., one of the 18–20 extant genera of the conifer family Podocarpaceae (Farjon, 2010; Page, 2019), comprises two extant species with small rainforest ranges: *Acmopyle pancheri* (Brogn. et Gris) Pilg., confined to New Caledonia, and *Acmopyle sahniana* Buchholz et N.E. Gray, restricted to the Fiji Islands (Figure 1; Buchholz and Gray, 1947; Farjon, 2010; Knopf et al., 2012). Both species are extremely rare and under threat (*A. pancheri* is near threatened; *A. sahniana* is critically endangered; https://www.iucnredlist.org, accessed 21 August 2022) because of habitat loss due to climate change and human impact (Doyle, 1998; Jaffré et al., 2010; Thomas, 2010, 2013). The macrofossil record that has been associated with the genus is markedly more diverse and widely distributed through post-Gondwanan landmasses, including reports since the Cretaceous from Patagonia (southern Argentina and Chile), Antarctica, Australia, New Zealand, and India (Table 1, Figure 1; Banerji and Ghosh, 2006, 2008). However, the reliable fossil record of *Acmopyle* is restricted to Australia, starting in the Paleocene (e.g., Hill and Brodribb, 1999;

https://doi.org/10.1002/ajb2.16221

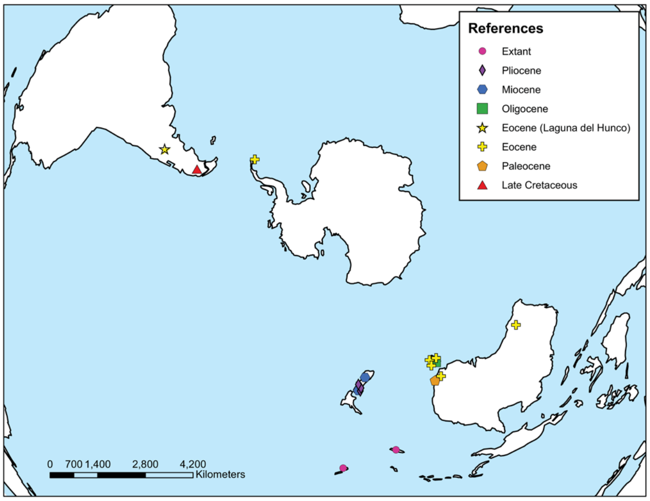

FIGURE 1 Map of south polar view, showing the distribution of extant and fossil species of *Acmopyle* summarized in Table 1. Made with Natural Earth (https://www.naturalearthdata.com), using the Lambert Azimuthal Equal Area projection in ESRI ArcMap 10.6 (ESRI, California, USA).

Table 1). Most of these records correspond to vegetative remains, usually adpressions of leafy shoots with cuticles attached (Table 1).

The phylogenetic position of living *Acmopyle* is stable, always recovered as an early diverging group of the tropical clade of Podocarpaceae and positioned on a long branch (Conran et al., 2000; Kelch, 2002; Sinclair et al., 2002; Biffin et al., 2011, 2012; Knopf et al., 2012; Leslie et al., 2012, 2018; Lu et al., 2014; Andruchow-Colombo et al., 2019a, b). The long branch, combined with the genus's low extant diversity, comparatively high extinct diversity, and broader past geographic range, suggests a relictual status for *Acmopyle*.

Acmopyle is heterophyllous, presenting two or three distinct leaf types (Farjon, 2010). Its most common leaf morphology consists of highly coriaceous, robust, expanded leaves that are bilaterally flattened (Sahni, 1920; Buchholz and Gray, 1947; de Laubenfels, 1969). All its leaves exhibit a helical phyllotaxy, but the bilateral leaves show a secondary arrangement in two ranks over the shoots (Sahni, 1920; Hill and Brodribb, 2003), which can show one or multiple growing seasons depending on the species (de Laubenfels, 1969). Each of the seasonal shoots has been proposed to correspond to a photosynthetic unit, functionally similar to a compound leaf (Brodribb and Hill, 1997). Similar leaf morphologies are also observed in two other genera of Podocarpaceae, *Dacrycarpus* (Endl.) de Laub. and *Falcatifolium* de Laub. (de Laubenfels, 1969, 1972; Farjon, 2010). *Dacrycarpus* species have two distinct leaf types, the generally more common scale-like morphology and the bilateral leaf morphology. The bilateral leaf type of *Dacrycarpus* is similar to the leaves of *Acmopyle* in both external morphology and anatomy, but the leaves are markedly more slender in *Dacrycarpus* and diverge from the branch at a lower angle, among other differences (Sahni, 1920; de Laubenfels, 1969, 1972; Farjon, 2010; Knopf et al., 2012; Wilf, 2012). *Falcatifolium* bilateral leaves are falcate in profile and more expanded and laminar than the coriaceous and robust, non-falcate *Acmopyle* leaves (de Laubenfels, 1969, 1972; Farjon, 2010).

In this contribution, we describe a new fossil species of *Acmopyle* from the early Eocene Laguna del Hunco site, Patagonia, Argentina, based on 42 impressions and compressions of vegetative material. Among the described specimens, some show putative reproductive buds, unique preservation (among all extinct members of the genus) of accessory transfusion tissue (ATT), and evidence of insect damage. The ATT is an extra-venous water-conducting tissue composed of tracheids arranged perpendicularly to the midvein (Sahni, 1920). The position and large diameter of these tracheids make them highly susceptible to collapse and dysfunction under water stress (Brodribb and Holbrook, 2005; Blackman et al., 2010). Extra-venous conducting tissues that transport water perpendicularly from the midvein are known for most genera of Podocarpaceae with moderately expanded to expanded leaves (Sahni, 1920; Brodribb and Holbrook, 2005; Knopf et al., 2012). The reliance of *Acmopyle* on the ATT for lateral fluid transport makes it one of the most drought-sensitive of all podocarps, restricting its habitat to ever-wet rainforests and making it more susceptible to climate change (Brodribb and Hill, 1998, 2004; Brodribb and Holbrook, 2005; Brodribb, 2011). To test the systematic placement of the fossil, we include the new species in a total evidence phylogenetic analysis with extant and extinct members of Podocarpaceae and Araucariaceae and evaluate the relationships among the extant species of *Acmopyle* and the new fossil species. Likewise, we revise the fossil record assigned to the genus, with particular emphasis on a previously described fossil species from another Patagonian locality, which is here concluded to belong to *Dacrycarpus*. The new *Acmopyle* species defined here, from the early Eocene of Argentina and preserving ATT, expands the past range of *Acmopyle* to West Gondwana, while providing physical evidence of a wet (aseasonal) rainforest environment persisting in Patagonia during the Early Eocene Climatic Optimum (EECO).

MATERIALS AND METHODS

Geologic setting

The fossils reported here were collected at the Tufolitas Laguna del Hunco (LH) site, Huitrera Formation, Chubut, Argentina (paleolatitude ca. 47°S). The LH site preserves lacustrine mudstones, sandstones, volcanic ashes, and other sediments that infilled the Piedra Parada caldera, a part of the middle Chubut River volcanic complex within the Pilcaniyeu volcanic belt (Petersen, 1946; Aragón and Romero, 1984; Aragón and

TABLE 1 Fossil and extant species referred to *Acmopyle*.

Species	Age	Locality	Organs known	Bilateral leaf dimensions (L × H, mm)	L/H	Leaf insertion angle	Short shoot dimensions (L × W, mm)	Citation
?*A. antarctica*	Late Cretaceous	Cerro Guido, Chile	LS	9.5–11.5 × 1–2	7–8	55–60°	32 (inc.) × 18	Menéndez, 1972; this study
A. florinii	Late Paleocene	Luke Bungarby, Australia	LS, C	NR	8–10	NR	40 × 20	Hill and Carpenter (1991); Hill (1995)
A. antarctica[1]	Eocene	La Meseta Formation, Antarctica	LS	16 × 2	NR	48°–68°	60 × NR	Florin (1940); Cantrill et al. (2011)
***A. grayae* sp. nov.**	**Early Eocene**	**Laguna del Hunco, Argentina**	**LS, C**	**7–17 × 1.5–3**	**4–9**	**51°–84°**	**45–103 × 13–36**	**This study**
Dacrycarpus engelhardti comb. nov. (formerly *A. engelhardti*)[2]	Middle Eocene	Río Pichileufú, Argentina	LS	5–16 × 1–2.5	5–9	ca. 48°	NP × 9–24	Berry, 1938; Florin, 1940; this study
aff. *A. setiger*/*A. florinii*	Early Eocene	Mount Hotham, Australia	LS, C	5 × NR	NR	NR	NR	Carpenter et al. (2004)
A. setiger	Early Eocene Middle to Late Eocene	Buckland, Tasmania, Australia Pidinga Formation, Australia	L, C C	4–8 × 2	NR	NP	NP	Townrow (1965); Hill and Carpenter (1991); Carpenter and Pole (1995); Mill and Hill (2004)
A. glabra	Early Eocene Early Oligocene	Regatta Point, Tasmania, Australia Cethana, Tasmania, Australia	LS, C LS, C	NR	6–8	NR	40 × 16	Hill and Carpenter (1991); Pole (2007)
A. compactus	Middle to Late Eocene	Hasties, Tasmania, Australia	L,[f] C	NR × 2	NR	NP	NP	Pole (1992)
A. tasmanica	Middle to Late Eocene	Loch Aber, Tasmania, Australia	L, C	7 × 1.5	NR	NR	NR	Hill and Carpenter (1991)
A. masonii	Early Miocene	Manuherikia Group, New Zealand	LS,[f] C	NP	NP	NP	NP	Pole (1997)
A. kirrileeae	Late Miocene to Early Pliocene	Glenhope Formation, New Zealand	L, C	NP	NP	NP	NP	Pole (2007)
Acmopyle sp.	Middle to Late Pliocene Middle Pliocene	Arapito Road, New Zealand Waitahu River, New Zealand	C C	NP	NP	NP	NP	Pole (2007)

(Continues)

TABLE 1 (Continued)

Species	Age	Locality	Organs known	Bilateral leaf dimensions (L × H, mm)	L/H	Leaf insertion angle	Short shoot dimensions (L × W, mm)	Citation
A. biformis	Late Pliocene to Early Quaternary	Grey River, New Zealand	LS, C	2.3–5 × 1–1.7	NR	NP	NP	Pole (2007)
A. pancherii	Extant	New Caledonia	Whole plant	10–30 × 1.5–3	NR	60°–90°	30–150 × ?	Sahni (1920); de Laubenfels (1969); Stockey and Ko (1988); Hill and Carpenter (1991); Farjon (2010)
A. sahniana	Extant	Fiji Islands	Whole plant	10–25 × (0.6–)2–4(–4.8)	NR	60°–90°	20–60(–120) × ?	Buchholz and Gray (1947); de Laubenfels (1972); Hill and Carpenter (1991); Farjon (2010)

Notes: Under "Species":

L = isolated leaves; LS = leafy shoots; C = cuticle; PC = pollen cones; SC = seed cones; and S = seeds. "L/H" = leaf length/height ratio. "Leaf insertion angle" = leaf insertion angle of bilateral leaves. "Short shoot dimensions" = short shoot length × width in millimeters. NR = not reported by the original authors; NP = not preserved; inc. = incomplete. No bilaterally flattened leaves are known for *A. masonii*, *A. kirrileeae*, or *Acmopyle* sp. Measurements of?*A. antarctica* were taken from images of the original material described by Menéndez (1972), provided by Dr. E. I. Vera.

[1] Referred to *Elatocladus* by Cantrill et al., 2011.

[2] see Appendix 1 for new taxonomic treatment of this species. Under "Organs known".

[f] = only fragments preserved.

Mazzoni, 1997; Iannelli et al., 2017; Gosses et al., 2021). The principal fossiliferous section of LH is located in the northeastern part of the caldera, represented by 170 m of stratigraphic section and >30 distinct fossil quarries, as detailed elsewhere (Wilf et al., 2003; Gosses et al., 2021). Radiometric dating of sanidine crystals from a tuff interbedded in the middle of the fossiliferous sequence yielded an ^{40}Ar-^{39}Ar age of 52.22 ± 0.22 Ma (Ypresian; Wilf et al., 2003, 2017), while sanidines from the uppermost strata of the underlying Ignimbrita Barda Colorada produced an ^{40}Ar-^{39}Ar age of 52.54 ± 0.17 Ma (Gosses et al., 2021), showing that fossil deposition occurred during the Early Eocene Climatic Optimum (e.g., Westerhold et al., 2020).

Paleoflora

With >185 macrofloral species currently estimated (based on the number of distinct leaf morphotypes), the Laguna del Hunco flora is one of the most diverse Eocene floras known worldwide (Wilf et al., 2005a). Many of the nearest living relatives of systematically studied taxa are found in Australasian and southeast Asian rainforests (i.e., Wilf et al., 2009, 2013; Kooyman et al., 2014; Merkhofer et al., 2015). The flora is angiosperm-dominated, including taxa such as *Eucalyptus* L'Hér. (Gandolfo et al., 2011; Hermsen et al., 2012), *Castanopsis* (D. Don) Spach (Wilf et al., 2019), *Gymnostoma* L.A.S. Johnson (Zamaloa et al., 2006), *Ceratopetalum* Sm. (Gandolfo and Hermsen, 2017), and *Orites* R. Br. (Romero et al., 1988), but it also includes ferns such as *Todea* Willd. ex Bernh. (Carvalho et al., 2013) and *Dicksonia* M.R. Schomb. (mentioned but not illustrated in Carvalho et al., 2013; see also Barreda et al., 2020; Machado et al., 2023), as well as abundant and diverse gymnosperms including Araucariaceae (Wilf et al., 2014; Rossetto-Harris et al., 2020), Podocarpaceae (Wilf et al., 2005a, 2017; Wilf, 2012, 2020; Andruchow-Colombo et al., 2019a; Barreda et al., 2020; Pujana et al., 2020), Cupressaceae (Wilf et al., 2009), Ginkgoales (Villar de Seoane et al., 2015), and Zamiaceae (Wilf et al., 2016). Podocarp genera previously reported from the flora include *Dacrycarpus* (Endl.) de Laub., *Huncocladus* A. Andruchow-Colombo, Wilf, I. Escapa (relative of *Phyllocladus*), *Podocarpus* Labill., and *Retrophyllum* C.N. Page (Wilf, 2012; Wilf et al., 2017; Andruchow-Colombo et al., 2019a, 2021), along with additional podocarp taxa represented in the palynoflora and wood flora (Barreda et al., 2020; Pujana et al., 2020).

Berry (1925) first described the fossil flora from Laguna del Hunco, including 36 species, but he did not report any gymnosperms, which Petersen (1946) first observed at the site (Araucariaceae only). *Acmopyle* was informally recognized from Laguna del Hunco much later, from more recent collections (Wilf et al., 2005a, 2009; Wilf, 2012). Some of the material described here has been previously referred informally to Podocarpaceae and *Acmopyle* (see below), including the fossil species *Acmopyle engelhardti* (Berry) Florin, a species originally defined from the middle Eocene (ca. 47.7 Ma) Río Pichileufú flora, Río Negro, Argentina (Berry, 1938; Florin, 1940; Wilf et al., 2009, 2017; Wilf, 2012).

A total of 41 specimens with *Acmopyle* characteristics were collected from Laguna del Hunco quarries AL1, LH1, LH4, LH6, LH13, LH15, LH22, LH23, LH25, LH27, and LH29 (Wilf et al., 2003, 2009), with the highest abundance at LH13 (13 specimens) and LH27 (nine specimens). The fossils are curated and housed permanently in the Paleobotanical Collection of the Museo Paleontológico Egidio Feruglio (MEF, Trelew, Chubut, Argentina; repository acronym MPEF-Pb).

Fossil preparation and illustration

The fossils are preserved as compressions and impressions, in some cases with extremely fragmentary or coalified cuticle remains that were studied in situ. Images of macromorphology were taken with Nikon D90, D700, and D850 DSLRs (Nikon, Tokyo, Japan) with 60 mm and 105 mm macro lenses, using polarizing filters and baffles. Microscopic imaging was done with a Nikon DS-Ri1 camera attached to a Nikon SMZ1500 stereomicroscope and to a Nikon LV100 compound microscope at Pennsylvania State University (University Park, Pennsylvania, USA). Epifluorescence imaging on these microscopes used an Endow GFP Longpass Emission green filter (exciter HQ470/40, dichroic Q495LP BS, emitter HQ500LP, Chroma Technology no. 41018; Chroma Technology, Rockingham, Vermont, USA). Images were processed with Adobe Lightroom Classic 10.2 (Adobe, Mountain View, California, USA) for whole-image white balance and contrast enhancements and with Adobe Photoshop 22.3.1 for assembling the plates.

Living material preparation and illustration

Images of herbarium material of the extant *Acmopyle pancheri* and *A. sahniana* were taken in the collections of the Australian National Herbarium (CANB, Canberra, Australia), the National Herbarium of New South Wales (NSW, Sydney, Australia), and in the Herbarium of the Royal Botanic Gardens Kew (K. Richmond, United Kingdom) with several Nikon DSLR cameras and 60 mm and 105 mm macro lenses with polarizing filters.

We studied the anatomical arrangement of the internal leaf conducting tissue in the two extant species of *Acmopyle*. Samples were obtained from 25-yr-old trees growing at the University of Tasmania (Sandy Bay, Tasmania, Australia). Paradermal sections of leaves were made by carefully removing the upper epidermal layer with a razor blade, then clearing the leaf with a commercial household bleach (50 g L^{-1} sodium hypochlorite and 13 g L^{-1} sodium hydroxide) until all pigment was removed. Sections were rinsed, stained with 1% toluidine blue, and mounted on microscope slides in phenol glycerine jelly, then photographed using a camera (Digital Sight DS-L1; Nikon, Melville, New York, USA) mounted on a microscope (DM 1000; Leica, Nussloch, Germany).

With the objective of showing the collapse of the ATT elements in water stress conditions, cross sections of the ATT of *Acmopyle pancheri* under normal water availability and of *A. sahniana* under mild water stress (−2 Mpa) were performed. Cross-sectional anatomy of ATT cells was revealed by making sections of leaves cut perpendicular to the radially oriented ATT cells. Leaves were placed in a small aluminum specimen holder and submerged in liquid nitrogen. The ATT runs perpendicular to the midrib, so leaves were cut 1 mm from the margin, parallel to the midrib, to expose the ATT tracheids in cross section. Sectioned leaves were examined at 20× magnification under a fluorescence microscope (Axioscop; Carl Zeiss, Oberkochen, Germany) while still partially submerged in liquid nitrogen, and photographed using a digital camera (Axiocam; Carl Zeiss) attached to a photographic tube. Images of reproductive shoots were taken from the same material producing cones in 2022 in a glasshouse at the University of Tasmania.

Terminology

Most previous authors used the term *width* to describe mature leaves of *Acmopyle*; however, given the bilaterally flattened nature of the mature foliage, we refer to this measurement as the leaf *height*. It is important to distinguish these two measurements (i.e., leaf width vs. leaf height) because Podocarpaceae has genera with bifacially flattened (e.g., *Podocarpus*, *Prumnopitys* Phil.; Farjon, 2010), bilaterally flattened (i.e., *Acmopyle* and *Falcatifolium* de Laub.), or both types of foliage (e.g., *Dacrycarpus*). Both of these leaf types are planate, but bilateral leaves are flattened in the vertical plane, whereas bifacial leaves are flattened in the horizontal plane (see Sahni, 1920: text and fig. 2). Increasing height (distance between the central point of adaxial and abaxial surfaces) and increasing width (distance between the lateral margins) comprise two completely different paths taken to increment the leaf surface, and therefore these two measurements are non-homologous.

Phylogenetic analysis

The combined matrix includes 92 extant and fossil taxa and 8103 characters from morphology and DNA. The taxon sampling includes 88 extant and four fossil species (Appendix S1, Table S1). The extant taxa include 56 species from the 18 extant genera of Podocarpaceae, 21 species from the three extant genera of Araucariaceae as the taxonomic outgroup, and *Cryptomeria japonica* (Cupressaceae) for rooting the tree (Appendix S1, Table S1). The four fossil species included are *Kirketapel salamanquensis* (Andruchow-Colombo et al., 2019b; early Paleocene of Chubut, Argentina), *Huncocladus laubenfelsii* (Andruchow-Colombo et al., 2019a; early Eocene of Chubut, Argentina), the new early Eocene Patagonian *Acmopyle* species described in the present study (early Eocene of Chubut, Argentina), and *Lepidothamnus diemenensis* (Pole, 1992; middle to late Eocene of Tasmania, Australia).

The morphological block encompasses 61 characters of leaf and seed cone morphology; of those, characters 0–58 were taken from Andruchow-Colombo et al. (2019a), with minor modifications in character scoring (Appendix S2). Characters 59 (*Morphology of bilaterally flattened leaves*) and 60 (*Phylloclades II*) are new (Appendix S2). The nexus file for the morphological matrix is available at Morphobank (https://morphobank.org; project no. P4224).

The molecular blocks include 8047 characters (25.05% informative for parsimony) from two chloroplast (*matK* and *rbcL*) and two nuclear (*PHYP* and *LFY*) regions that were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank; for accession numbers, see Appendix S1, Table S1). Sequences were compiled, aligned, and formatted with the program GenBank-to-TNT (Goloboff and Catalano, 2012) using MAFFT version 7.487 (Katoh et al., 2002; Katoh and Toh, 2008; Katoh and Standley, 2013), using default parameters.

The phylogenetic analysis of the combined matrix was performed under the maximum parsimony optimality criterion with equal weights, using the software TNT 1.5 (Goloboff et al., 2008; Goloboff and Catalano, 2016). All characters were treated as non-additive except for one leaf character and three seed cone characters (19, *Florin ring*; 29, *Mature seed cone size*; 51, *Young ovule orientation*; and 52, *Mature seed orientation*; Appendix S2), which were treated as additive following Andruchow-Colombo et al. (2019a, b). Tree searches were performed by an initial round of 30 random addition sequences, followed by tree-bisection-reconnection (TBR) rearrangements and with a combination of the algorithms Sectorial Searches and Tree Fusing (Goloboff, 1999) under the default parameters in TNT, setting an allocated memory space for 20,000 trees. Searches were set to stop after finding the minimum length 50 times (i.e., from 50 independent replicates). After that initial search, a second round of TBR was performed with the trees in memory. Unstable taxa were pruned from the strict consensus (command *ne//*) to show their alternative positions over the consensus topology. Absolute frequencies of Symmetric Resampling, excluding the unstable taxa, were calculated for group support (300 replicates, change probability $p = 0.33$; Goloboff et al., 2003).

Group synapomorphies (common to all most parsimonious trees) were calculated with the TNT implemented command *apo*. Multistate characters of interest were mapped on all most-parsimonious trees, and the common result of all the individual mappings is shown on the strict consensus tree. Character mapping was performed in TNT (menu options *Optimize* > *Characters* > *Common Mapping*), and its results are provided as supplemental information (Appendix S3).

RESULTS

Systematic Paleontology

Family—Podocarpaceae Endl., Synopsis Coniferarum. 203: 1847.

Genus—*Acmopyle* Pilg., in Engler, Pflanzenr. IV.5 [18]: 117. 1903.

Type species—*Acmopyle pancheri* (Brogn. Et Gris) Pilg., in Engler, Pflanzenr. IV.5 [18]: 117. 1903.

Species—*Acmopyle grayae* A. Andruchow-Colombo, G. Rossetto-Harris, T. J. Brodribb, Gandolfo, et Wilf sp. nov. (Figures 2; 3; 4A–I; 5; 6A–C; and 7).

Etymology—This new species is named in honor of Dr. Netta E. Gray (1913–1970), for her significant advances in understanding comparative morphology and taxonomic delimitations of genera in Podocarpaceae (see https://www.conifers.org/topics/Botanists.php).

Holotype here designated—MPEF-Pb 16508a, b (LH13 locality; Figure 2).

Paratypes—MPEF-Pb 16500–16501 (AL1 locality); MPEF-Pb 16502 (LH01); MPEF-Pb 468a, b (LH04); MPEF-Pb 16503a,b–16504 (LH06); MPEF-Pb 16506–16507, MPEF-Pb 16509a,b–16510a,b, MPEF-Pb 16511–16518a,b, MPEF-Pb 16519 (LH13); MPEF-Pb 16520 (LH15); MPEF-Pb 16521 (LH22); MPEF-Pb 16522–16523a,b (LH23); MPEF-Pb 16525a,b (LH25); MPEF-Pb 16528–16530, MPEF-Pb 16533–16534a,b, MPEF-Pb 16535a,b, MPEF-Pb 16537a,b, MPEF-Pb 16539–16540a,b (LH27), MPEF-Pb 16541 (LH29).

Referred material—MPEF-Pb 16526, MPEF-Pb 16527, MPEF-Pb 16531, MPEF-Pb 16532, MPEF-Pb 16536a,b, MPEF-Pb 16538 (LH27).

Diagnosis—Leafy shoots heterophyllous with three leaf types: bifacial scale-like, transitional, and bilaterally flattened mature leaves. Leafy shoots with up to four scale-like leaves at the base, followed by up to five transitional leaves, and by 19–42 bilateral leaves per side of the branch. Complete leafy-shoot length 44–103 mm; maximum width 13–36 mm, located at 17%–49% of the total length of the leafy shoot. Scale-like leaves in helical phyllotaxy; mostly bifacially flattened, adpressed, triangular; base decurrent, apex acute; length 2–3 mm, width 0.5–1 mm. Transitional leaves in a helical to pseudodistichous phyllotaxy; tetragonal in cross section to bilaterally flattened, straight to slightly spreading; length 2–6 mm, height 0.5–2 mm. Mature leaves with opposite to alternate arrangement on the shoots; lanceolate to falcate; with a slightly asymmetric general outline and strongly asymmetrical apex; single veined; relatively expanded; bilaterally flattened; length 4–17 mm, height 1–3 mm; insertion angle of 50°–85°; leaf base decurrent with its lower side extending basally 1–4 mm and its upper side with a constriction; leaf apex keeled, asymmetrical, apiculate, straight or slightly curved upward. Accessory transfusion tissue present in the mesophyll, emerging from both sides of the midvein, is composed of thin and elongated, closely spaced, subparallel elements. Two stomatal bands on one of the leaf surfaces, parallel to and occurring immediately alongside the midvein; stomatal bands 0.2–0.4 mm wide, bearing multiple stomatal rows. Stomatal orientation parallel to the major leaf axis.

FIGURE 2 Holotype of *Acmopyle grayae* sp. nov., MPEF-Pb 16508a. (**A**) General view of the holotype, representing a complete expanded photosynthetic unit (EPU). (**B**) Detail of the basal third of the EPU, showing its width increase and the three type of leaves: basal scale-like leaves, transitional leaves, and mature leaves; several leaves and leaf portions were chewed by herbivores. (**C**) Detail of the middle third of the EPU, showing only mature leaves that are nearly invariable in dimensions. (**D**) Detail of the apical third of the EPU, showing its width decrease and two leaf types: mature and transitional leaves, and a globose apex. (**E**, **F**) Details of the globose apex of the EPU, which is covered in scale-like leaves. Four transitional leaves are marked in E with white arrowheads. (**G**) Detail of mature leaves, showing decurrent base constricted on the apical side, midvein, two compressed stomatal bands adjacent to midvein, and asymmetrical, acute apiculate apex. (**H**) Detail of the EPU base, showing three scale-like leaves (black arrowheads) and at least two transitional leaves (white arrowheads), as well as one chewed leaf (red arrowhead). Scale bars: A = 9 mm; B, C, D = 5 mm; G = 2.5 mm; E, H = 2 mm; F = 1 mm.

Description

Leafy shoot morphology

Naked branch segments are 0.5–1.5 mm wide and ≤103 mm long. Leafy shoots are heterophyllous, bearing three types of leaves: small and scale-like, mostly bifacially flattened (Figure 2H, black arrowheads); transitional leaves that are tetragonal in cross section to bilaterally flattened (Figure 2H, white arrowheads); and robust, expanded, bilaterally flattened mature leaves (Figures 2–4). Transitional leaves show an intermediate length between the scale-

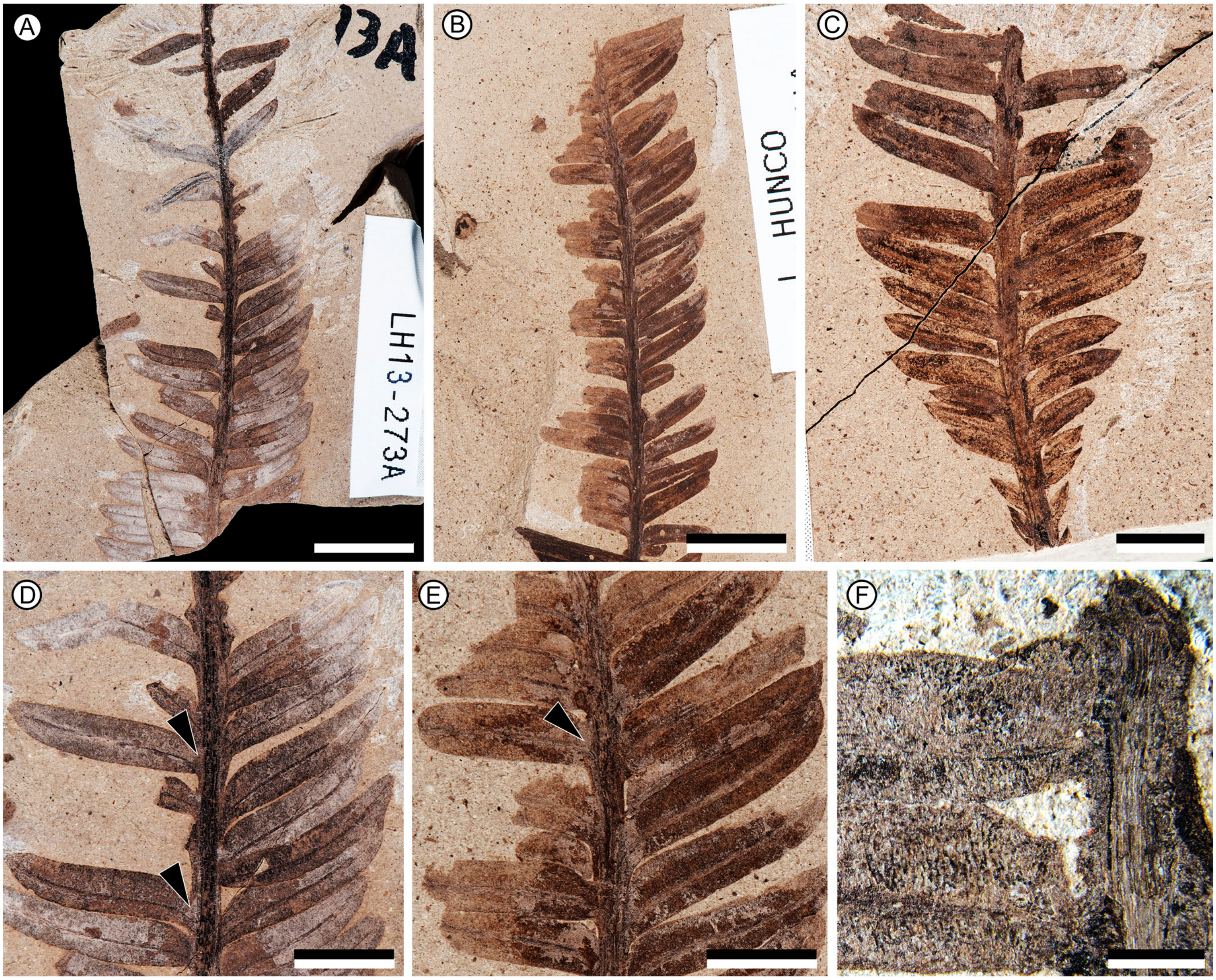

FIGURE 3 Vasculature details of *Acmopyle grayae* sp. nov. (**A**) General morphology of MPEF-Pb 16507. (**B**) General morphology of MPEF-Pb 16510a. (**C**) General morphology of MPEF-Pb 16511. (**D**) Detail of MPEF-Pb 16507, showing the connection between the leaf and shoot vasculature (black arrowheads). Note several leaves chewed at the left mid-portion of the branch. (**E**) Detail of MPEF-Pb 16510a, showing the connection between the leaf and shoot vasculature (black arrowhead). (**F**) Detail of MPEF-Pb 16511, showing leaf and shoot vasculature with coalified mesophyll and ATT (cf. Figure 6). Scale bars: A, B = 10 mm; C, D = 5 mm; E = 4 mm; F = 2 mm.

like and the expanded leaves. Several specimens show evidence of the shoot vasculature (Figure 3), in some cases possibly preserving vascular elements (Figure 3F). Additionally, some specimens preserve the connection between the shoot and leaf vasculature (Figure 3D, E).

At the base of the shoot, there are up to four scale-like leaves followed by up to five transitional leaves (Figures 2A, B, H; 3C; 4A, B, D, H; and 5I, J). There are 19–42 (mean = 30; SD = 10; n = 5) bilateral leaves per side of the branch, forming most of the leafy-shoot structure (Figure 4A–I). The bilateral leaves increase in length toward the medial zone of the shoot and then decrease in length toward the shoot apex, where the foliage shifts rapidly to transitional leaves (Figures 2D, E; and 4A, B). In one specimen, the leafy shoot culminates in a globose structure of 1.3 mm diameter, covered by scaly leaves (Figure 2D–F). In other specimens we observe different morphologies of the apical region of the leafy shoot, including possible reproductive structures; these are (1) a cluster of transitional leaves (Figure 5C–E); (2) a short continuation of the axis covered by scale-like leaves that appears to be broken before its apex (Figures 4A and 5A, B); (3) a continuation of the axis covered by transitional leaves and showing a terminal globose apex of 1.5 mm in diameter that appears to be covered in scaly leaves (Figures 4B and 5F); and (4) two mature terminal leaves (Figures 4I and 5G).

The complete leafy-shoot structure, as described above, is 45–103 mm long (mean = 77 mm; SD = 26.0 mm; n = 5) and has a maximum width of 13–36 mm (mean = 22 mm; SD = 8 mm; n = 5) located at 18%–48% of the leafy-shoot total length (mean = 39%; SD = 13; n = 5).

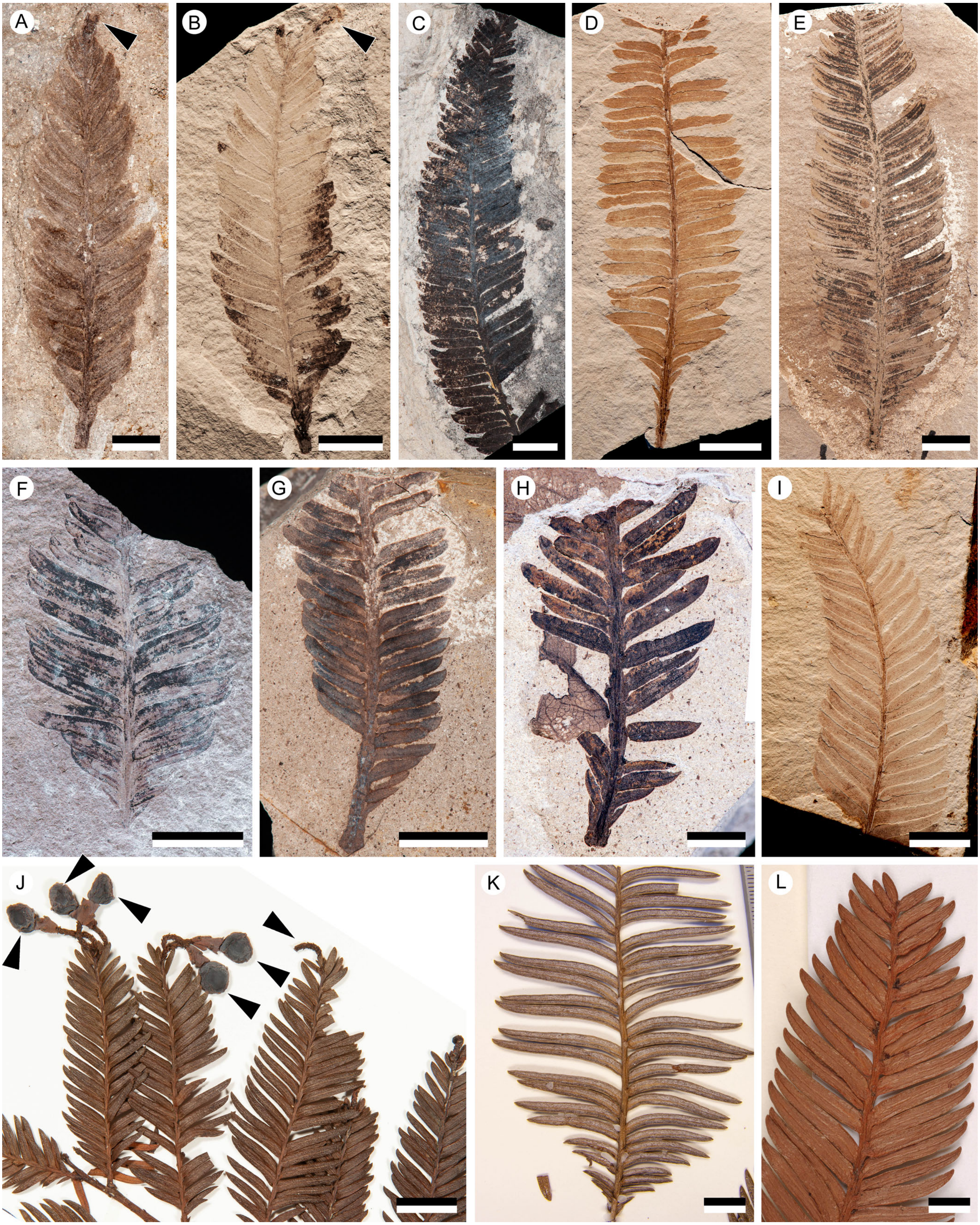

FIGURE 4 (See caption on next page).

Leaf morphology

The scale-like leaves are mostly bifacially flattened, adpressed, and triangular, with a decurrent base and an acute apex (Figure 2H). They are present at the shoot base and apex in helical phyllotaxy (Figure 2B, H); they are generally smaller than the other two leaf types, measuring 2–3 mm long (mean = 2 mm; SD = 1 mm; *n* = 10) and 0.5–1 mm wide (mean = 0.7 mm; SD = 0.3 mm; *n* = 10).

The transitional leaves are tetragonal in cross section to bilaterally flattened, straight (i.e., not curved inward) to slightly spreading (Figures 2H, 3C, and 5J), and generally larger than the scale-like leaves but smaller than the fully mature leaves, measuring 2–6 mm long (mean = 4 mm; SD = 2 mm; *n* = 16) and 0.5–2 mm high (mean = 1 mm; SD = 1 mm; *n* = 16). Their phyllotaxis is helical to pseudodistichous (Figures 2H, 3C, and 5I, J).

Mature leaves show a helical primary phylotaxy with the leaf bases bending, resulting in an opposite to alternate secondary arrangement on the shoots (Figure 5I, J). Leaf insertion angles are 51°–84° (mean = 64°; SD = 7°; *n* = 42; Figures 2–4). Leaves are strongly coriaceous in appearance (Figure 4G), expanded, lanceolate to falcate (Figures 2C; 3A, D; and 4F, G), showing bilateral flattening (Figures 3D and 5I) and a single vein (Figures 2, 3C–F, and 6A–C). The leaf base is decurrent, with its lower side extending basally onto the shoot for 1–4 mm (mean = 2 mm; SD = 1; *n* = 38; Figures 3E and 5I, H, M) and with its upper side showing a constriction where the leaf meets the branch (Figure 5L). The leaf apex is slightly to very asymmetric (Figures 2G, 3C–E, and 4H), keeled, apiculate, and straight or slightly curved upward (Figures 2C, D; and 3C). Mature leaves are 4–17 mm long (mean = 9 mm; SD = 4 mm; *n* = 39) and 1–3 mm high (mean = 2 mm; SD = 0.4 mm; *n* = 42).

Several specimens preserve anatomical details, showing remains of vasculature in branches (Figure 3A–F) and mature leaves (Figure 3A, B, D, E), and coalified mesophyll, within which are darkly coalified sublinear, subparallel features that we interpret as remains of accessory transfusion tissue (ATT). The ATT emerges close to perpendicular (71°–103°, mean = 86°; SD = 11°; *n* = 10) from the midvein toward the leaf margins (Figures 4H and 6A–C), reaching 52%–85% of the distance from the midvein to the margin (*n* = 3; Figure 6A), and is composed of thin, elongated, closely spaced elements (Figures 4H and 6A–C). The width of the ATT elements is 35–52 μm (mean = 44 μm; SD = 7 μm; *n* = 7 elements), and the spacing between neighbor elements is 12–30 μm (mean = 22 μm; SD = 7 μm; *n* = 9); all these measurements were taken from a single leaf with the best preservation of the ATT tissue (Figures 4H and 6A–C).

Epidermal morphology

Some specimens show two broad stomatal bands on their leaves varying between 0.2 mm and 0.4 mm in width (mean = 0.3 mm; SD = 0.1 mm; *n* = 10 leaves of a single branch; Figure 7A–C). Stomatal bands begin directly on the midvein sides (i.e., they are not separated from it by a non-stomatal zone). Each band shows several rows of coalified stomata (Figure 7D) oriented parallel to the major leaf axis and possibly sharing subsidiary cells, given their proximity to each other (Figure 7D).

Remarks

Scale-like leaves show a more fragile appearance than the other leaf types and are usually preserved partially or only as leaf scars in some specimens (Figure 5J). Our interpretation of the coalified sublinear, subparallel features as ATT is based on their distinctive comb-like configuration, position, size, and spacing, all of which closely resemble the ATT documented in living *Acmopyle* species, especially *A. sahniana* (Figure 6D). Specimens here assigned to *Acmopyle grayae* sp. nov. were previously referred informally to "Podocarpaceae (planated foliage)," morphotype TY007 (Wilf et al., 2005a: table A2), *Acmopyle* (Wilf et al., 2009: 2047), and *Acmopyle engelhardti* (see below; Wilf, 2012: 568; Merkhofer et al., 2015: table 1; Wilf et al., 2017: pp. 1354, 1363). Specimens of *A. grayae* show a particular distribution of the three leaf types on their shoots, from base to apex: scale-like leaves, transitional foliage, mature leaves, transitional foliage (Figure 2A), and occasionally either a globose apex (Figure 2E) or a short axis covered in scale-like leaves (Figure 5A, B). This pattern is compatible with determinate or seasonal growth (as seen in extant *Acmopyle*; de Laubenfels, 1969).

Insect/herbivore damage

Several specimens show evidence of insect-feeding damage (Figures 2H and 7A, E–K), including margin feeding (DT12), apex feeding (DT13), hole feeding (DT01), and possible circular punctures (DT46 or DT47) as defined by Labandeira et al. (2007). Margin feeding included circular

FIGURE 4 General morphology of the expanded photosynthetic units of *Acmopyle*. (A–I) *Acmopyle grayae* sp. nov. (J–L) *Acmopyle pancheri*. (A, B) Complete expanded photosynthetic units (EPUs), showing partially preserved reproductive structures at their apices (black arrowheads). (C–I) Partially complete EPUs. (**A**) MPEF-Pb 16506. (**B**) MPEF-Pb 16520a. (**C**) MPEF-Pb 16533. (**D**) MPEF-Pb 16535a. (**E**) MPEF-Pb 16536b. (**F**) MPEF-Pb 16504. (**G**) 16516. (**H**) MPEF-Pb 16514; black arrowhead indicates the leaf that preserves ATT (see Figure 6A–C). (**I**) MPEF-Pb 16537a. (**J**) Branch bearing several EPUs with mature apical seed cones (black arrowheads; CANB 593572). (**K**) Basal portion of an EPU, K 000287741. (**L**) Apical portion of an EPU, NSW 368939. Scale bars: A, H = 5 mm; B, C = 8 mm; D, E, F, G, I, K = 10 mm; J = 20 mm; L = 6 mm.

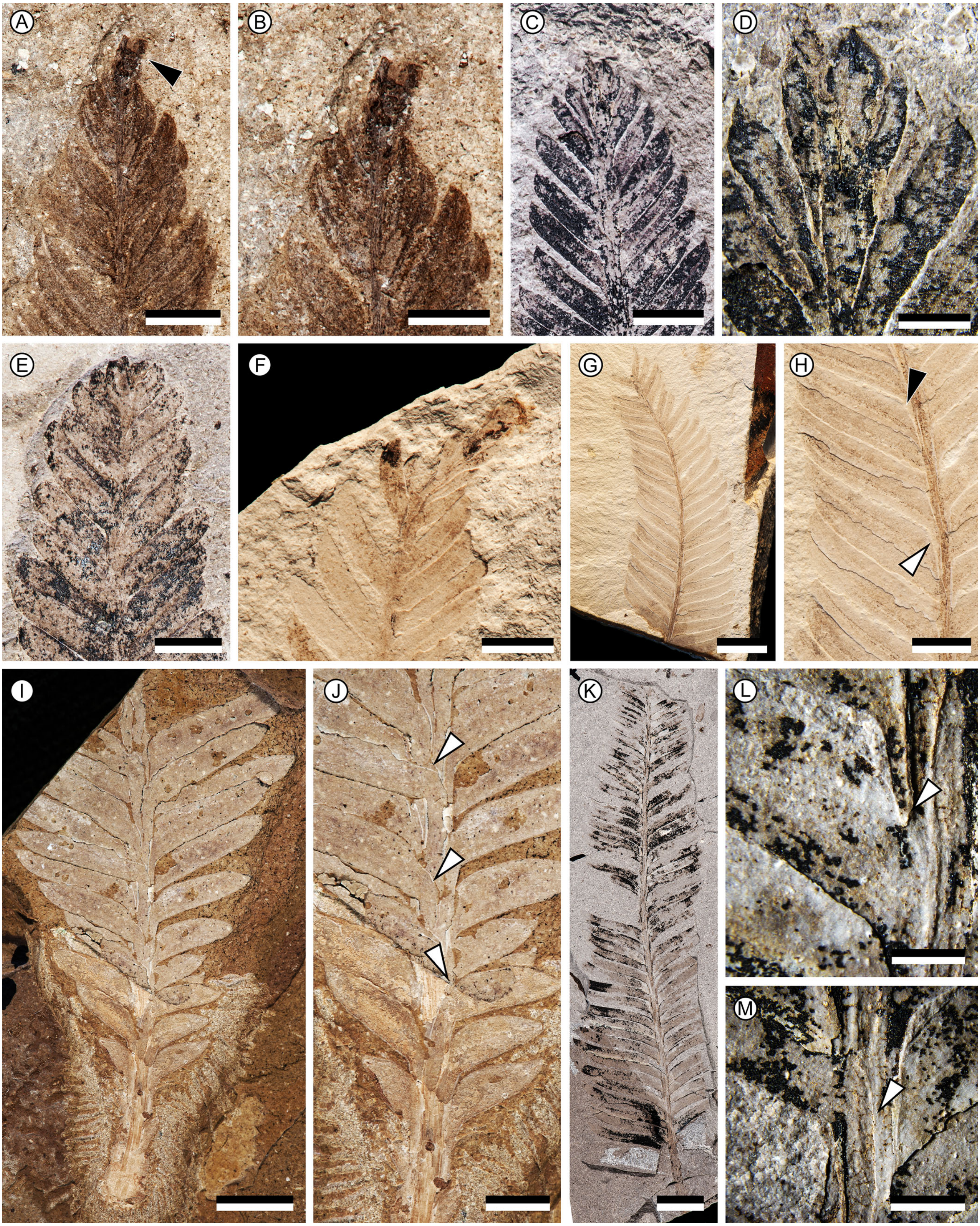

FIGURE 5 (See caption on next page).

and mostly shallow excisions (DT12) at the lateral margins of leaves (Figure 7A, white arrowhead; F, G, I, black arrowheads) and in one case close to the leaf apex (Figure 7A, K, white arrowheads). Apex feeding (DT13) consumed the apex and midvein of several leaves (Figures 2H, red arrowhead; and 7I, J, white arrowheads), in some cases removing most of the blade (Figure 7J). Hole feeding was found in one specimen, a circular perforation of 0.3 mm diameter with a dark reaction rim (DT01; Figure 7H, black arrowhead). Additionally, one specimen shows two small circular scars of 0.2 mm (Figure 7A, white rectangle, E) that are compatible with either DT46 or DT47.

Phylogenetic analysis

The phylogenetic analysis generated 1488 maximum parsimony trees (MPT) of 6390 steps; the strict consensus of all MPT is shown in Figure 8. The analysis recovered the prumnopityoid and tropical main clades of Podocarpaceae. The prumnopityoid clade comprises *Prumnopitys* as sister to the scale-leaved clade (*sensu* Andruchow-Colombo et al., 2019b), which includes three of the analyzed fossil species (*Huncocladus laubenfelsii*, *Kirketapel salamanquensis*, and *Lepidothamnus diemenensis*). *Huncocladus* is recovered as sister to the clade that comprises extant *Phyllocladus* species as in Andruchow-Colombo et al. (2019a). *Kirketapel* and *L. diemenensis* show more alternative positions than in their original analysis (Figure 8, black and white circles respectively; Andruchow-Colombo et al., 2019b). All alternative positions of *Kirketapel* are within the total group of the scale-leaved clade (Figure 8, black circles). *Lepidothamnus diemenensis* resolves as sister to the extant *L. fonkii* or as sister to the clade composed of *Parasitaxus*, *Manoao*, and *Lagarostrobos* (Figure 8, white circles).

Within the tropical clade, the genera *Saxegothaea*, *Microcachrys*, *Acmopyle*, and *Pherosphaera* are recovered as successive sister taxa to a clade formed by the dacrydioid and podocarpoid lineages; the latter comprises *Podocarpus* as sister to the polypodiopsis clade (Figure 8). *Acmopyle grayae* sp. nov. is recovered in a trichotomy with extant *Acmopyle pancheri* and *A. sahniana* (Figure 8, black and white star).

Morphological synapomorphies common to all most parsimonious trees that support the placement of *Acmopyle grayae* as part of the total group of *Acmopyle* are the opposite to subopposite leaf arrangement (character 0, state 2) and the bilaterally flattened mature leaf type (character 3, state 0; Appendix S3, Figure S1; morphological matrix at P4224 at https://morphobank.org). Other characters that support the grouping of *A. grayae* with the extant *Acmopyle* species are the broad morphology of mature leaves (character 1, state 2; Appendix S3, Figure S2) and the robust appearance of the bilaterally flattened leaves (character 59, state 1; Appendix S3, Figure S3). Additionally, there are ≤78 molecular synapomorphies for the *Acmopyle* total-group node that are common to some of the most parsimonious trees. These are distributed between the *matK* (17 synapomorphies, 21.8%), *rbcL* (10 synapomorphies, 12.8%), and *PHYP* (51 synapomorphies, 65.4%) blocks.

DISCUSSION

Assignment to *Acmopyle*

The fossil specimens from Laguna del Hunco are referred to the genus *Acmopyle* because of the presence of numerous shared vegetative features, including leafy shoots with determinate growth and three distinct leaf types (i.e., scale-like, transitional, and broad bilateral mature leaves); distichous mature leaf arrangement; lanceolate, robust, and coriaceous bilaterally flattened leaf morphology; leaves with broad insertion angle (ca. 64°), decurrent base, and apiculate apex; and the presence and distribution of the accessory transfusion tissue in the leaves (Sahni, 1920; de Laubenfels, 1969; Bush and Doyle, 1997; Farjon, 2010). Sahni (1920: text figure 3) wrote that the mature bilateral leaves of *Acmopyle pancheri* "emerge in positions of the branch other than its sides and bend (but do not torsion), reaching in that way the lateral position in the branch," meaning that the genus shows a primary helical phyllotaxy, but the leaves reorient in a secondary, pseudo-distichous arrangement by bending their leaf bases. This feature is preserved in the LH specimens (Figure 5I, J).

Two other genera of Podocarpaceae with leafy-shoot morphologies comparable to that of the new species are *Dacrycarpus* and *Falcatifolium* (de Laubenfels, 1969; Farjon, 2010). Both genera have bilaterally flattened, relatively broad foliage forms that are arranged in a pseudo-distichous manner, constituting expanded photosynthetic units (EPU; Brodribb and Hill, 1997; Biffin et al., 2012). In *Acmopyle* the broad bilateral leaves are markedly more common than other leaf types, whereas in

FIGURE 5 Detailed morphology of *Acmopyle grayae* sp. nov. (A–G) Apices of the expanded photosynthetic unit (EPU). (H–M) Leaf base morphology. (A, B) MPEF-Pb 16506. (C, D) MPEF-Pb 16528. (E) MPEF-Pb 16521. (F) MPEF-Pb 16533. (G, H) MPEF-Pb 16537a. (I, J) MPEF-Pb 16501. (K–M) MPEF-Pb 16503a. (**A**, **B**) Detail of an EPU apex, showing the peduncle of a potential reproductive structure (arrowhead in A). (**C–E**) Detail of two EPU apical regions, showing transitional leaves helically arranged. (**F**) Detail of an apical region ending in a peduncle bearing transitional leaves and ending in a globose structure. (**G**) General view of a specimen. (**H**) Detail of the leaf insertion in the specimen shown in G; black arrowhead indicates the constriction in the upper side of the leaf insertion; white arrowhead indicates the leaf base expanding downward decurrently onto the twig. (**I**) General view of a specimen. (**J**) Detail of the specimen in I, showing leaf torsion (white arrowheads). (**K**) General view of a specimen. (**L**) Detail of the specimen in K, showing the constriction in the upper side of the leaf insertion (white arrowhead). (**M**) Detail of the specimen in K, showing the leaf base expanding downward decurrently onto the twig (white arrowhead). Scale bars: A = 4 mm; B, C, J = 2.5 mm; D, L = 1 mm; E = 3 mm; F, H = 4 mm; G, K = 10 mm; I = 5 mm; M = 1.5 mm.

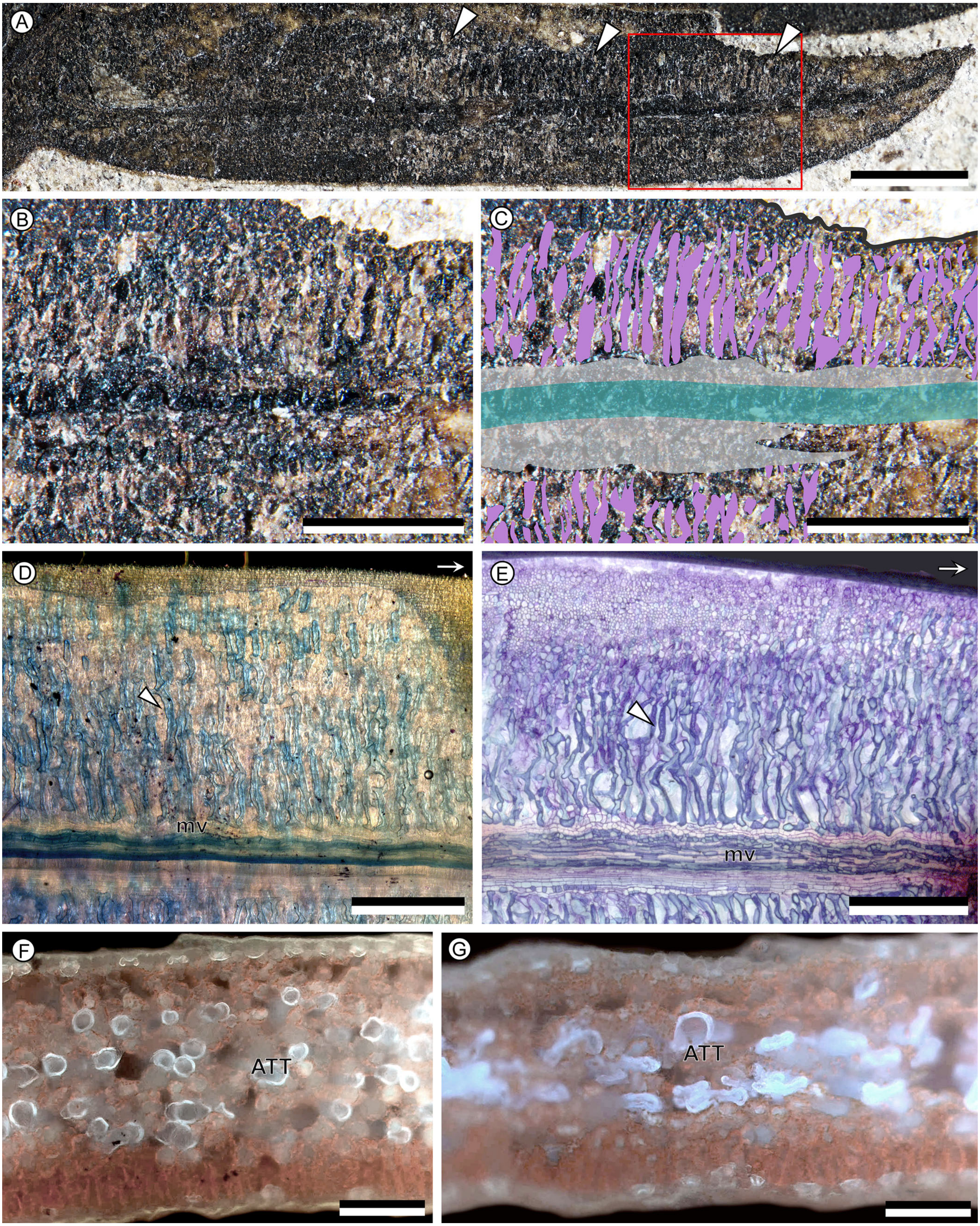

FIGURE 6 (See caption on next page).

Dacrycarpus bifacially flattened, scale-like morphologies tend to predominate in the foliage type (Farjon, 2010). However, bilateral *Dacrycarpus* foliage is common in several living species (e.g., *D. imbricatus*) and appears in the fossil record of the genus (e.g., Hill and Brodribb, 1999; Wilf, 2012). *Acmopyle* species, including the Patagonian fossil described here, have thick stomatal bands alongside the midvein, whereas species of *Dacrycarpus* are characterized by thin, linear stomatal bands well offset from the midvein (Wells and Hill, 1989), as observed in the fossil species *Dacrycarpus puertae*, which is also from Laguna del Hunco (Wilf, 2012). Furthermore, the bilateral leaves of *Acmopyle* are generally taller (1.0–2.7 vs. 0.6–2.0 mm height) and more robust than those of *Dacrycarpus* and show a generally higher angle of leaf insertion (ca. 60°–90° compared to ca. 45°; see Table 1; Farjon, 2010). These differences are also observed between the new species and co-occurring *D. puertae* and help to readily distinguish them (e.g., Wilf, 2012: fig. 19). Differences between *Acmopyle* and *Falcatifolium* include lanceolate to slightly falcate, coriaceous leaves in *Acmopyle*, compared to strongly falcate and thinner (i.e., more laminar) leaves in *Falcatifolium* (Farjon, 2010). Additionally, *Falcatifolium* has homomorphic leaves in their lateral vegetative shoots, whereas *Acmopyle* is heteromorphic (Farjon, 2010). The new fossil species coincides with *Acmopyle* in all the distinctive features mentioned that separate these three genera.

Possible developmental stages of reproductive structures

Acmopyle grayae sp. nov. specimens show a wide range of leafy shoot apex morphologies, some of which may represent dormant vegetative apices or various stages of ovuliferous cone development (Figures 2D–F; 4A, B, I; and 5A–C, E–G). As described above, the observed shoot apex diversity includes a globose structure covered in scaly leaves (Figures 2D–F and 9A; labeled HS3); a cluster of transitional leaves (Figures 5C–E and 9A; labeled HS1 and HS2); a short (broken) continuation of the axis covered with scale-like leaves (Figures 4A; 5A, B; and 9A; labeled HS4); a continuation of the axis covered with transitional leaves terminating in a globose apex covered with scale-like leaves (Figures 4B, 5F, and 9A; labeled HS5); and two mature leaves (Figures 4I and 5G). Most vegetative apices of extant *Acmopyle* leafy shoots reported in the literature and directly observed from fresh and herbarium material (A.A.C. and G.R.H., personal observation, March 2022) culminate in either fertile shoots or two or three bilaterally flattened terminal leaves (Figure 4L; de Laubenfels, 1969), the latter similar to a fossil specimen from Laguna del Hunco (Figure 4I). Some of the leaf-cluster apical morphologies observed in *A. grayae* (Figure 5C–E) could also be compatible with a dormant vegetative apex. In this sense, *A. sahniana* has been reported to frequently produce multiseasonal growth of its leafy shoots (de Laubenfels, 1969; Bush and Doyle, 1997), whereas this is more rare in shoots of *A. pancheri* (T.B., personal observation). However, there are no illustrations of dormant apices of *A. sahniana* expanded photosynthetic units in the literature, nor were these found in the herbarium and living material examined (A.A.C. and G.R.H., personal observation, March 2022); therefore, a precise morphological comparison is not possible at this time.

We suggest that some of the morphologies observed in *A. grayae* are compatible with early developmental stages of seed cones because they are similar to those reported for the extant *A. pancheri* (Mill et al., 2001; Figure 9A). We compare three of the apical morphologies observed in *A. grayae* to the early developmental stages illustrated by Mill et al. (2001) in their study of *A. pancheri*, namely the globose terminal structure covered with scale-like leaves and the two states that show continuations of the axis covered with scale-like and transitional leaves (Figure 9A; labeled HS3, HS4, and HS5). In this scenario, the leaf clusters might represent either a dormant vegetative apex or early stages of megasporangiate-cone development (Figure 9A; labeled HS1, HS2). The globose apical morphology observed in the holotype of *A. grayae* (Figure 2D–F) could also be compatible with an early-aborted ovuliferous cone (Figure 9B).

Was *Acmopyle* present at Río Pichileufú?

Acmopyle (*Podocarpus*) *engelhardti* (Berry) Florin was described on the basis of leafy shoots, constituting expanded photosynthetic units bearing bilaterally flattened leaves, that were collected at the middle Eocene Río Pichileufú locality (Río Negro, Patagonia, Argentina; Berry, 1938; Florin, 1940; Wilf et al., 2005a). Upon observation of Berry's type and cohort material (Figure 10) deposited at the Smithsonian

FIGURE 6 Accessory transfusion tissue in the fossil *Acmopyle grayae* sp. nov. (A–C), and in the extant *A. sahniana* (D) and *A. pancheri* (E–G). (**A**–**C**) Coalified accessory transfusion tissue (ATT) in *Acmopyle grayae* sp. nov, MPEF-Pb 16514. (**A**) Complete leaf, showing the ATT preserved in its midapical and subapical portions, particularly on the upper side, preserved as numerous, thin, closely spaced, coalified linear, subparallel features nearly perpendicular to the midvein (arrowheads). (**B**) Detail of a portion of the leaf illustrated in A (red rectangle in A), showing the ATT. (**C**) Portion of the leaf illustrated in B with ATT elements outlined in lilac. (**D**, **E**) Paradermal sections of leaves of *Acmopyle sahniana* (D) and *A. pancheri* (E), showing the morphology and distribution of the ATT elements (white arrowheads) emerging along both sides of the midvein (mv); arrow indicates the direction of the apex. The ATT of *A. sahniana* (D) is more linear and thus more similar to the fossils (A, B) than that of *A. pancheri*. (**F**, **G**) Longitudinal section of leaves of *Acmopyle*, showing the transversal section of the ATT with the lignin autofluorescing in light blue, and chlorophyll in red. (**F**) ATT of *A. pancheri* under normal conditions of water availability for the plant. (**G**) ATT of *A. sahniana* collapsed due to water stress (−2 Mpa). Scale bars: A = 1 mm; B–E = 500 μm; F, G = 200 μm.

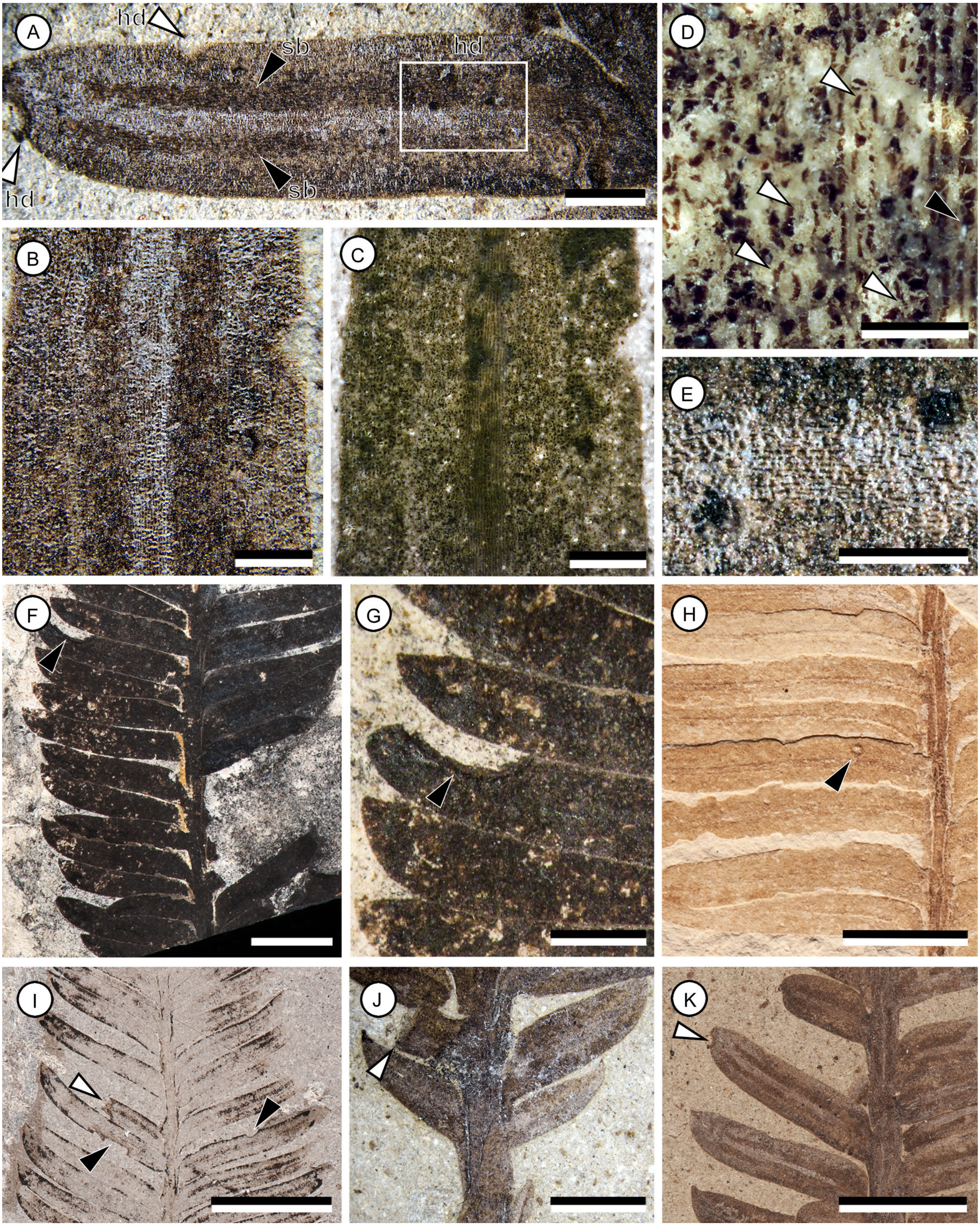

FIGURE 7 (See caption on next page).

National Museum of Natural History (Washington, D.C., USA; repository acronym USNM) and new material collected at the original locality (deposited at Museo Paleontólogico de Bariloche [MAP-BAR], Río Negro, Argentina), we determined that no type or other Río Pichileufú material of *A. engelhardti* belongs in *Acmopyle*. All but one of these Río Pichileufú "*Acmopyle*" fossils actually represent shoots of *Dacrycarpus* bearing both bifacially and bilaterally flattened foliage. The remaining type specimen belongs to *Araucaria pichileufensis* Berry (Figure 10C; Berry, 1938: pl. 13 fig. 2; Rossetto-Harris et al., 2020).

The affinity of the Río Pichileufú material with *Dacrycarpus* and not *Acmopyle* is clearly shown in several features detailed in Appendix 1, such as the presence of well-developed bifacial leaves, the comparatively small and slender bilateral leaves, the mixed phyllotaxy between pseudo-distichous and helical (Figure 10A–C), the relatively low angles of leaf insertion (ca. 48° compared with ca. 60°–90° in *Acmopyle* and ca. 45° in *Dacrycarpus*; Farjon, 2010), and their thin stomatal bands that are separated from the midvein (Figure 10A). Interestingly, the genus *Dacrycarpus* was previously identified at Río Pichileufú, with five fossil specimens assigned to *Dacrycarpus puertae* P. Wilf (Wilf, 2012: 572, second column). *Dacrycarpus puertae* was originally based and typified on Laguna del Hunco material, which includes leafy shoots with epidermal and anatomical preservation, as well as attached pollen and ovulate cones (Wilf, 2012). However, the *Dacrycarpus* material from Río Pichileufú only includes leafy shoots. We propose the new combination *Dacrycarpus engelhardti* (Berry) P. Wilf et A. Andruchow-Colombo for all the *Dacrycarpus* material at Río Pichileufú (Figure 10; Appendix 1). In summary, there is no evidence of presence of *Acmopyle* at Río Pichileufú.

All previous informal references of Laguna del Hunco *Acmopyle* material to "*A.*" *engelhardti* (see above) are therefore also incorrect at species level, making necessary our new species *A. grayae* for all *Acmopyle* specimens from Laguna del Hunco.

Fossil record of *Acmopyle*

The fossil record referred to the genus *Acmopyle* is based only on vegetative remains (i.e., leafy shoots, isolated leaves, and in situ or dispersed cuticles). The genus has been reported from multiple Southern Hemisphere localities, with putative records starting from the Late Cretaceous (Table 1). Additionally, Banerji and Ghosh (2006, 2008) erected the extinct genus *Podocarpospermum* Banerji et Gosh, from the Early Cretaceous of the Rajmahal Basin, Jharkhand, India, based on permineralized, isolated ovules/seeds covered by an epimatium, which they associated with *Acmopyle*.

We compare *Acmopyle grayae* sp. nov. to other *Acmopyle* fossil species (other than "*A.*" *engelhardti*, discussed above) described from the Cretaceous–Pliocene (Table 1). In many cases, previous studies reported a restricted list of character measurements or lack the detailed preservation of the new LH fossils, and consequently the comparisons are limited (Table 1). From the Late Cretaceous (Campanian or lower Maastrichtian; E. I. Vera, personal communication) of Cerro Guido, Chile, *?Acmopyle antarctica* is a single leafy shoot specimen with leaves within the leaf length, length/height ratio, and insertion angle ranges of *A. grayae* but of slightly less height (Table 1; Menéndez, 1972, and additional measurements from the present study). Overall, this specimen is generally smaller than most of those referred to *A. grayae*; however, the poor preservation of the Chilean specimens does not allow further comparisons.

Acmopyle florinii R. Hill et Carpenter from the late Paleocene of New South Wales, Australia, is similar in the overall length and width of the short shoot, and the angle of insertion (ca. 60°, measured by us from the published images; Hill and Carpenter, 1991) falls within the lower part of the range of *A. grayae*; however, *A. florinii* remains show a coalified layer that makes it difficult to detect additional characters for comparison. Although there is an overlap in the leaf size and insertion angle between this Paleocene species and *A. grayae*, these are defining characters at the generic, rather than the specific, level. For reference, both extant species of *Acmopyle* also show a range overlap for these characters (Table 1).

Acmopyle antarctica Florin was described by Florin (1940) from the early Eocene of Antarctica. More recently, Cantrill et al. (2011: 321) disputed this genus assessment, based on the lack of cuticle on the Antarctic specimens and a different interpretation of its leaf macromorphology; those authors reassigned Florin's *A. antarctica* to the form-genus

FIGURE 7 Epidermal morphology of *Acmopyle grayae* sp. nov. (A–D) and insect-feeding damage (A, E–K). (A–E) MPEF-Pb 16510b. (**A**) General view of a leaf, showing the two coalified lateral stomatal bands (sb, black arrowheads) under light microscopy and insect damage scars (hd, white arrowheads and rectangle), detail of margin-feeding area (left white arrowhead), DT12 margin feeding (right white arrowhead), as well as two small circular DT46 or DT47 piercing-and-sucking scars (white rectangle, detailed in E). (**B**, **C**) Details of the leaf in A under light microscopy (B) and epifluorescence (C), showing coalified stomatal bands and epidermal cells of the midvein (C); the apex of the leaf is upward in both images. (**D**) Details of a stomatal zone under epifluorescence, showing the parallel-oriented remnants of stomata (white arrowheads) and the elongated epidermal cells over the midvein (black arrowhead). (**E**) Detail of the two DT46 or DT47 scars shown in A. (**F**, **G**) MPEF-Pb 16520a, showing DT12 margin feeding (black arrowhead). (**H**) MPEF-Pb 16535a, showing a cuspate DT12 perforation with a dark reaction rim (black arrowhead). (**I**) MPEF-Pb 16503b, showing DT13 apical feeding (white arrowhead) and DT12 margin feeding (black arrowheads). (**J**) MPEF-Pb 16508a, showing DT13 apical feeding revealing the leaf cross section (white arrowhead). (**K**) MPEF-Pb 16510b, showing DT12 margin feeding close to the apex (white arrowhead). Scale bars: A = 1 mm; B, C = 0.5 mm; D = 0.1 mm; E = 0.375 mm; F, H, K = 5 mm; G = 2 mm; I = 10 mm; J = 3 mm.

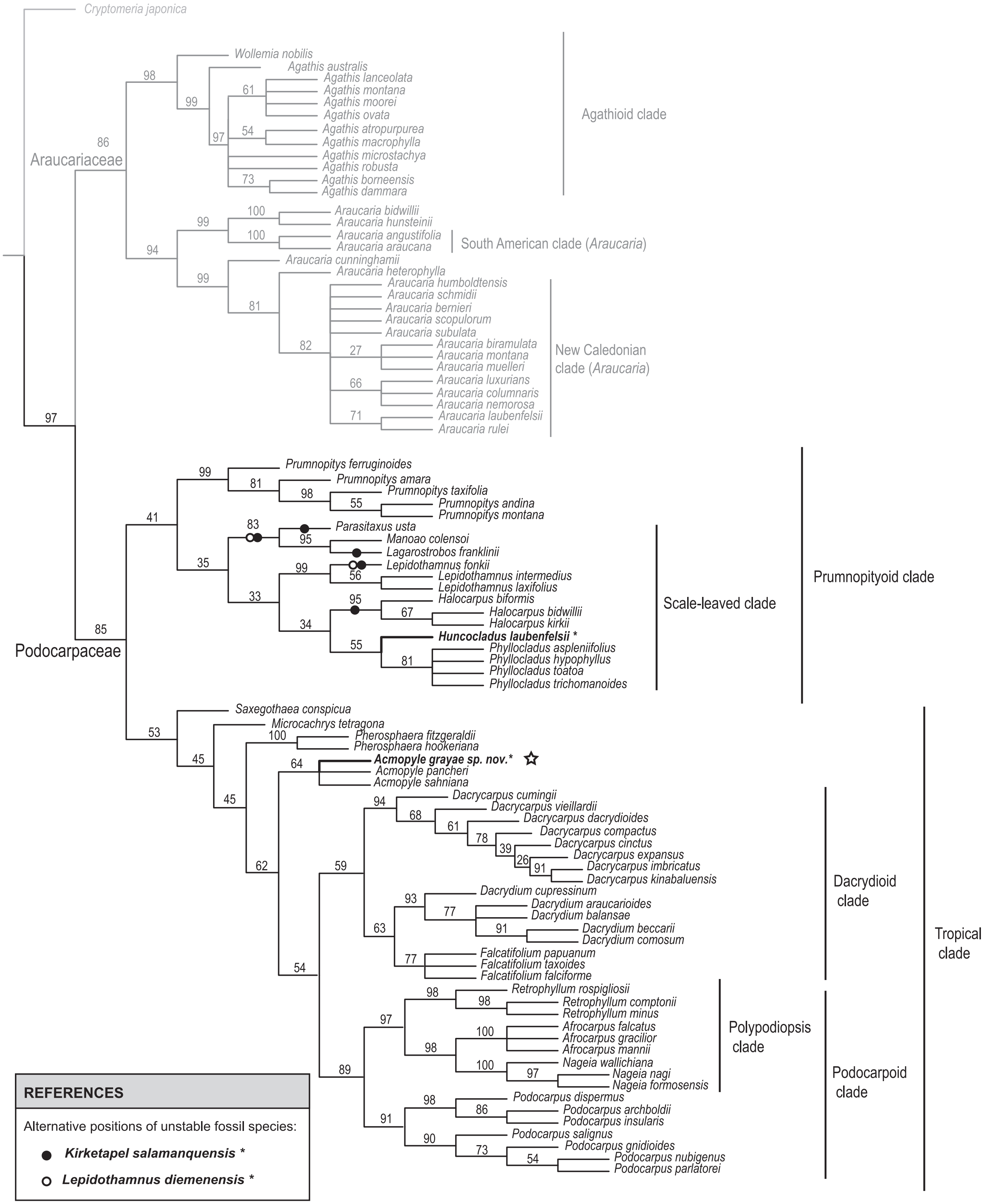

FIGURE 8 Strict consensus of all the most parsimonious trees obtained in the phylogenetic analysis. Family names are indicated next to the diversification nodes, and clade names are indicated to the right of the consensus tree. Fossil species are indicated by bold type and asterisk, and the new species is indicated with a star; alternative positions are indicated for two unstable fossil taxa. Symmetric resampling values (absolute frequencies) are indicated over the branches.

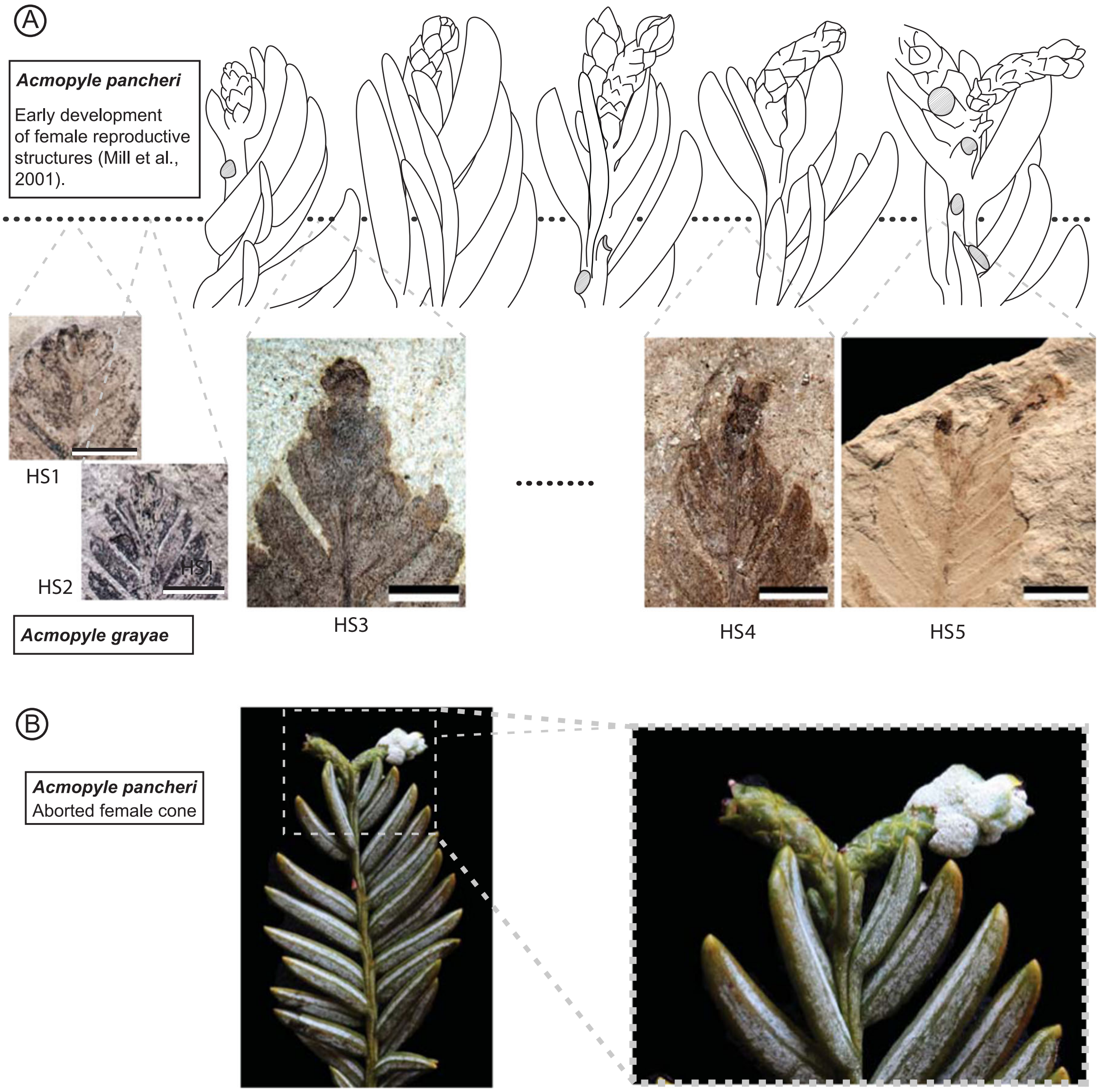

FIGURE 9 Morphological comparison of the apical structures of *Acmopyle grayae* sp. nov. and early developmental stages of the ovuliferous cones of *A. pancheri* (A) and aborted cones of *A. pancheri* (B). (**A**) Line drawings represent the sequence of ovuliferous cone development of *A. pancheri*, based on the ontogenetic stages illustrated by Mill et al. (2001); pictures of *A. grayae* are positioned below; gray broken lines indicate the tentative position of each of the fossil stages in the ontogenetic path above; HS1–HS5 stand for hypothetical stages 1–5, and represent the putative reproductive stages identified in the fossil material. The two morphologies on the left have not been reported for extant development of ovuliferous cones, which might correspond either to early reproductive stages or to vegetative growth. (**B**) Aborted ovuliferous cones in *A. pancheri* from a tree cultivated at the University of Tasmania.

Elatocladus Halle, within the new species *E. seymourensis* (Florin) D.J. Cantrill, A.M.P. Tosolini et J.E. Francis. It is of interest that this taxon has been alternatively interpreted as having bifacially (Cantrill et al., 2011) or bilaterally flattened leaves (Florin, 1940). According to our interpretation of figured specimens (Florin, 1940: text and fig. 2A–C; Cantrill et al., 2011: fig. 4E, F), this species has bilaterally flattened leaves; and, because the form genus *Elatocladus* is based on bifacially flattened leaves (Halle, 1913), we find that the Antarctic species cannot be placed in it. Because of these observations and the general morphology and robustness of the leafy shoot shown by these specimens, we refer to them using Florin's original name, *Acmopyle antarctica*. *Acmopyle antarctica* and *A. grayae* leaves are of similar size. However, although their insertion angles overlap, the angles of *A. antarctica* are not as obtuse as those of *A. grayae* (Florin, 1940; Cantrill et al., 2011; Table 1).

The early Eocene aff. *Acmopyle setiger*/*A. florinii* from Victoria, Australia, is mainly described on the basis of dispersed cuticles, but there is one shoot fragment showing 5 mm long leaves that are markedly smaller than those of *A. grayae* (Carpenter et al., 2004; Table 1). *Acmopyle setiger* (Townrow) R.S. Hill & R.J. Carp. ex R.R. Mill & R.S. Hill, *Acmopyle compactus* Pole, and *Acmopyle tasmanica* R. Hill & Carpenter from the Eocene of Tasmania are mainly distinguished on the basis of cuticular features (Pole, 1992),

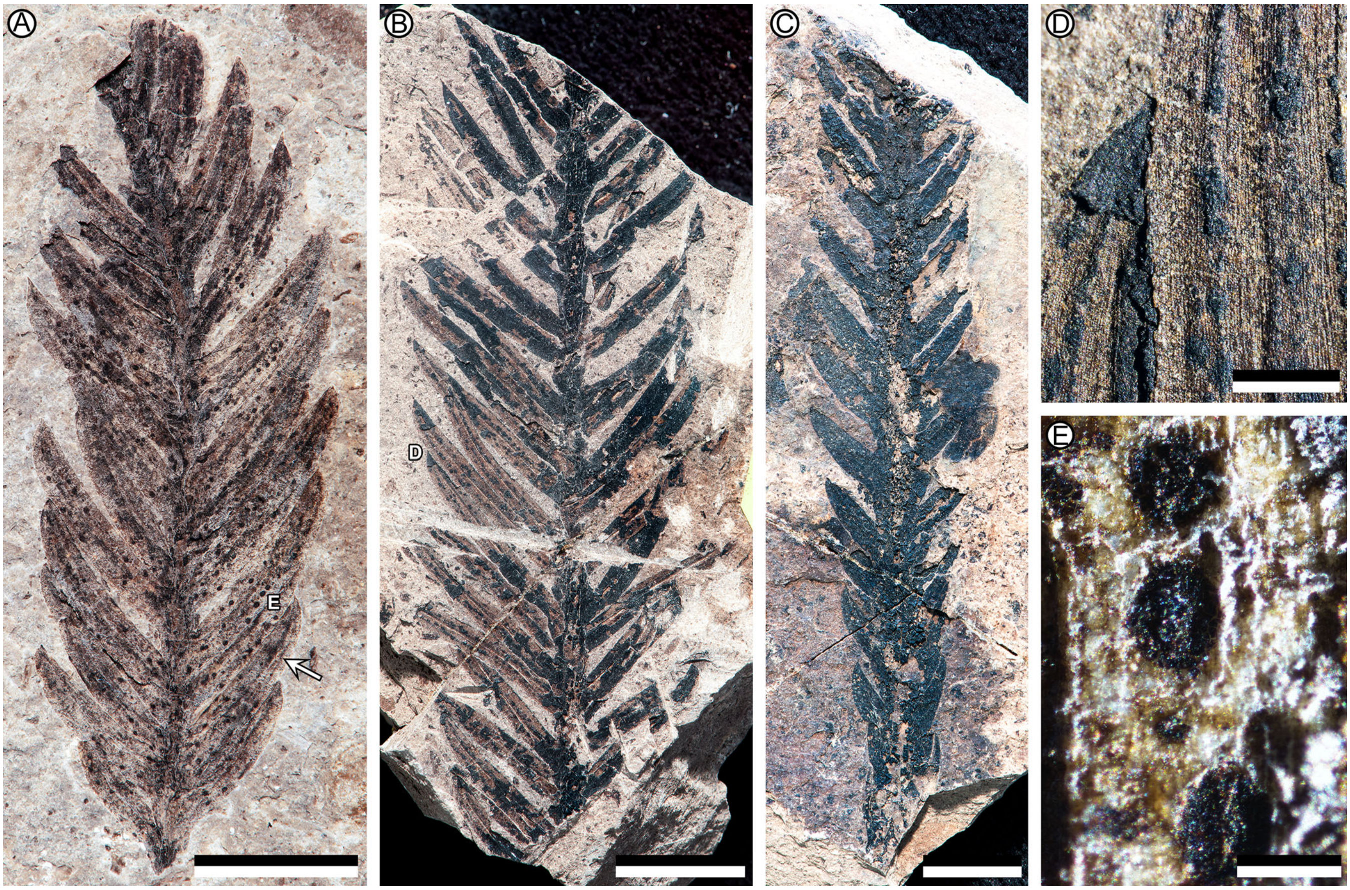

FIGURE 10 *Dacrycarpus engelhardti* (Berry) Wilf et A. Andruchow-Colombo comb. nov., type material of bilaterally flattened foliage originally illustrated as *Podocarpus engelhardti* Berry (1938: figs. 1–3) and *Acmopyle engelhardti* by Florin (1940: text figure 3). Foliage in A and B with extensive, coalified, circular piercing-and-sucking lesions from fluid-feeding insects. (**A**) USNM 40385a, lectotype selected by Florin (1940); arrow indicates linear stomatal band offset from the midvein, typical of *Dacrycarpus* species. (**B**) USNM 40385b, with extensively degraded cuticle (light brown) overlying coalified mesophyll. (**C**) USNM 40385c, referred here to *Araucaria pichileufensis* Berry (see also Rossetto-Harris et al., 2020). (**D**) Detail of cuticle remnants overlying coalified mesophyll in two adjacent leaves, from the location marked in panel B. (**E**) Detail of piercing-and-sucking lesions, from the location marked in panel A. Scale bars: A, C = 7 mm; B = 10 mm; D = 1 mm; E = 250 μm.

and therefore comparisons to the new Patagonian species are limited. Among those with leaf macromorphology preserved, *A. setiger*, *A. glabra*, and *A. tasmanica* have leaves shorter than or on the low end of the range of those of *A. grayae* (Table 1; Hill and Carpenter, 1991).

Acmopyle masonii Pole from the early Miocene (Pole, 1997) and *A. kirrileeae* from the late Miocene to early Pliocene (Pole, 2007) of New Zealand are based only on scale-like leaf fragments and cuticular remains. *Acmopyle biformis* Pole of the late Pliocene to early Quaternary from New Zealand is based on fragmentary leafy shoots with leaves distinctly smaller than those of *A. grayae* (Table 1), a nearly ovate shape, and an obtuse apex (Pole, 2007).

Even though these comparisons are not exhaustive, they suggest that the Patagonian fossils cannot be included in any previously described fossil species. This, combined with the unique suite of characters observed in *A. grayae*, strongly supports the designation of the new species.

In summary, the fossil record for *Acmopyle* is recognized possibly since the Late Cretaceous of Chilean Patagonia (Menéndez, 1972; uncertain record as stated by its original author) and certainly since the late Paleocene of Australia (Hill and Carpenter, 1991). *Acmopyle* then became widespread during the Eocene through the Southern Hemisphere, with multiple records in Australia, possibly in Antarctica (Florin, 1940; Cantrill et al., 2011), and now also in Argentinean Patagonia. The genus was also reported in younger (Miocene to Quaternary) deposits of New Zealand (Figure 1, Table 1); however, these records are here considered less reliable due to their lack of preservation of the expanded, bilaterally flattened mature leaves characteristic of this genus. The new record of *Acmopyle* from Patagonia represents the first confirmed record for the genus in South America and the last known representative in the region, where this drought-sensitive taxon would not have survived past the EECO and its regional rainforest conditions.

Insect damage

Many studies on plant-insect interactions for Cenozoic Patagonian floras are based on angiosperms (e.g., Wilf et al., 2005b; Sarzetti et al., 2009; Donovan et al., 2017, 2018; Gandolfo and Zamaloa, 2021). Information on gymnosperm herbivory encompasses detailed records for *Agathis* (Araucariaceae; Wilf et al., 2014; Donovan et al., 2020, 2023) and observations for *Papuacedrus* (Cupressaceae; Wilf et al., 2009), *Retrophyllum* (Podocarpaceae; Wilf et al., 2017), and now *Acmopyle grayae*. For the fossil Podocarpaceae species *Retrophyllum oxyphyllum*, also from the Laguna del Hunco site, Wilf et al. (2017) reported hole feeding (DT01 and DT03), margin feeding (DT12, DT13, and DT14), and piercing and sucking (DT138). The Laguna del Hunco podocarps *A. grayae* and *R. oxyphyllum* share the common hole-feeding (DT01) and a margin-feeding (DT12) damage types, and they both show evidence of piercing and sucking damage, albeit with different morphological characteristics (Figure 7A, E–K; Wilf et al., 2017). To our knowledge, there is no modern analog known for the piercing and sucking damage observed in the fossils (P.W. and T.B., personal observation).

Phylogenetic analysis

Acmopyle grayae sp. nov. is the first phylogenetically corroborated fossil for the genus (Figure 8). The synapomorphies and other supporting characters for the grouping of the LH species with *Acmopyle* belong to leaf macromorphology (i.e., 0. Opposite to subopposite leaf arrangement; 1. Broad morphology of mature leaves; 3. The bilaterally flattened mature leaf type; and 59. The robust appearance of the bilaterally flattened leaves), which is highly characteristic of the genus (Sahni, 1920; de Laubenfels, 1969; Farjon, 2010). No internal structure was recovered for the *Acmopyle* clade, due to the lack of characters in the matrix that vary within the group. However, features not included in the phylogenetic matrix are more similar between the Patagonian fossil and the Fijian *A. sahniana*, such as the morphology of ATT elements (relatively straight in *A. sahniana* and *A. grayae* and more sinuous in *A. pancheri*; Figure 6A–E) and the possible multi-seasonal growth of the expanded photosynthetic unit in *A. grayae*, as suggested by the putative presence of terminal buds (Figure 5C–E). Multi-seasonal growth occurs normally in *A. sahniana* (de Laubenfels, 1969) and is rare in *A. pancheri* (T.B., personal observation).

The position of other fossils in the phylogenetic analysis presented here mostly concurs with previous phylogenetic work (Andruchow-Colombo et al., 2019a, b). The only exception is a change in the position of the extinct Patagonian species *Kirketapel salamanquensis* (Andruchow-Colombo et al., 2019b). *Kirketapel* was originally recovered as sister to the monotypic *Lagarostrobos* or *Parasitaxus* (Andruchow-Colombo et al., 2019b), but here it resolves in multiple alternative positions within the scale-leaved clade (Figure 8, black circles). However, Andruchow-Colombo et al. (2019b) discussed that *Kirketapel* might be more closely related to other sub-lineages within the scale-leaved clade or even belong to its stem group, a hypothesis corroborated by the present analysis.

Paleoecological and biogeographic interpretations

The presence of *Acmopyle* within the conifer assemblage at Laguna del Hunco supports the prevailing interpretation of a trans-Antarctic, ever-wet rainforest that reached Patagonia in the early Eocene, under a greenhouse climate during the terminal stage of Gondwana (Wilf et al., 2013; Kooyman et al., 2014). The genus occurs at Laguna del Hunco with a large number of genera that today are restricted to ever-wet rainforests of Australasia and Malesia (see above), many of which have fossil records in Australia, New Zealand, Antarctica, or elsewhere in Patagonia, corroborating their paleo-Antarctic history and vast former ranges (Hill, 1994; Kooyman et al., 2014).

The presence of a single vein (rather than multivenation) can hinder leaf expansion because water has to travel from the midvein to the margins (i.e., lateral transport) outside the vascular system. However, with the exception of *Nageia*, all extant broad-leaved genera of Podocarpaceae are single-veined (Farjon, 2010). Many of these genera achieve lateral fluid transport through specialized extra-venous water-conducting tissues, such as the accessory transfussion tissue (ATT, Sahni, 1920; Buchholz and Gray, 1948; Schoonraad and van der Schuff, 1974; Brodribb and Holbrook, 2005; Knopf et al., 2012; Arbicheva et al., 2021). Unlike the xylary vascular system, these extra-venous tissues are unsupported in the mesophyll and are susceptible to collapse and dysfunction under water stress (Figure 6F, G; Sahni, 1920; Brodribb and Hill, 1998; Brodribb and Holbrook, 2005; Brodribb, 2011). *Acmopyle* was experimentally shown to be one of the most physiologically sensitive genera to drought among conifers in general and Podocarpaceae in particular (Brodribb and Hill, 1998), which could be attributed to the presence of ATT (Figure 6). Their extreme drought intolerance is considered the cause of the extinction of *Acmopyle* from Australia and, presumably, of its very limited and threatened modern range (Brodribb and Hill, 1998, 2004). Thus, the presence of *Acmopyle* in the Laguna del Hunco flora, as well as its absence from later, more seasonal intervals in Patagonia (such as the middle Eocene of Río Pichileufú), reinforce the hypothesis of perhumid conditions during the early Eocene in Patagonia from direct physiological adaptations observed in the *Acmopyle* fossils.

Living *A. pancheri* are understory or small canopy trees in the perhumid rainforests of New Caledonia, growing under ca. 1800 mm of mean annual precipitation, at ca. 20.0°C mean annual temperature, and at elevations ranging from sea level to 1200 m (Farjon, 2010; Biffin et al., 2012). Other conifers that grow in the ultramafic Southern Massif of New Caledonia in association with *A. pancheri* include species of *Agathis* and *Araucaria* section *Eutacta* (Araucariaceae), relatives of the two most abundant conifer species present in the Laguna del Hunco fossil flora (Wilf et al., 2005a, 2014; Farjon, 2010; Jaffré et al., 2010; Pujana et al., 2020; Rossetto-Harris et al., 2020). The critically endangered *A. sahniana*, endemic to cloud rainforest ridges and summits of Mount Vakarogasiu, Viti Levu, Fiji, grows under 2820 mm mean annual precipitation, 22.3°C mean annual temperature, and at elevations between 600 m and 1050 m (Farjon, 2010; Biffin et al., 2012). Other conifer genera found on Viti Levu include *Podocarpus*, *Dacrycarpus*, *Retrophyllum*, and *Agathis* (Doyle, 1998), all present in the Laguna del Hunco paleoassemblage (Wilf, 2012; Wilf et al., 2014, 2017).

The new Patagonian species *Acmopyle grayae* is the only known reliable record of the genus reported in South America (Table 1). Climatic cooling, increased seasonality, and isolation of South America as consequences of the deepening and widening of the Drake Passage that began just following ~50 Ma caused the biogeographic isolation of South America and extirpation of the genus from Patagonia after the early Eocene (Lawver et al., 2011; Dunn et al., 2015; Barreda et al., 2020; Reguero and Goin, 2021; van de Lagemaat et al., 2021). Like many paleo-Antarctic lineages (Kooyman et al., 2014), *Acmopyle* survived in Australia at least until the middle to late Eocene (Hill and Carpenter, 1991; Table 1; Figure 1) and potentially in New Zealand from the Miocene to the early Quaternary (Pole, 1997, 2007; Table 1; Figure 1) by tracking its preferred high-rainfall habitats. *Acmopyle* eventually went extinct in Australia because of declining temperatures and seasonal rainfall. However, its early presence in Australia and potentially in New Zealand allowed *Acmopyle* to successfully disperse to refugia in the island rainforests of New Caledonia and Fiji (Figure 1; Hill and Brodribb, 1999; Lee et al., 2001; Brodribb and Hill, 2004).

CONCLUSIONS

We erect *Acmopyle grayae* sp. nov. on the basis of 41 specimens of leafy shoots with preservation of accessory transfusion tissue (ATT) and several apical structures collected from the early Eocene Laguna del Hunco locality (Chubut, Patagonia, Argentina). In addition, the species exhibits external feeding and fluid-feeding insect-herbivore damage. The referral of the Patagonian material to the genus *Acmopyle* is based on numerous vegetative features (i.e., leafy shoots with defined growth and three distinct leaf types, distichous mature leaf arrangement; leaf shape and morphology; insertion angle; base and apex morphology; stomatal distribution; presence and distribution of the accessory transfusion tissue in the leaves), and it is supported by a total evidence phylogenetic analysis that placed it in a polytomy together with the two extant species of *Acmopyle*. We interpret the diverse apical morphologies as preserving various stages of development of reproductive structures and a few potential dormant apices.

Upon examination of historical and new material, we establish that the previously described *Acmopyle engelhardti*, from the middle Eocene Río Pichileufú locality (Patagonia, Argentina), belongs within *Dacrycarpus*, and we erect the new combination *Dacrycarpus engelhardti* (see above and Appendix 1). Therefore, *A. grayae* from Laguna del Hunco represents the only reliable record of the genus in South America.

The record of *Acmopyle* in the early Eocene of Argentina provides physiologically grounded evidence of the ever-wet rainforest environments of Patagonia during the Early Eocene Climatic Optimum and final stages of Gondwana, due to the demonstrated presence of ATT in its leaves that rapidly collapse under drought in the living analog species. This is one of the reasons why *Acmopyle*, after tens of millions of years of survival over vast former ranges demonstrated from fossils, is now extremely vulnerable to climate change that could affect seasonal rainfall distribution in its highly restricted current ranges.

AUTHOR CONTRIBUTIONS

A.A.C. described the material and assembled plates, built the combined matrix and performed phylogenetic analyses, and led the discussion and writing of the manuscript. G.R.H. measured fossil specimens and participated in the discussion and writing of the manuscript. M.A.G. provided funding for the project, led and participated in the field trips where the fossils were collected, and participated in writing the manuscript. T.B. prepared and imaged paradermal sections (Figure 6D–G) and imaged aborted seed cones (Figure 9B) of extant *Acmopyle*. P.W. provided funding for the project, led and participated in the field trips where the fossils were collected, photographed the fossil and herbarium specimens, and participated in writing the manuscript. All authors gave comments, suggestions, and corrections or wrote sections of the manuscript drafts.

ACKNOWLEDGMENTS

We thank P. Puerta, M. Caffa, L. Canessa, R. Cúneo, I. Davies, E. J. Hermsen, A. Iglesias, N. Jud, M. Donovan, K. Johnson, S. Wing, and many others for field and laboratory assistance; E. Ruigomez, J. Wingerath, A. P. Carabajal, L. Reiner, I. H. Escapa, and A. Iglesias, for curation; the editor ase well as the reviewers, R. S. Hill and E. I. Vera, for their comments and suggestions that greatly improved the manuscript; E. I. Vera for providing images of the original material of ? *A. antarctica*; Secretaría de Cultura de Chubut and Secretaría de Cultura de Río Negro for assistance with loans; and the Nahueltripay family and Secretaría de Cultura de Chubut for land access. We thank the Australian National Herbarium and the Centre for Australian National Biodiversity Research for granting permission for our images of herbarium specimens to be used here. Financial support has been provided by the National Science Foundation, including grants DEB-1556666, DEB-0919071, DEB-0918932, DEB-1556136, EAR-1925552, and EAR-1925755, as well as by the National Scientific and Technical Research Council (CONICET, Argentina), the International Association for Plant Taxonomy (IAPT, Slovakia), the Systematics Association (SA, UK), the Linnean Society of

London (UK), and the Botanical Society of America (BSA, USA).

DATA AVAILABILITY STATEMENT

Morphological matrix analyzed available at Morphobank (Project P4224): http://morphobank.org/permalink/?P4224.

A high-resolution image archive of the fossils reported in this article is available open-access at Figshare: https://doi.org/10.6084/m9.figshare.19545040.

ORCID

Ana Andruchow-Colombo http://orcid.org/0000-0003-2942-5603
Gabriella Rossetto-Harris http://orcid.org/0000-0002-4968-6491
Timothy J. Brodribb http://orcid.org/0000-0002-4964-6107
María A. Gandolfo http://orcid.org/0000-0002-5430-1808
Peter Wilf http://orcid.org/0000-0001-6813-1937

REFERENCES

Andruchow-Colombo, A., P. Wilf, and I. H. Escapa. 2019a. A South American fossil relative of *Phyllocladus*: *Huncocladus laubenfelsii* gen. et sp. nov. (Podocarpaceae), from the early Eocene of Laguna del Hunco, Patagonia, Argentina. *Australian Systematic Botany* 32: 290–309.

Andruchow-Colombo, A., I. H. Escapa, R. J. Carpenter, R. S. Hill, A. Iglesias, A.M. Abarzua, and P. Wilf. 2019b. Oldest record of the scale-leaved clade of Podocarpaceae, early Paleocene of Patagonia, Argentina. *Alcheringa* 43: 127–145.

Andruchow-Colombo, A., P. Wilf, and I. H. Escapa. 2021. Reaffirming the phyllocladoid affinities of *Huncocladus laubenfelsii* (Podocarpaceae) from the early Eocene of Patagonia: a comment on Dörken et al. (2021). *Botanical Journal of the Linnean Society* 197: 554–557.

Aragón, E., and M. M. Mazzoni. 1997. Geología y estratigrafía del complejo volcánico piroclástico del Río Chubut medio (Eoceno), Chubut, Argentina. *Revista de la Asociación Geológica Argentina* 52: 243–256.

Aragón, E., and E. J. Romero. 1984. Geología, paleoambientes y paleobotánica de yacimientos Terciarios del occidente de Río Negro, Neuquén y Chubut. *Actas del IX Congreso Geológico Argentino, San Carlos de Bariloche* 4: 475–507.

Arbicheva, A., A. Pautov, and A. Saldaña. 2021. Water storage and transport in leaves of vesselless trees in the temperate rainforest of south-central Chile. *Grayana Botanica* 78: 141–155.

Banerji, J., and A. K. Ghosh. 2006. *Podospermum* gen. *et* sp. nov., an *Acmopyle*-like dispersed silicified ovule/seed from Lower Cretaceous Intertrappean Beds of the Rajmahal Basin, India. *Cretaceous Research* 27: 707–711.

Banerji, J., and A. K. Ghosh. 2008. *Podocarpospermum*, a replacement name for the *Acmopyle*-like ovule/seed from *Podospermum* Banerji and Ghosh from Lower Cretaceous intertrappean beds of the Rajmahal Basin, India. *Cretaceous Research* 29: 561–562.

Barreda, V. D., L. Palazzesi, R. R. Pujana, C. Panti, M. J. Tapia, D. A. Fernández, and S. Noetinger. 2020. The Gondwanan heritage of the Eocene–Miocene Patagonian floras. *Journal of South American Earth Sciences* 107: 103022.

Berry, E. W. 1925. A Miocene flora from Patagonia. *Johns Hopkins University Studies in Geology* 6: 183–251.

Berry, E. W. 1938. Tertiary flora from the Río Pichileufú, Argentina. *Geological Society of America Special Papers* 12: 1–149.

Biffin, E., J. G. Conran, and A. J. Lowe. 2011. Podocarp evolution: a molecular phylogenetic perspective. *In* B. L. Turner and L. A. Cernusak [eds.], Ecology of the Podocarpaceae in Tropical Forests, 1–20. Smithsonian Institution Scholarly Press, Washington, D.C., USA.

Biffin, E., T. J. Brodribb, R. S. Hill, P. Thomas, and A. J. Lowe. 2012. Leaf evolution in Southern Hemisphere conifers tracks the angiosperm ecological radiation. *Proceedings of the Royal Society B* 279: 341–348.

Blackman, C. J., T. J. Brodribb, and G. J. Jordan. 2010. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist* 188: 1113–1123.

Brodribb, T. J. 2011. A functional analysis of podocarp ecology. *In* B. L. Turner and L. A. Cernusak [eds.], Ecology of the Podocarpaceae in Tropical Forests, 165–175. Smithsonian Institution Scholarly Press, Washington, D.C., USA.

Brodribb, T. J., and R. S. Hill. 1997. Light response characteristics of a morphologically diverse group of southern hemisphere conifers as measured by chlorophyll fluorescence. *Oecologia* 110: 10–17.

Brodribb, T., and R. S. Hill. 1998. The photosynthetic drought physiology of a diverse group of southern hemisphere conifer species is correlated with minimum seasonal rainfall. *Functional Ecology* 12: 465–4710.

Brodribb, T., and R. S. Hill. 2004. The rise and fall of the Podocarpaceae in Australia ‐ a physiological explanation. *In* A. R. Hemsley and I. Poole [eds.], The Evolution of Plant Physiology: from Whole Plants to Ecosystems, 381–399. Academic Press, London, UK.

Brodribb, T. J., and N. M. Holbrook. 2005. Water stress deforms tracheids peripheral to the leaf vein of a tropical conifer. *Plant Physiology* 137: 1139–1146.

Buchholz, J. T., and N. E. Gray. 1947. A Fijian *Acmopyle*. *Journal of the Arnold Arboretum* 28: 141–143.

Buchholz, J. T., and N. E. Gray. 1948. A Taxonomic Revision of Podocarpus I. the Sections of the Genus and Their Subdivisons With Special Reference to Leaf Anatomy. *Journal of the Arnold Arboretum* 29: 49–63.

Bush, E. W., and M. F. Doyle. 1997. Taxonomic description of *Acmopyle sahaniana* (Podocarpaceae): additions, revisions, discussion. *Harvard Papers in Botany* 2: 229–233.

Cantrill, D. J., A. M. P. Tosolini, and J. E. Francis. 2011. Paleocene flora from Seymour Island, Antarctica: revision of Dusén's (1908) pteridophyte and conifer taxa. *Alcheringa* 35: 309–328.

Carpenter, R. J., A. Iglesias, and P. Wilf. 2018. Early Cenozoic Vegetation in Patagonia: New Insights from Organically Preserved Plant Fossils (Ligorio Márquez Formation, Argentina). *International Journal of Plant Sciences* 179: 115–135.

Carpenter, R. J., and M. Pole. 1995. Eocene plant fossils from the Lefroy and Cowan paleodrainages, Western Australia. *Australian Systematic Botany* 8: 1107–1154.

Carpenter, R. J., R. S. Hill, D. R. Greenwood, A. D. Partridge, and M. A. Banks. 2004. No snow in the mountains: early Eocene plant fossils from Hotham Heights, Victoria, Australia. *Australian Journal of Botany* 52: 685–718.

Carvalho, M. R., P. Wilf, E. J. Hermsen, M. A. Gandolfo, N. R. Cúneo, and K. R. Johnson. 2013. First record of Todea (Osmundaceae) in South America, from the early Eocene paleorainforests of Laguna del Hunco (Patagonia, Argentina). *American Journal of Botany* 100: 1831–1848.

Conran, J. G., G. M. Wood, P. G. Martin, J. M. Dowd, C. J. Quinn, P. A. Gadek, and R. A. Price. 2000. Generic relationships within and between the gymnosperm families and Phyllocladaceae based on an analysis of the chloroplast gene *rbcL*. *Australian Journal of Botany* 48: 715–724.

de Laubenfels, D. 1969. A revision of the Malesian and Pacific rainforest conifers, I. Podocarpaceae, in part. *Journal of the Arnold Arboretum* 50: 274–369

de Laubenfels, D. J. 1972. Flore de la Nouvelle-Caledonie et Dependances. Vol. 4. Gymnospermes. Muséum National d'Histoire Naturelle, Paris, France.

Donovan, M. P., A. Iglesias, P. Wilf, C. C. Labandeira, and N. R. Cúneo. 2017. Rapid recovery of Patagonian plant-insect associations after the end-Cretaceous extinction. *Nature Ecology & Evolution* 1: 0012.

Donovan, M. P., A. Iglesias A., P. Wilf, C. C. Labandeira, and N. R. Cúneo. 2018. Diverse plant-insect associations from the latest Cretaceous and early Paleocene of Patagonia, Argentina. *Ameghiniana* 55: 303–338.

Donovan, M. P., P. Wilf, A. Iglesias, N. R. Cúneo, and C. C. Labandeira. 2020. Persistent biotic interactions of a Gondwanan conifer from Cretaceous Patagonia to modern Malesia. *Communications Biology* 3: 708.

Donovan, M. P., P. Wilf, A. Iglesias, N. R. Cúneo, and C. C. Labandeira. 2023. Insect herbivore and fungal communities on Agathis (Araucariaceae) from the latest Cretaceous to Recent. *PhytoKeys* 226: 109–158.

Doyle, M. F. 1998. Gymnosperms of the SW Pacific –1. Fiji. Endemic and indigenous species: changes in nomenclature, key, annotated checklist, and discussion. *Harvard Papers in Botany* 3: 101–106.

Dunn, R. E., C. A. E. Strömberg, R. H. Madden, M. J. Kohn, and A. A. Carlini. 2015. Linked canopy, climate, and faunal change in the Cenozoic of Patagonia. *Science* 347: 258–261.

Farjon, A. 2010. A Handbook of the World's Conifers. Brill, Leiden, Netherlands.

Florin, R. 1940. Die heutige und frühere verbreitung der koniferengattung *Acmopyle* Pilger. *Svensk Botanisk Tidskrift* 34: 117–140.

Gandolfo, M. A., and M. C. Zamaloa. 2021. Macroevolutionary changes in the fossil plant record: Key examples from the Cretaceous-Paleogene of Patagonia, Argentina. *Journal of South American Earth Sciences* 110: 103250.

Gandolfo, M. A., and M. C. Hermsen. 2017. *Ceratopetalum* (Cunoniaceae) fruits of Australasian affinity from the early Eocene Laguna del Hunco flora, Patagonia, Argentina. *Annals of Botany* 119: 507–516.

Gandolfo, M. A., E. J. Hermsen, M. C. Zamaloa, K. C. Nixon, C. C. González, P. Wilf, N. R. Cúneo, et al. 2011. Oldest Known *Eucalyptus* Macrofossils Are from South America. *PLoS One* 6: e21084.

Goloboff, P. A. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428.

Goloboff, P. A., and S. A. Catalano. 2012. GB-to-TNT: facilitating creation of matrices from GenBank and diagnosis of results in TNT. *Cladistics* 28: 503–513.

Goloboff, P. A., and S. A. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* 32: 221–238.

Goloboff, P. A., J. S. Farris, M. Källersjö, B. Oxelman, M. J. Ramírez, and C. A. Szumik. 2003. Improvements to resampling measures of group support. *Cladistics* 19: 324–332.

Goloboff, P. A., J. S. Farris, and K. C. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.

Gosses, J., A. R. Carroll, B. T. Bruck, B. S. Singer, B. R. Jicha, E. Aragón, A. P. Walters, et al. 2021. Facies interpretation and geochronology of diverse Eocene floras and faunas, northwest Chubut Province, Patagonia, Argentina. *GSA Bulletin* 133: 740–752.

Halle, T. G., 1913. The Mesozoic flora of Graham Land. In O. Nordenskjöld [ed.] Wissenschaftliche Ergebnisse der Schwedischen Südpolar—Expedition 1901–1903, Geologie und Paläontologie, 1–123. Norstedt and Söner, Stockholm, Sweden.

Hermsen, E. J., M. A. Gandolfo, and M. C. Zamaloa. 2012. The fossil record of *Eucalyptus* in Patagonia. *American Journal of Botany* 99: 1356–1374.

Hill, R. S. 1994. The history of selected Australian taxa. In R. S. Hill (ed.). History of the Australian Vegetation: Cretaceous to Recent, 390–419. Cambridge University Press, Cambridge, UK.

Hill, R. S. 1995. Conifer origin, evolution and diversification in the Southern Hemisphere. *In* Enright N. J. and R. S. Hill [eds.], Ecology of the Southern Conifers, 10–29. Smithsonian Institution Press, Washington, D.C., USA.

Hill, R. S. and T. J. Brodribb. 1999. Southern Conifers in Time and Space. *Australian Journal of Botany* 47: 639–696.

Hill, R. S., and T. J. Brodribb. 2003. Evolution of conifer foliage in the Southern Hemisphere. *Acta Horticulturae* 615: 53–58.

Hill, R. S., and R. J. Carpenter. 1991. Evolution of *Acmopyle* and *Dacrycarpus* (Podocarpaceae) foliage as inferred from macrofossils in south-eastern Australia. *Australian Systematic Botany* 4: 449–479.

Iannelli, S. B., V. D. Litvak, L. Fernández Paz, A. Folguera, M. E. Ramos, and V. A. Ramos. 2017. Evolution of Eocene to Oligocene arc-related volcanism in the North Patagonian Andes (39–41°S), prior to the break-up of the Farallon plate. *Tectonophysics* 696–697: 70–87.

Jaffré, T., J. Munzinger, and P. P. Lowry II. 2010. Threats to the conifer species found on New Caledonia's ultramafic massifs and proposals for urgently needed measures to improve their protection. *Biodiversity and Conservation* 19: 1485–1502.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Katoh, K., and H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.

Katoh, K., K. Misawa, K. I. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Kelch, D. G. 2002. Phylogenetic assessment of the monotypic genera *Sundacarpus* and *Manoao* (Coniferales: Podocarpaceae) utilising evidence from 18S rDNA sequences. *Australian Systematic Botany* 15: 29–35.

Knopf, P., C. Schulz, D. P. Little, T. Stützel, and D. W. Stevenson. 2012. Relationships within Podocarpaceae based on DNA sequence, anatomical, morphological, and biogeographical data. *Cladistics* 28: 271–299.

Kooyman, R. M., P. Wilf, V. D. Barreda, R. J. Carpenter, G. J. Jordan, J. K. Sniderman, A. Allen, et al. 2014. Paleo-Antarctic rainforest into the modern Old World tropics: The rich past and threatened future of the "southern wet forest survivors." *American Journal of Botany* 101: 2121–2135.

Labandeira, C. C., P. Wilf, K. R. Johnson, and F. Marsh. 2007. Guide to insect (and other) damage types on compressed plant fossils. Version 3.0. Smithsonian Institution, Washington, D.C., USA.

Lawver, L. A., L. M. Gahagan, and I. W. D. Dalziel. 2011. A different look at gateways: Drake Passage and Australia/Antarctica. *In* J. B. Anderson and J. S. Wellner [eds.], Tectonic, climatic, and cryospheric evolution of the Antarctic Peninsula, 5–33. John Wiley and Sons, Washington, DC, USA.

Lee, D. E., W. G. Lee, and N. Mortimer. 2001. Where and why have all the flowers gone? Depletion and turnover in the New Zealand Cenozoic angiosperm flora in relation to palaeogeography and climate. *Australian Journal of Botany* 49: 341–356.

Leslie, A. B., J. M. Beaulieu, H. S. Rai, P. R. Crane, M. J. Donoghue, and S. Mathews. 2012. Hemisphere-scale differences in conifer evolutionary dynamics. *Proceedings of the National Academy of Sciences* 109: 16217–16221.

Leslie, A. B., J. Beaulieu, G. Holman, C. S. Campbell, W. Mei, L. R. Raubeson, and S. Mathews. 2018. An overview of extant conifer evolution from the perspective of the fossil record. *American Journal of Botany* 105: 1531–1544.

Lu, Y., J. H. Ran, D. M. Guo, Z. Y. Yang, and X. Q. Wang. 2014. Phylogeny and divergence times of gymnosperms inferred from single-copy nuclear genes. *PLoS One* 9: e107679.

Machado, M. A., M. G. Passalía, E. I. Vera, and A. Yañez. 2023. Ferns from the Arroyo Chacay flora (Huitrera Formation, Eocene) Río Negro Province, Argentina. *Review of Palaeobotany and Palynology* 313: 104892.

Menéndez, C. A. 1972. Paleophytologia Kurtziana III. 9. La flora del Cretácico Superior de Cerro Guido, Chile (3–7). *Ameghiniana* 9: 289–297.

Merkhofer, L., P. Wilf, M. T. Haas, R. M. Kooyman, L. Sack, C. Scoffoni, and N. R. Cúneo. 2015. Resolving Australian analogs for an Eocene Patagonian paleorainforest using leaf size and floristics. *American Journal of Botany* 102: 1160–1173.

Mill, R. R., and R. S. Hill. 2004. Validations of the names of seven Podocarpaceae macrofossils. *Taxon* 53: 1043–1046.

Mill, R. R., M. Möller, F. Christie, S. M. Glidewell, D. Masson, and B. Williamson. 2001. Morphology, anatomy and ontogeny of female cones in *Acmopyle pancheri* (Brongn. and Gris) Pilg. (Podocarpaceae). *Annals of Botany* 88: 55–67.

Page, C. N. 2019. New and maintained genera in the taxonomic alliance of *Prumnopitys* s.l. (Podocarpaceae), and circumscription of a new genus: *Pectinopitys*. *New Zealand Journal of Botany* 57: 137–153.

Petersen, C. S. 1946. Estudios geológicos en la región del Río Chubut medio. *Dirección de Minas y Geología Boletín* 59: 1–137.

Pole, M. 1992. Eocene vegetation from Hasties, north-eastern Tasmania. *Australian Systematic Botany* 5: 431–475.

Pole, M. 1997. Miocene conifers from the Manuherikia group, New Zealand. *Journal of the Royal Society of New Zealand* 27: 355–370.

Pole, M. 2007. Plant-macrofossil assemblages during Pliocene uplift, South Island, New Zealand. *Australian Journal of Botany* 55: 118–142.

Pujana, R. R., P. Wilf, and M. A. Gandolfo. 2020. Conifer wood assemblage dominated by Podocarpaceae, early Eocene of Laguna del Hunco, central Argentinean Patagonia. *PhytoKeys* 156: 81–102.

Reguero, M. A., and F. J. Goin. 2021. Paleogeography and biogeography of the Gondwanan final breakup and its terrestrial vertebrates: New insights from southern South America and the "double Noah's Ark" Antarctic Peninsula. *Journal of South American Earth Sciences* 108: 103358.

Romero, E. J., M. Dibbern, and M. A. Gandolfo 1988. Revisión de *Lomatia bivascularis* (Berry) Frenguelli (Proteaceae) del yacimiento de la Laguna del Hunco (Paleoceno), Pcia. del Chubut. *Actas del IV Congreso Argentino de Paleontología y Bioestratigrafía, Mendoza* 3: 125–130.

Rossetto-Harris, G., P. Wilf, I. H. Escapa, and A. Andruchow-Colombo. 2020. Eocene *Araucaria* sect. *Eutacta* from Patagonia and floristic turnover during the initial isolation of South America. *American Journal of Botany* 107: 806–832.

Sahni, B. 1920. VII.—On the structure and affinities of *Acmopyle Pancheri*, Pilger. *Philosophical Transactions of the Royal Society of London, Series B* 210: 253–310.

Sarzetti, L. C., C. C. Labandeira, J. Muzón, P. Wilf, N. R. Cúneo, K. R. Johnson, and J. F. Genise 2009. Odonatan endophytic oviposition from the Eocene of Patagonia: The ichnogenus *Paleoovoidus* and implications for behavioral stasis. *Journal of Paleontology* 83: 431–447.

Schoonraad, E., and H. P. van der Schuff 1974. Anatomy of leaves of the genus *Podocarpus* in South Africa. *Phytomorphology* 24: 75–85.

Sinclair, W. T., R. R. Mill, M. F. Gardner, P. Woltz, T. Jaffré, J. Preston, M. L. Hollingsworth, et al. 2002. Evolutionary relationships of the New Caledonian heterotrophic conifer, *Parasitaxus usta* (Podocarpaceae), inferred from chloroplast trnL-F intron/spacer and nuclear rDNA ITS2 sequences. *Plant Systematics and Evolution* 233: 79–104.

Stockey, R. A., and H. Ko. 1988. Cuticle micromorphology of some New Caledonian podocarps. *Botanical Gazette* 149: 240–252.

Thomas, P. 2010. *Acmopyle pancheri. The IUCN Red List of Threatened Species* 2010: e.T31005A9598852. Website: https://doi.org/10.2305/IUCN.UK.2010-2.RLTS.T31005A9598852.en [accessed 25 March 2022].

Thomas, P. 2013. *Acmopyle sahniana. The IUCN Red List of Threatened Species* 2013: e.T31052A2801815 Website: https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T31052A2801815.en [accessed 25 March 2022].

Townrow, J. A. 1965. Notes on some Tasmanian pines I. Some Lower Tertiary podocarps. *Papers and Proceedings of the Royal Society of Tasmania* 99: 87–107.

van de Lagemaat, S. H. A., M. L. A. Swart, B. Vaes, M. E. Kosters, L. M. Boschman, A. Burton-Johnson, P. K. Bijl, et al. 2021. Subduction initiation in the Scotia Sea region and opening of the Drake Passage: When and why? *Earth-Science Reviews* 215: 103551.

Villar de Seoane, L., N. R. Cúneo, I. H. Escapa, P. Wilf, and M. A. Gandolfo. 2015. *Ginkgoites patagonica* (Berry) comb. nov. from the Eocene of Patagonia, last ginkgoalean record in South America. *International Journal of Plant Sciences* 176: 346–363.

Wells, P. M., and R. S. Hill. 1989. Leaf Morphology of the Imbricate-leaved Podocarpaceae. *Australian Systematic Botany* 2: 369–386.

Westerhold, T., N. Marwan, A. J. Drury, D. Liebrand, C. Agnini, E. Anagnostou, J. S. Barnet, et al. 2020. An astronomically dated record of Earth's climate and its predictability over the last 66 million years. *Science* 369: 1383–1387.

Wilf, P. 2012. Rainforest conifers of Eocene Patagonia: attached cones and foliage of the extant Southeast Asian and Australasian genus *Dacrycarpus* (Podocarpaceae). *American Journal of Botany* 99: 562–584.

Wilf, P. 2020. Eocene "*Chusquea*" fossil from Patagonia is a conifer, not a bamboo. *PhytoKeys* 139: 77–89.

Wilf, P., N. R. Cúneo, K. R. Johnson, J. F. Hicks, S.L. Wing, and J. D. Obradovich. 2003. High plant diversity in Eocene South America: Evidence from Patagonia. *Science* 300: 122–125.

Wilf, P., K. R. Johnson, N. R. Cúneo, M. E. Smith, B. S. Singer, and M. A. Gandolfo. 2005a. Eocene plant diversity at Laguna del Hunco and Río Pichileufú, Patagonia, Argentina. *The American Naturalist* 165: 634–650.

Wilf, P., C. C. Labandeira, K. R. Johnson., and N. R. Cúneo. 2005b. Richness of plant–insect associations in Eocene Patagonia: A legacy for South American biodiversity. *Proceedings of the National Academy of Sciences* 102: 8944–8948.

Wilf, P., S. A. Little, A. Iglesias, M. C. Zamaloa, M. A. Gandolfo, N. R. Cúneo, and K. R. Johnson. 2009. *Papuacedrus* (Cupressaceae) in Eocene Patagonia: a new fossil link to Australasian rainforests. *American Journal of Botany* 96: 2031–2047.

Wilf, P., N. R. Cúneo, I. H. Escapa, D. Pol, and M. O. Woodburne. 2013. Splendid and seldom isolated: the paleobiogeography of Patagonia. *Annual Review of Earth and Planetary Sciences* 41: 561–603.

Wilf, P., I. H. Escapa, N. R. Cúneo, R. M. Kooyman, K. R. Johnson, and A. Iglesias. 2014. First South American *Agathis* (Araucariaceae), Eocene of Patagonia. *American Journal of Botany* 101: 156–179.

Wilf, P., D. W. Stevenson, and N. R. Cúneo. 2016. The last Patagonian cycad, *Austrozamia stockeyi* gen. et sp. nov., early Eocene of Laguna del Hunco, Chubut, Argentina. *Botany* 94: 817–829.

Wilf, P., M. P. Donovan, N. R. Cúneo, and M. A. Gandolfo. 2017. The fossil flip-leaves (*Retrophyllum*, Podocarpaceae) of southern South America. *American Journal of Botany* 104: 1344–1369.

Wilf, P., K. C. Nixon, M. A. Gandolfo, and N. R. Cúneo. 2019. Eocene Fagaceae from Patagonia and Gondwanan legacy in Asian rainforests. *Science* 364: eaaw5139.

Zamaloa, M. C., M. A. Gandolfo, C. C. González, E. J. Romero, N. R. Cúneo, and P. Wilf. 2006. Casuarinaceae from the Eocene of Patagonia, Argentina. *International Journal of Plant Sciences* 167: 1279–1289.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Table S1 with taxa included in the phylogenetic analysis and corresponding *GenBank* accession numbers.

Appendix S2. Morphological character list.

Appendix S3. Figures S1–S3 with common mappings (i.e., character mappings common to all most parsimonious trees, and then illustrated in the strict consensus).

How to cite this article: Andruchow-Colombo, A., G. Rossetto-Harris, T. J. Brodribb, M. A. Gandolfo, and P. Wilf. 2023. A new fossil *Acmopyle* with accessory transfusion tissue and potential reproductive buds: Direct evidence for ever-wet rainforests in Eocene Patagonia. *American Journal of Botany* e16221. https://doi.org/10.1002/ajb2.16221

APPENDIX 1. Nomenclatural acts.

Genus*—*Dacrycarpus (Endl.) de Laubenfels, *Journal of the Arnold Arboretum* 50: 315 (1969).

Species*—*Dacrycarpus engelhardti (Berry) Wilf et A. Andruchow-Colombo comb. nov. (Figure 10A, B, D, E).

Dacrycarpus puertae Wilf, *American Journal of Botany* 69: 572 (2012) *pro parte*, cited Río Pichileufú material only.

Acmopyle engelhardti (Berry) Florin, *Svensk Botanisk Tidskrift* 34: 127 (1940, *pro parte*: excluding text figure 3c = USNM 40385c).

"*Fitzroya tertiaria*" Berry, *sensu Geological Society of America Special Paper* 12: 60 (1938), cited Río Pichileufú material only.

Basionym*—*Podocarpus engelhardti Berry, *Geological Society of America Special Paper* 12: 63 (1938, *pro parte*: excluding pl. 13 fig. 2 = USNM 40385c), cited Río Pichileufú material only.

Lectotype—USNM 40385a (Figure 10A, E), as specified by Florin (1940: 127). National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Syntype—USNM 40385b (Figure 10B, D).

Additional material—Berry (1938) cohort collection, eight specimens: USNM 40385d–g (four distinct specimens), USNM 40381 part and counterpart (Berry, 1938: "*Fitzroya tertiaria*"; see Wilf, 2012), USNM 543561 (Wilf, 2012: fig. 16), USNM 771221. Collections at MAP-BAR, 19 specimens: MAP-BAR 610-20, 4204, 4746, 4747, 5001-20, 5604, 5606, 5621, 8528, 8529, 8531, 8533, 8537–8540, 8542–8544.

Notes—Berry (1938: plate 13, figs. 1–3) described and illustrated the three specimens from Río Pichileufú shown in Figure 10 as *Podocarpus engelhardti*, while incorrectly including Eocene material from Chile in that species. Florin (1940) later revised the Río Pichileufú material to *Acmopyle engelhardti* and selected the lectotype (USNM 40385a; Figure 10A), leaving the remaining two specimens as syntypes.

Wilf (2012) recognized *Dacrycarpus* bifacial and bilateral foliage from both Laguna del Hunco and Río Pichileufú, including foliage with attached seed and pollen cones at Laguna del Hunco, and found that Berry (1938) had erroneously described the bifacial *Dacrycarpus* foliage from Río Pichileufú as "*Fitzroya tertiaria*," the name of a Cenomanian conifer from the Mata Amarilla Formation. Wilf (2012) consolidated all the *Dacrycarpus* material that he recognized at the time from Laguna del Hunco and Río Pichileufú under *Dacrycarpus puertae*, whose holotype is a branch with several bilateral shoots and attached seed cones from Laguna del Hunco (Wilf, 2012). Wilf (2012) also re-separated the Chilean material as *Dacrycarpus chilensis* (Engelhardt) Wilf, which was also recently reported from the Paleogene Ligorio Márquez Formation in Santa Cruz, Argentina (Carpenter et al., 2018).

For the present contribution, we reexamined the type (Figure 10) and cohort material of *Acmopyle engelhardti* from Río Pichileufú held at USNM and all recently collected material similar to this species from the site, a total of 28 specimens as listed above. We found that one of Berry's (1938: pl. 13 fig. 2; here, Figure 10C; USNM 40385c) figured specimens belongs to *Araucaria pichileufensis* and is here assigned to that species (see Rossetto-Harris et al., 2020). All the other specimens have diagnostic features of *Dacrycarpus*, including well-developed bifacial and bilateral leaves, which occur in a single shoot (USNM 771221). The bifacial leaves occur in extensive sections of the shoot, and not only in small numbers at the shoot base as in *Acmopyle* (up to leaves; Figures 2B and 4D). The bilateral foliage has markedly lower leaf divergence angles and narrower and shorter leaves than *Acmopyle*, and the stomata lie in single, slender lines parallel to and offset on either side of the midvein (as seen in Florin's lectotype; Figure 10A, Table 1). All these features are characteristic of *Dacrycarpus* and not *Acmopyle*. Thus, we conclude that *Acmopyle* was not present at Río Pichileufú, where it was historically reported (Florin, 1940), even as we describe a new species of the genus here from Laguna del Hunco. This finding also explains why bilateral foliage of *Dacrycarpus* was apparently rare at Río Pichileufú (Wilf, 2012): it was present but misclassified as *Acmopyle*.

The bilateral leaves of *D. engelhardti* and *D. puertae* overlap in length, height, and length:height aspect ratio. However, these three measurements reach higher values in *D. engelhardti* than in *D. puertae* (18.5 mm vs. 13.9 mm maximum length, 3.2 mm vs. 1.7 mm maximum height, and 11.4:1 vs. 10.0:1 for maximum length:height ratio). Moreover, the holotype of *D. puertae* comes from a different site that is ca. 4.5 million years older, Laguna del Hunco, and is far more complete than the Río Pichileufú specimens, consisting of leafy branches with attached seed cones, and the Laguna del Hunco locality also preserves leafy branches bearing pollen cones (Wilf, 2012). Therefore, we decided not to disrupt the nomenclature for the Laguna del Hunco material. Instead, we here consolidate all the Río Pichileufú *Dacrycarpus* material in a separate species, using the basionym from the same site, as *D. engelhardti*. Further work, ideally informed by the eventual discovery of attached reproductive structures of *D. engelhardti*, will be required to clarify more morphological differences between *D. puertae* and *D. engelhardti*. In summary, we recognize three fossil *Dacrycarpus* species for South America: *D. puertae* from Laguna del Hunco, *D. engelhardti* from Río Pichileufú, and *D. chilensis* from several localities in Chile and Santa Cruz, Argentina.

Finally, the specimen USNM 40383c, previously considered to represent *Dacrycarpus* (Rossetto-Harris et al., 2020), is here considered as indeterminate Podocarpaceae.