

Pollen cone structure of the *Libocedrus s.l.* (Callitroideae, Cupressaceae) and its systematic implications for a controversial genus complex

Abstract

A *Papuacedrus arfakensis* tree in the living collection of one of the authors (HN) first formed pollen cones in 2018. This event was used for a detailed morpho-anatomical study of the pollen cones, with two major aims: first, a detailed documentation of the pollen cone structure of *P. arfakensis*; second, a comparison of those data with those of the other three genera of the *Libocedrus s.l.* group to check whether there are any unique pollen cone features that clearly distinguish and separate the four genera *Austrocedrus*, *Libocedrus*, *Papuacedrus* and *Pilgerodendron* from each other.

According to our data, pollen cones within the *Libocedrus s.l.* group only show minor variation. They differ only slightly in the number of microsporangioophores per cone, the number of microsporangia per microsporangioophore and the dimensions of the microsporangia. Previously two major diagnostic differences were recognised in the group; the spiral or whorled arrangement of the microsporangioophores of *Papuacedrus*, and the larger number of microsporangia per microsporangioophore in *Pilgerodendron* than in the other taxa. These differences were emphasized and used to split *Libocedrus s.l.* in four distinct genera. However, in our material, all pollen cones of *Papuacedrus* had a decussate arrangement of microsporangioophores, as is also the case for all other taxa within the *Libocedrus s.l.* group. In our *Pilgerodendron* cones the number of microsporangia per microsporangioophore was not significantly increased compared to the other genera. Thus, the pollen cone structure is more or less similar throughout the entire *Libocedrus s.l.* group, as was previously shown also for the seed cone structure and for the majority of vegetative traits. Thus, the differences in the reproductive as well as vegetative structures are not sufficient enough to justify the systematic treatment of *Austrocedrus*, *Libocedrus*, *Papuacedrus* and *Pilgerodendron* as four distinct genera.

Keywords: pollen cones, morphology, systematic, microsporangia, microsporangioophores.

Introduction

In 2018 a tree of *Papuacedrus arfakensis* cultivated in the living collection of HUBERTUS NIMSCH (Bollschweil, St. Ulrich, Freiburg im Breisgau, Germany) entered its reproductive phase and cones were produced for the first time. So far, only pollen cones have been produced. This fits well with the normal gender development in monoecious Cupressaceae. When individuals enter the reproductive phase, they exclusively produce pollen cones first, and then with increasing age later seed cones. It remains to be seen how the situation will develop in the next years: does the tree remain exclusively male, or will there be seed cones in addition?

This event is used now as an opportunity to investigate the structure of *Papuacedrus arfakensis* pollen cones in detail, and to compare them with pollen cones of other taxa belonging to the *Libocedrus s.l.* group (*Austrocedrus*, *Libocedrus* and *Pilgerodendron*). The aim of this study is not only a simple comparison of the pollen cone structure, but additionally to verify if the results are reliable enough to give statements about the systematic relationships within the *Libocedrus s.l.* group, which are still controversial and not finally resolved.

The evergreen coniferous genus *Papuacedrus* H.L.Li is native to Papua New Guinea, New Guinea and the East-Moluccas, where it occurs between (620-)900-3600(-3800) m above sea level (FARJON 2005). This genus belongs to the Cypress family (Cupressaceae). Within the Cupressaceae, *Papuacedrus* is a member of the exclusively southern hemisphere subfamily Callitroideae. The

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genus comprises only two taxa. Depending on the systematic view, they are treated either as two distinct species or both are treated as two subspecies (compare FARJON 2005, 2020; ECKENWALDER 2009; DÖRKEN & NIMSCH 2018, 2019). *Papuacedrus* and the three genera *Austrocedrus* FLORIN & BOUTELJE (one taxon; Chile and Argentina), *Libocedrus* ENDL. (five taxa; New Zealand and New Caledonia) and *Pilgerodendron* FLORIN (one taxon; southern Argentina and Chile) form a small, but complex cluster, comprising just few quite similar species. However, their systematic relationships are still controversial. Due to a very similar structure in the vegetative and also the reproductive parts, which show nearly no significant differences between the taxa, a systematic re-inclusion of all taxa into an enlarged genus *Libocedrus s.l.* could be justifiable, as mentioned by JAGEL & DÖRKEN 2015 who investigated the seed cones of the *Libocedrus s.l.* group.

Of the four genera belonging to the *Libocedrus s.l.* group only *Austrocedrus* is hardy enough in Central Europe to survive outdoors in protected and climatic mild conditions. In the forest arboretum Freiburg-Günterstal (Germany), *Pilgerodendron* is also cultivated; it has survived there outdoors, well protected by the canopy of the forest trees, for the last 20 years. However, all four genera are only rarely cultivated in common where they can be studied together, with *Austrocedrus* and *Pilgerodendron* the most “frequently“ grown. In comparison, *Libocedrus* is only rarely cultivated. JOHNS (1995) describes *Papuacedrus* outside of Papua New Guinea as “*it is not known to be cultivated outside New Guinea*”. There are however, just a few, rare individuals, cultivated as pot plants in some special collections. Thus, it is not surprising that in particular for *Papuacedrus* only comparatively little data exists, especially about its reproductive structures. Even such basic features such as the distribution of the genders is still an open question. JOHNS (1995) describes the genus as basically dioecious, however he reports about single monoecious specimens which were found in its natural habitat on the Owen Stanley Mountains in Papua New Guinea. In contrast, KRÜSSMANN (1983), DE LAUBENFELS (1988), PAGE (1990) and ECKENWALDER (2009) describe the genus as monoecious. Also FARJON (2010) describes *Papuacedrus* as monoecious, however with an important additional note “*monoecious, often appearing dioecious*”. This is in accordance with the majority of existing herbarium specimens of *Papuacedrus*, where male and female vouchers were often collected from the same individual (JOHNS 1995). It could be shown that if the gender distribution is actually dioecious, then the majority of individuals are functionally often male, and the functionally exclusively female individuals represent the exception (JOHNS 1995). It could be conceivable that the general gender distribution is dioecious, however cones of both gender are not produced every year, so that there are male and female years, leading to the impression of dioecy. However, to solve this question without doubt additionally further *in situ* studies are necessary. Depending on the few *ex situ* cultivated individuals and the fact that most of them do not produce cones, reliable statements about the true gender distribution are not possible.

Material und Methods

Material

To investigate the morpho-anatomical structure, 20 mature pollen cones of each of the following species were collected shortly before pollen release. Material of *Papuacedrus arfakensis* was from the private living collection of HUBERTUS NIMSCH, Bollschweil, St. Ulrich, Germany; material of *Austrocedrus chilensis*, *Libocedrus bidwillii* and *Pilgerodendron uviferum* was collected in the Palmengarten, Frankfurt am Main, Germany; *A. chilensis* outdoors, *L. bidwillii* and *P. uviferum* in the sub-antarctic house. Herbarium vouchers of *Papuacedrus papuana* seed cones were provided by WOLF STIEGLITZ (curator of the cone collection in the Bot. Garden Wuppertal, Germany).

Methods

Freshly collected material was photographed and then fixed in FAA (100 ml FAA = 90 ml ethanol 70% + 5 ml acetic acid 96% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The anatomy was studied from sections using the classical paraffin technique and subsequent astrablue/safranin staining (GERLACH 1984). Macrophotography was done with a digital camera (CANON POWERSHOT IS2) and microphotography with a digital microscope (KEYENCE VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright-field illumination (KEYENCE VH-S5).

Special terms

Microsporangiophore: because the identity of the coniferous pollen sac carrying structure is not finally resolved, the terms “sporophyll” or “microsporophyll” are not used, because homologies that are *a priori* applied to it should be avoided. Thus, the neutral term “microsporangiophore” (carrier of the pollen sacs) is used instead.

Microsporangium (pollen sac): structure developed on a microsporangiophore producing the pollen grains.

Pollen cone: the pollen producing “male” cones; in the majority of conifers unbranched structures usually with numerous pollen sacs (microsporangia) carrying structures (microsporangiophores). Pollen cones of all conifers are non-woody and dry out during or shortly after pollen release; dry and empty pollen cones are quickly abscised.

Seed cone: the ovule producing “female” cones. Within all conifers they are compound, inflorescence-like structures or can be regarded as being derived from such once; in the majority of species they become woody. After seed release the empty seed cones of some species remain, at time for several years, on the tree, while in others they abscise quickly or slowly.

Scutellum: small phylloid, distal structure, developed on the stalk of a microsporangiophore.

Cone: the more or less compact reproductive structures of conifers. The “male” cones producing the pollen are called pollen cones, the “female” cones producing the ovules, are called seed cones.

Results

Pollen cones of *Papuacedrus arfakensis*

The pollen cones are simple, unbranched, cone structures. They are developed densely on the previous year’s small lateral, scale-leaved branchlets. The pollen cones are exclusively terminal; lateral axillary cones were not found (Fig. 1A-B). They are in an upright or plagiotropic position (Fig. 1A-B). The stalk of the cone is very short and does not elongate even at maturity, so the pollen cones are more or less sessile. (Fig. 1C). The mature pollen cones are about 5-15 mm long and 2-3 mm in diameter.

The pollen cone consists of a central cone axis, which bears 4-8(-10) pairs of microsporangiophores (Fig. 1C). There are 1-2 pairs of green, sterile, scaly transitional leaves developed below the first pair of fertile microsporangiophores (Fig. 1C). Bracts are absent in the cone (Fig. 1C). Microsporangiophores are decussately arranged (Figs 3 & 4). However some cones had an apparent spiral or whorled arrangement of microsporangiophores (Figs 1C, 1E & 2A). This non-decussate arrangement was mostly observed in small pollen cones with a very short cone axis, or in larger cones showing a very high number of microsporangiophores. In both cases, there is a lack of space on the cone axis and the microsporangiophores are densely arranged. Thus, maturing microsporangia push the stalks of adjacent microsporangiophores laterally out of their original position so that it appears that the microsporangiophores are in a spiral or whorled arrangement. However, the insertion points of the stalks at the cone axis clearly indicate the original decussate formation on the cone axis as illustrated clearly in Figs 3 & 4. Thus, the apparently spiral or whorled arrangement is just a result of bent microsporangiophore stalks which are so strongly curved that the distal parts (scutellum and microsporangia) get shifted out of their original decussate position. This clearly demonstrates the importance of anatomical sections as illustrated in Fig. 3 & 4. Without such anatomical sections, the actual decussate arrangement could not be proven in mature cones showing non-decussate microsporangiophores. When more material is available, additional developmental studies of earliest ontogenetic stages should be carried out, to show clearly the actual position of the microsporangiophore primordia.

There is a concentric vascular bundle in centre of the cone axis with inner xylem and an outer phloem. The xylem and phloem are not separated by any cambium, and endodermis is also absent. In the centre a distinct mark is developed. The concentric vascular bundle strand gets strongly interrupted by the leaf traces of the lateral microsporangiophores (Fig. 2B). The central vascular bundle strand is surrounded by a monomorphic, parenchymatic cortex, which is rich in cellulose. Sclerenchyma and resin ducts are absent (Fig. 2B).

All microsporangiophores are hyposporangiate, and consist of a central stalk, an adaxial terminal, phylloid-like green scutellum and abaxial microsporangia (Figs 1C, 1E & 2C-F). The scutellum has a triangular to slightly rhombic shape and a skinny, hyaline margin. It is about 1.8-2.3 mm long and 1.5-2.5 mm in diameter (Figs 1E & 2C-D). The scutelli of the distal microsporangiophores are however significantly smaller (Figs 2A & 2D); in some cases they were entirely reduced. These distal microsporangiophores consist only of the stalk and the abaxial microsporangia (Fig. 2A). On the lower side of the stalk there are (2-)3-4(-5) roundish, yellow microsporangia, which are developed in a single row (Tab.1). The microsporangiophores in the middle of the cone have the highest number of microsporangia (Fig. 2C). Distal microsporangiophores often develop just 2 microsporangia (Fig. 2D). The microsporangia are about 0.4-0.8(-1) mm in diameter (Figs 2C-F & 5). The distal scutellum and the microsporangia are always free and not fused to each other (Figs 2E & 5A). Mature microsporangia open via a stomium that represents a predefined line of dehiscence, which consists of flat and thin walled parenchymatic cells (Fig. 5B). The other cells of the microsporangia wall are large sized with distinct U-shaped wall thickenings (Fig. 5C).

There is only one collateral vascular bundle supplying the microsporangiophore, with the xylem located adaxially, and the phloem abaxially. This vascular strand is not branched. The vascular bundle has a closed structure; the xylem and phloem are not separated by any cambium. A vascular bundle sheath, controlling the exchange between the bundle strand and the surrounding tissue, is also absent. Within the majority of microsporangiophores, there is a resin duct below the vascular bundle (Fig. 2F).

In early developmental stages, the scutelli are imbricate and cover the developing microsporangia. Thus, they play an important role in protecting the young microsporangia, as protecting bud scales surrounding the cone are generally absent (Fig. 1D). Just shortly before pollen release, the cone axis elongates so that the microsporangia become exposed. The elongation of the cone axis is however not caused by cell division, but the consequence of pumping water into the cells, which elongates the cells. This elongation ensures that the microsporangia become best exposed to the ambient airflow and that pollen grains are released best out of the microsporangia (Fig. 1E). After pollen release, the pollen cones dry out quickly and are soon abscised.

Pollen cones of *Austrocedrus*, *Libocedrus* and *Pilgerodendron*

As well as *Papuacedrus arfakensis*, pollen cones of *Austrocedrus chilensis*, *Libocedrus bidwillii* and *Pilgerodendron uviferum* were investigated. The results clearly indicate that their structure is broadly similar to that of *Papuacedrus*. They only differ slightly in the number of microsporangiophores per cone, in the number of microsporangia per microsporangiophore, and in the diameter of microsporangia (Table 1).

Table 1: Morphometric data of pollen cones developed in *Austrocedrus chilensis*, *Libocedrus bidwillii*, *Papuacedrus arfakensis* and *Pilgerodendron uviferum*; data based on 20 investigated pollen cones per species (d = decussate; h = hyposporangiate; t = terminal).

Taxa	pollen cones			Microsporangiophores			microsporangia		
	position	length [mm]	diameter [mm]	structure	arrangement	number per cone	number per microsporangiophore	diameter [mm]	arrangement
<i>A. chilensis</i>	t	5-15	2-3	h	d	10-12(-20)	(2-)3-4(-5)	0.5-0.6(-0.8)	single row
<i>L. bidwillii</i>	t	5-15	2-3	h	d	8-12(-20)	(2-)3-4(-6)	0.5-0.6(-0.8)	single row
<i>Pa. arfakensis</i>	t	5-15	2-3	h	d	8-16(-20)	(2-)3-4(-5)	0.4-0.8(-1)	single row
<i>Pi. uviferum</i>	t	5-15	2-3	h	d	8-12(-24)	(2-)2-4(-6)	0.5-0.6(-1)	single row

Discussion

As already discussed in JAGEL & DÖRKEN (2015), the existing genus concept of the *Libocedrus s.l.* group seems to be more likely geographically motivated than being based on reliable distinct morpho-anatomical differences. The vegetative structure of *Pilgerodendron* (Figs 6D & 7D) differs from the other three genera by a different foliage and arrangement of lateral branchlets. In *Pilgerodendron* the decussate small needle leaves are monomorphic and are spreading distinctly from the shoot axis (Fig. 7D). Furthermore, the shoots are not two-dimensional flattened but three-dimensionally arranged (Fig. 6D; DÖRKEN & NIMSCH 2018, 2019). In *Austrocedrus* (Figs 6A & 7A), *Libocedrus* (Figs 6B & 7B) and *Papuacedrus* (Figs 6C & 7C) the leaves are scaly and show a distinct leaf dimorphism with lateral and facial leaves, which are strongly adpressed to the shoot axis. Additionally the shoots are two-dimensionally flattened (DÖRKEN & NIMSCH 2018, 2019). Further structural differences in the vegetative body can be found in the wood anatomy (PEIRCE 1937). For example, within the *Libocedrus s.l.* group, *Papuacedrus* has the largest tracheids and also the largest cross-field pits (FARJON 2005). Furthermore, the four genera differ slightly in the formation and arrangement of stomata, the size of the epidermis cells and the formation of the cuticle (FLORIN 1930b; FLORIN 1951; FLORIN & BOUTELJE 1954), which could be interpreted as adaptations to the distinct and different local climatic conditions.

Even in times of molecular phylogenies, the reproductive structures are of great systematic importance. The structure of these should either be so similar, or so distinct, that they could allow a clear definition of a genus or separation from others. Previous investigations of seed cones have already shown that they are structurally quite similar within the *Libocedrus s.l.* group. The seed cones consist of two decussate pairs of cone scales. The distal pair is always significantly larger than the lower one (Fig. 8). Only the distal pair of cone scale is fertile and produces two ovules per cone scale. When only a single ovule is formed, the second one is mostly aborted in the earliest ontogenetic stages. In all taxa, the central columella, which represents the tip of the cone axis (DÖRKEN & JAGEL 2017), is visible as a small, pointed tip. The mature seed cones differ only marginally in the size and formation of the dorsal umbo developed on the back of the cone scales (Fig. 8), which can differ not only markedly between the currently commonly accepted different genera of the *Libocedrus s.l.* group, but also within species of a same genus. This is well illustrated by the accepted species in the genus *Libocedrus s.str.*, where strong variations are observed (TOMLINSON *et al.* 1993; CASTOR *et al.* 1996; JAGEL 2001; JAGEL & DÖRKEN 2015, NIMSCH & DÖRKEN 2020). For example in the New Caledonian *Libocedrus* species, the umbo of *L. chevalieri* is quite short and only slightly exceeds the cone scales (Fig. 10), compared to the very long umbo of *L. austrocaledonica* which significantly exceeds the length of the cone scale (Fig. 11). The lengths of the umbos of *L. yateensis* (Fig. 9), are more or less intermediate between *L. chevalieri* and *L. austrocaledonica* (NIMSCH & DÖRKEN 2020). Additionally, in *L. austrocaledonica* and *L. yateensis* the length of the umbos of the lower sterile pair of the cone scales is broadly similar to these of the upper fertile pair of the cone scales. In *L. chevalieri* however, the lengths of the umbos of the fertile and sterile pairs of cone scales are less similar (compare drawings in FARJON 2005, 2010 and descriptions in NIMSCH & DÖRKEN 2020).

The results of these pollen cone investigations also show no significant differences between the four currently accepted genera. The dimensions and the structure of mature pollen cones are more or less identical throughout all investigated species and show only marginal differences. Pollen cones of all four genera represent unbranched uniaxial structures, carrying exclusively decussately arranged hyposporangiate microsporangiophores. However, unlike our results, GIBBS (1917) and LI (1953) described microsporangiophores of *Papuacedrus* as spirally arranged, which if correct is not only a distinct and unique character within the *Libocedrus s.l.* group, but additionally also within the entire Cupressaceae *s.str.* (= subfamilies Cupressoideae and Callitroideae). In contrast to the Cupressaceae *s.l.* (= subfamilies Cunninghamioideae, Athrotaxoideae, Taiwanoideae, Sequoioideae and Taxodioideae), microsporangiophores of Cupressaceae *s.str.* are not spirally arranged. In the majority of species they are in decussate pairs; only in some *Juniperus* species (mostly in species of

section *Juniperus*, e.g. *J. communis*, and of section *Caryocedrus* (*J. drupacea*) and in *Actinostrobus* and *Callitris* species, they are arranged in alternating whorls of three (FARJON 2005, 2010; ECKENWALDER 2009; DÖRKEN 2019; DÖRKEN & STÜTZEL 2019), and e.g. in *Neocallitropsis* also whorls of 4 are developed (FARJON 2005, ECKENWALDER 2009). With the pollen cones investigated by FLORIN (1951) and FLORIN & BOUTELJE (1954), or in our material, a spiral arrangement of microsporangiophores in *Papuacedrus* was not found. However, FLORIN (1951), as well as FLORIN & BOUTELJE (1954) and KRÜSSMANN (1983), describe the microsporangiophores in whorls of four. FLORIN & BOUTELJE (1954: 26 & 30) describe them as “being arranged in whorls of four instead of decussately” (p. 26) and as “in pairs, of which two always appeared to be at the same level” (p. 30). Also RUSHFORTH (1987: 143) describes the position of microsporangiophores in whorls of four, and writes explicitly that they are not decussate: “set in whorls of 4, not in decussate pairs”. FARJON (2005: 433, 2010: 536) describes the position of microsporangiophores of *Papuacedrus* as decussate or in whorls of four “decussate or in whorls of 4”. However here differences between the two species *P. papuana* and *P. arfakensis* are mentioned, *P. papuana* – decussate or whorled, *P. arfakensis* exclusively whorled. In contrast to that, DE LAUBENFELS (1988) and ECKENWALDER (2009: 360) describes the position as decussate. DE LAUBENFELS (1988: 444) – with *papuana* under *Libocedrus* – writes: “The New Guinea species was separated on the basis of spirally placed microsporophylls. In fact, simple opposite decussate pollen cones occur alongside crowded cones whose microsporophylls appear to be whorled or perhaps spirally placed” as is also shown in a line drawing in that publication (1988: 446, Fig. 89, C). The same drawing showing opposite microsporangiophores is also presented in JOHNS (1995: 69). ECKENWALDER (2009: 360) writes “8-10 alternating pairs of pollen scales often arranged like four or five aligned quartets or so crowded as to appear irregular”. Our results are in accordance with the findings of DE LAUBENFELS (1988) and ECKENWALDER (2009). When having a closer look at the leaf traces entering the microsporangiophores, it is clearly visible that there are always only two and not four opposite leaf traces leaving the concentric bundle of the cone axis in the same plane and enter the stalk of each microsporangiophore (Figs 3 & 4). If the microsporangiophores would have been in whorls of four, then four vascular bundles should leave the concentric bundle of the cone axis in the same plane. This finding clearly show that the arrangement of microsporangiophores is in decussate pairs. However, the subsequent microsporangiophores are, due to a lack of space, often developed closely to the lower pair so that it seems that the microsporangiophores are arranged in whorls of four. DE LAUBENFELS (1988) and ECKENWALDER (2009) describe that in pollen cones with a very dense arrangement of microsporangiophores or a very short cone axis the microsporangiophores are apparently in a spiral or whorled arrangement. Our results strongly support these findings of DE LAUBENFELS (1988) and ECKENWALDER (2009). Pollen cones with a very short cone axis are more or less roundish or nearly globose. In consequence, space within these pollen cones is therefore very restricted and the microsporangiophores in these pollen cones are much more densely arranged than in pollen cones showing the typical longer cone axis. Due to the continuously increasing size of the developing microsporangia in combination with the lack of space, stalks of neighbored microsporangiophores can easily be pushed out of their original position, so that their arrangement finally seems to be spirally or whorled. However, a close look at the insertion points of the stalks at the cone axis clearly show the true arrangement, that is, also in these cones, decussate pairs (Figs 3 & 4).

In regard to the problematic decussate vs whorled arrangement of microsporangia FARJON (2005) pursued another interesting idea. In Cupressaceae the phyllotaxis in vegetative parts (= foliage) corresponds always to that of the reproductive units (= cones). Mature individuals of *Papuacedrus* have a decussate leaf arrangement however, in juveniles also whorls of four leaves occur. FARJON (2005) describes a correlation between the arrangement of leaves (decussate vs whorled), the type of foliage (juvenile vs mature) and the position of the microsporangiophores (decussate vs whorled). FARJON describes the position as basically decussate. A whorled arrangement of microsporangiophores can be only found on branchlets still showing the juvenile foliage with leaves arranged in whorls of four. Such a shift in the phyllotaxis can be also observed in numerous seedlings of *Cupressus* with juvenile needle leaves, which are arranged in whorls of four before

shifting to the mature type of foliage with decussate scale leaves (personal observations). In other taxa, e.g. *Callitris macleayana*, a similar shift can be observed; the juvenile needle leaves are arranged in whorls of four, the mature scale leaves however in whorls of three (DÖRKEN *et al.* 2019). When regarding the microsporangiophores as pollen producing leaves in the sense of a sporophyll, this is a strong argument supporting the idea that there is no difference in the leaf arrangement between vegetative and reproductive part, because the leaf arrangement on vegetative branchlets correspond to the arrangement of microsporophylls on the cone axis. This idea fits well to the situation here: the pollen cones with their decussate microsporangiophores were developed on exclusively scale leaved branches showing a decussate phyllotaxis.

If the arrangement of microsporangiophores is actually decussate as it is demonstrated in this study or if it is whorled as suggested by FLORIN (1951), FLORIN & BOUTELJE (1954), KRÜSSMANN (1983) and FARJON (2005, 2010), it can finally be solved by ontogenetic studies dealing with the earliest developmental stages and the formation of the primordia of the microsporangiophores at the cone axis.

In the studied pollen cones no evidence for a spiral arrangement of microsporangiophores as described by GIBBS (1917) and LI (1953) was found. Our results agree with FLORIN & BOUTELJE (1954) that the postulated relationship between the Taxodiaceae (at that time still regarded as a distinct family, today merged into the Cupressaceae as 5 subfamilies, Cunninghamioideae, Athrotaxoideae, Taiwanoideae, Sequoioideae and Taxodioideae) and *Papuacedrus*, as suggested by LI (1953), is not supported.

The pollen cones within the *Libocedrus s.l.* group only differ slightly in the number of microsporangiophores per cone, the number of microsporangia per microsporangiophore, and the size of the microsporangia (Tab. 1). In one of the latest conifer phylogenies (YANG *et al.* 2022) the four *Libocedrus s.l.* genera are split into 3 tribes – tribe 1: Papuacedreae Y.YANG, *trib. nov.* (only *Papuacedrus*), Tribe 2: Austrocedreae Y.YANG, *trib. nov.* (only *Austrocedrus*) and tribe 3: Libocedreae H.L.LI (*Libocedrus* and *Pilgerodendron*) with *Papuacedrus* as sister to *Austrocedrus* and *Libocedrus* sister to *Pilgerodendron*. A high number of microsporangiophores (8-30) in *Papuacedrus* is mentioned as one of the main morphological features distinguishing the Austrocedreae from the Papuacedreae. However, in our investigated material of *Papuacedrus arfakensis* the number of microsporangiophores per pollen cone was 8-16(-20) and is therefore in accordance with the other genera of the *Libocedrus s.l.* group (Tab. 1) and not significantly higher than in the other taxa (*Austrocedrus chilensis* 10-12(-20), *Libocedrus bidwillii* 8-12(-20), and *Pilgerodendron uviferum* 8-12(-24)). In our material the highest number of microsporangiophores per pollen cone (with up to 24) was actually found in *Pilgerodendron*. Additionally in this phylogeny, the microsporangiophores of *Papuacedrus* are described as “peltate microsporophylls”. In none of our investigated pollen cones peltate microsporangiophores were found; all had throughout a hyposporangiate structure as is the case for all other species of the *Libocedrus s.l.* group (Tab. 1). Thus, in our results, the structure of the pollen cones, and in particular the number of microsporangiophores per cone and the type of microsporangiophore, could not be used as a reliable argument to justify this split into two new described tribes Austrocedreae and Papuacedreae. This is notable in the respect that in other conifer genera the structural differences of pollen cones can be much more distinct. For example in *Juniperus* (Cupressaceae: Cupressoideae), the pollen cones of *J. drupacea* (Sect. *Caryocedrus*) are compound and “inflorescence”-like, while in all other *Juniperus* species, they are simple and therefore “flower”-like. Additionally the number of microsporangiophores and the number of inserted microsporangia per microsporangiophore vary distinctly and this not only between the distinct *Juniperus*-species, but also within a species and even within a single pollen cone (compare LEMOINE-SÉBASTIAN 1967 & DÖRKEN 2019).

FLORIN (1930a) and FARJON (2005, 2010) mention a significant higher amount of microsporangia per microsporangiophore in *Pilgerodendron*, which would be a distinct feature to distinguish *Pilgerodendron* from the rest of the *Libocedrus s.l.* species. FLORIN (1930a) mentions 6(-4-8),

exceptional up to 10 microsporangia per microsporangiphore, FARJON (2005, 2010) says 4-8(-10). This high number of microsporangia per microsporangiphore was not found in our investigated material. Our results with 3-4(-5) microsporangia per microsporangiphore are more like those of LI (1953), who mentioned 4-8 microsporangia per microsporangiphore. Despite FARJON (2005, 2010) mentioning a high number of microsporangia per microsporangiphore, the microsporangiphore he has drawn (FARJON 2005: 454, Fig. 122-7, up) with just 4 microsporangia, corresponds well with the situation in our available material. This high number of microsporangia per microsporangiphores mentioned by FLORIN (1930a) and FARJON (2005, 2010) is remarkable, because FLORIN describes *Pilgerodendron uviferum* pollen cones as 2 mm in diameter, FARJON as 2-2.5 mm. Our investigated microsporangia are 0.5-0.6(-1) mm in diameter. This means, if there are 10 microsporangia per microsporangiphore, a possible total cone diameter of up to 1 cm would be obtained. This high number of microsporangia per microsporangiphore in cones showing total cone diameter of maximum 2.5 mm as mentioned by FLORIN and FARJON could therefore only be realised if the size of each microsporangium were strongly reduced, or the hyposporangiate structure could be replaced by a perisporangiate one, with microsporangia developed all around the stalk of the microsporangiphore (as can be found in some Taxaceae like *Taxus* and *Pseudotaxus*; WILDE 1975; MUNDRY 2000; MUNDRY & MUNDRY 2001; DÖRKEN *et al.* 2011; DÖRKEN & NIMSCH 2016). However, perisporangiate microsporangiphores were not found in any of our investigated *P. uviferum* pollen cones. A further possibility to increase the number of microsporangia per microsporangiphore is an arrangement in more than one row as can be found in some *Juniperus* species (in Cupressaceae *s.str.*) (DÖRKEN 2019). For example in *J. drupacea* there are up to 11 microsporangia per microsporangiphore developed in 3 rows on the lower side of the stalk. But in our investigation of *P. uviferum* pollen cones, microsporangia were always inserted in a single abaxial row, parallel to the lower margin of the adaxial scutellum. Thus, it remains open, how the high number of microsporangia per microsporangiphores described in FLORIN (1930a) and FARJON (2005, 2010) were developed, without exceeding the cone diameter of 2-2.5 mm. This clearly indicates that further investigation is needed in this respect, ideally with material collected *in situ*.

Conclusion

Following the results of JAGEL & DÖRKEN (2015, seed cones) and the present study, the lack of distinct morpho-anatomical differences in the vegetative and reproductive structures in the four species *A. chilensis*, *Papuacedrus papuana*, *P. afarkenensis* and *Pilgerodendron uviferum*, they are returned to the genus *Libocedrus*:

- *Libocedrus chilensis* (D.DON) ENDL. (1847) [basionym *Thuja chilensis* D.DON (1832)]
- *Libocedrus papuana* F.MUELL. (1891)
- *Libocedrus arfakensis* GIBBS (1917)
- *Libocedrus uvifera* (D.DON) PILG. (1926) [basionym *Juniperus uvifera* D.DON (1828)]

The total number of species in the *Libocedrus* genus rises to 9 with:

- *Libocedrus bidwillii* HOOK. (1864)
- *Libocedrus austrocaledonica* BRONGN. & GRIS (1872)
- *Libocedrus plumosa* (D.DON) DRUCE (1917) [bas. *Dacrydium plumosum* D.DON (1828)]
- *Libocedrus chevalieri* J.BUCHHOLZ (1949)
- *Libocedrus yateensis* GUILLAUMIN (1949)

In this study the widely accepted genus names were used for clarity, pending circulation of the study. From now on, they should be regarded as synonyms.

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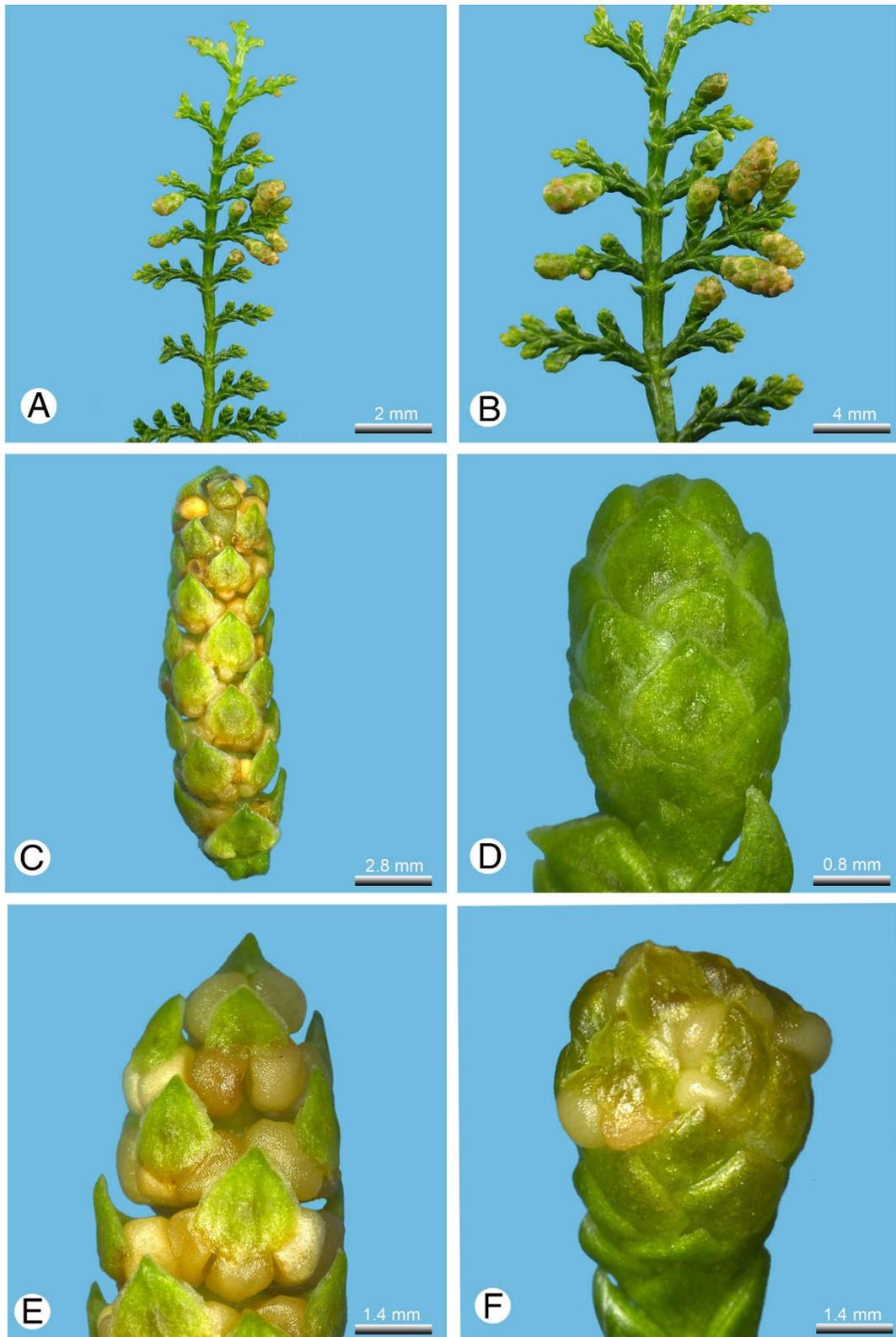
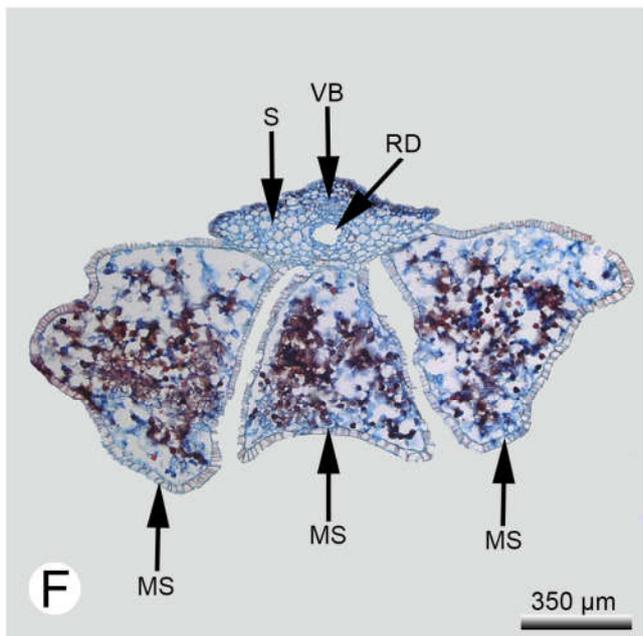
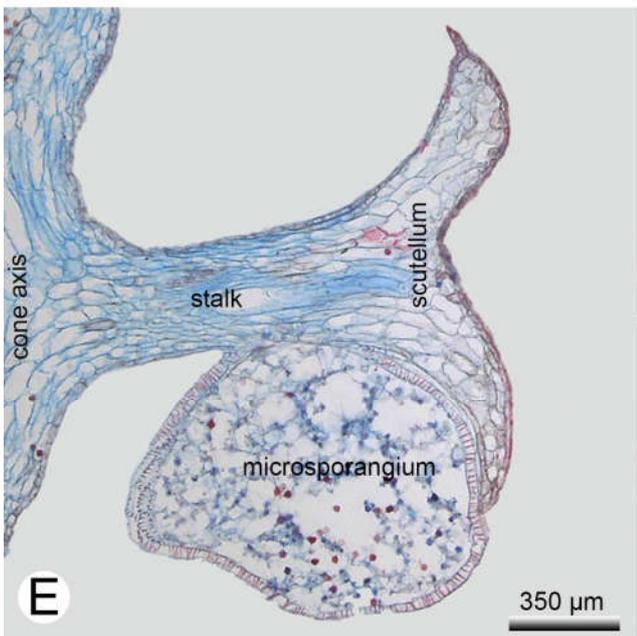
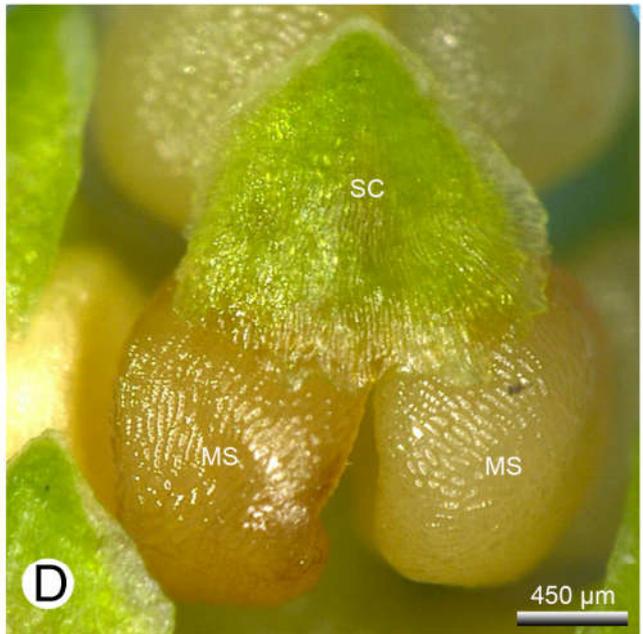
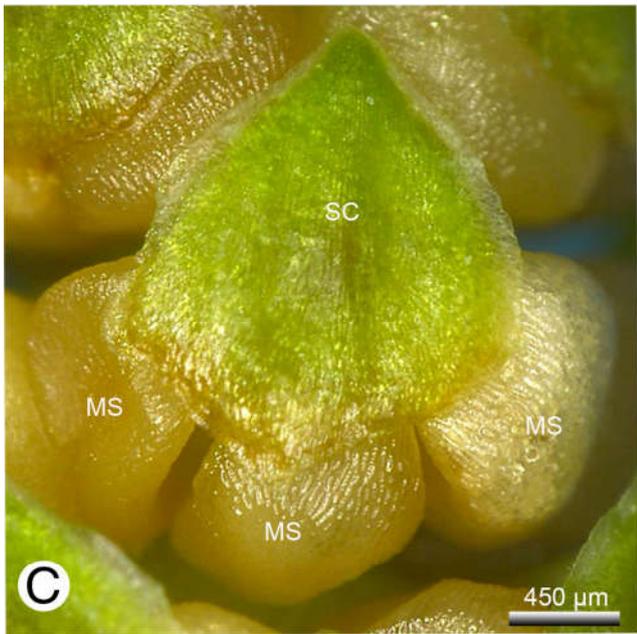
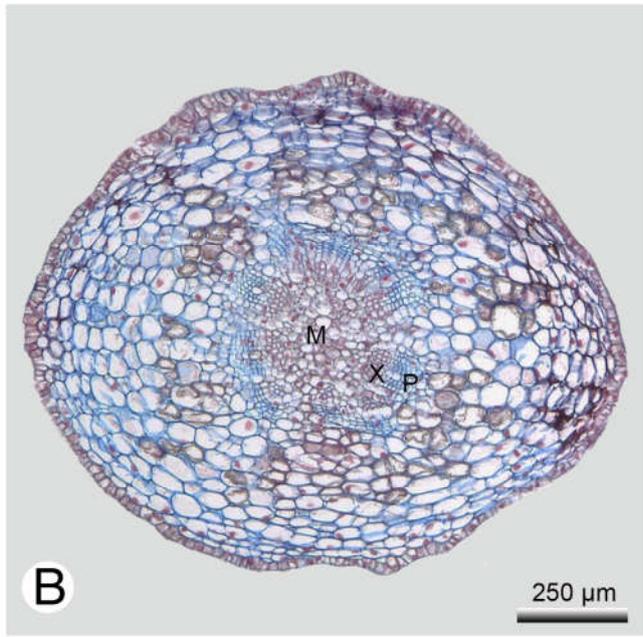
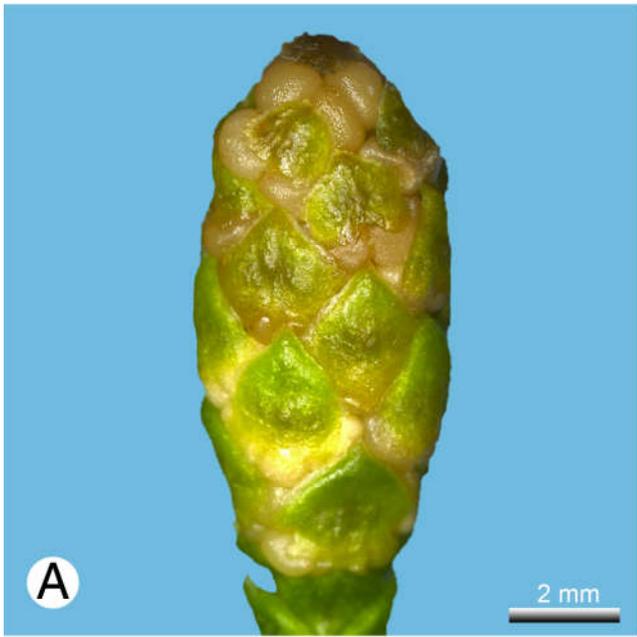


Fig. 1: *Papuacedrus arfakensis*, morphology of pollen cones (PC).

A: Fertile lateral branchlet; PC terminal on short, last year's branchlets. **B:** Detail of A. **C:** Mature PC with a high number of microsporangioophores; due to a lack of space on the cone axis the maturing microsporangia have pushed the stalks of adjacent microsporangioophores laterally out of their original position. **D:** Juvenile PC; scutelli imbricate and cover the microsporangia. **E:** Distal part of the mature PC illustrated in C, with spreading microsporangioophores. **F:** PC with a reduced cone axis; microsporangioophores seem to be in a spiral arrangement due to a lack of space within the cone because the maturing microsporangia push the stalks of the microsporangioophores out of their original position.

Images: V.M. DÖRKEN.



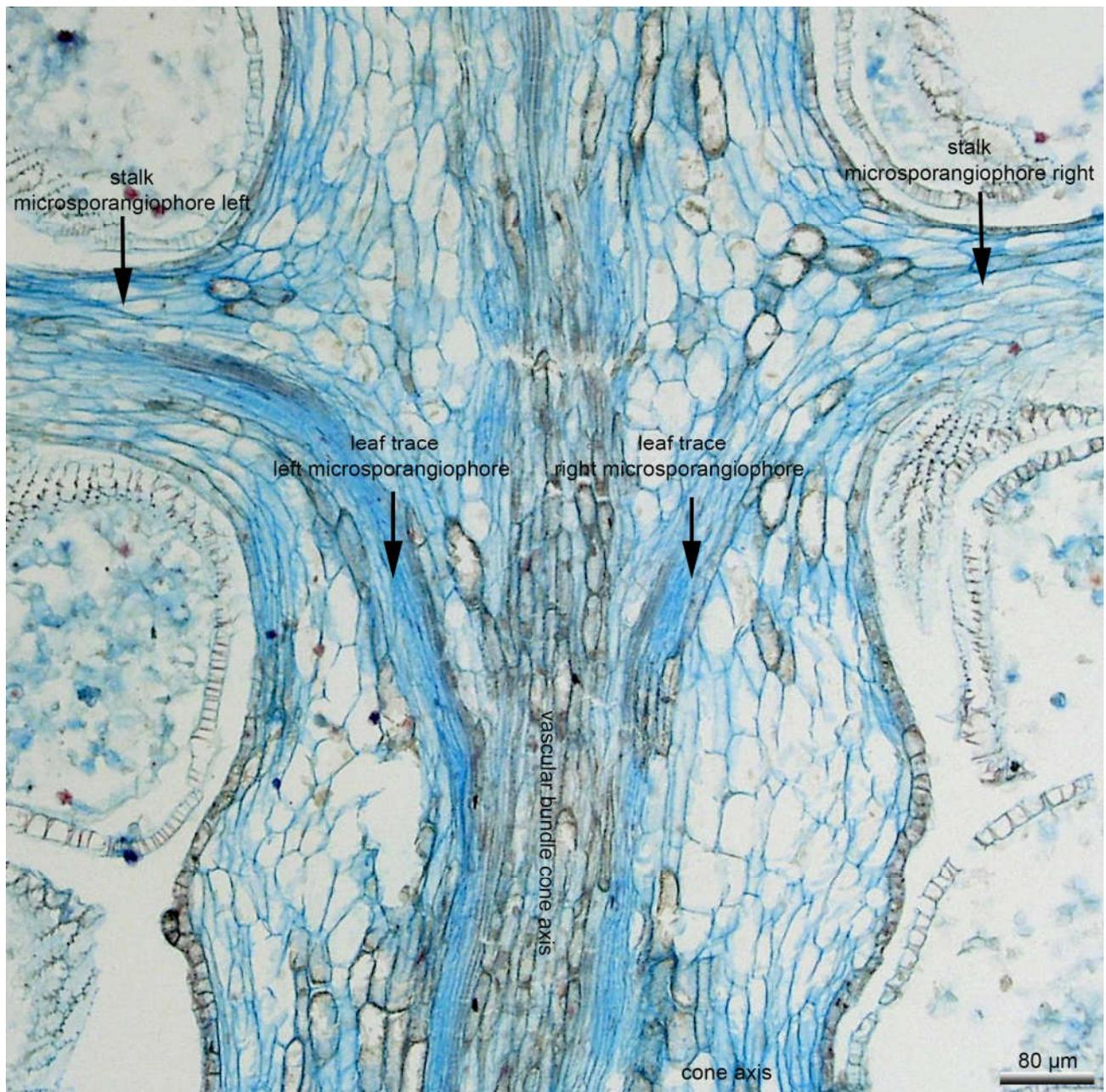


Fig. 3: *Papuacedrus arfakensis*, longitudinal section of a pollen cone showing the decussate microsporangiophores; the collateral leaf traces of the microsporangiophores are leaving the concentric stem bundle at the same level. Image: V.M. DÖRKEN.

Fig. 2 (p. 12): *Papuacedrus arfakensis*, morphology and anatomy of microsporangiophores.

A: Pollen cone with a short cone axis and densely arranged microsporangiophores; maturing microsporangia push the stalks of adjacent microsporangiophores laterally out of their original position so that it seems that the microsporangiophores are in a spiral or whorled arrangement; scutelli of distal microsporangiophores strongly or entirely reduced. B: Cross section in the middle of the cone axis, showing the concentric stem bundle which is strongly interrupted by the leaf traces of the microsporangiophores. C: Microsporangiophore from the middle of the cone; there are three microsporangia. D: Distal microsporangiophore with a small scutellum, only two microsporangia. E: Longitudinal section of a microsporangiophore. F: Cross section of a microsporangiophore; the stalk of the microsporangiophore is carrying three microsporangia.

M = mark; MS = microsporangium; P = phloem; RD = resin duct; S = stalk; SC = scutellum; VB = vascular bundle; X = xylem.

Images: V.M. DÖRKEN.

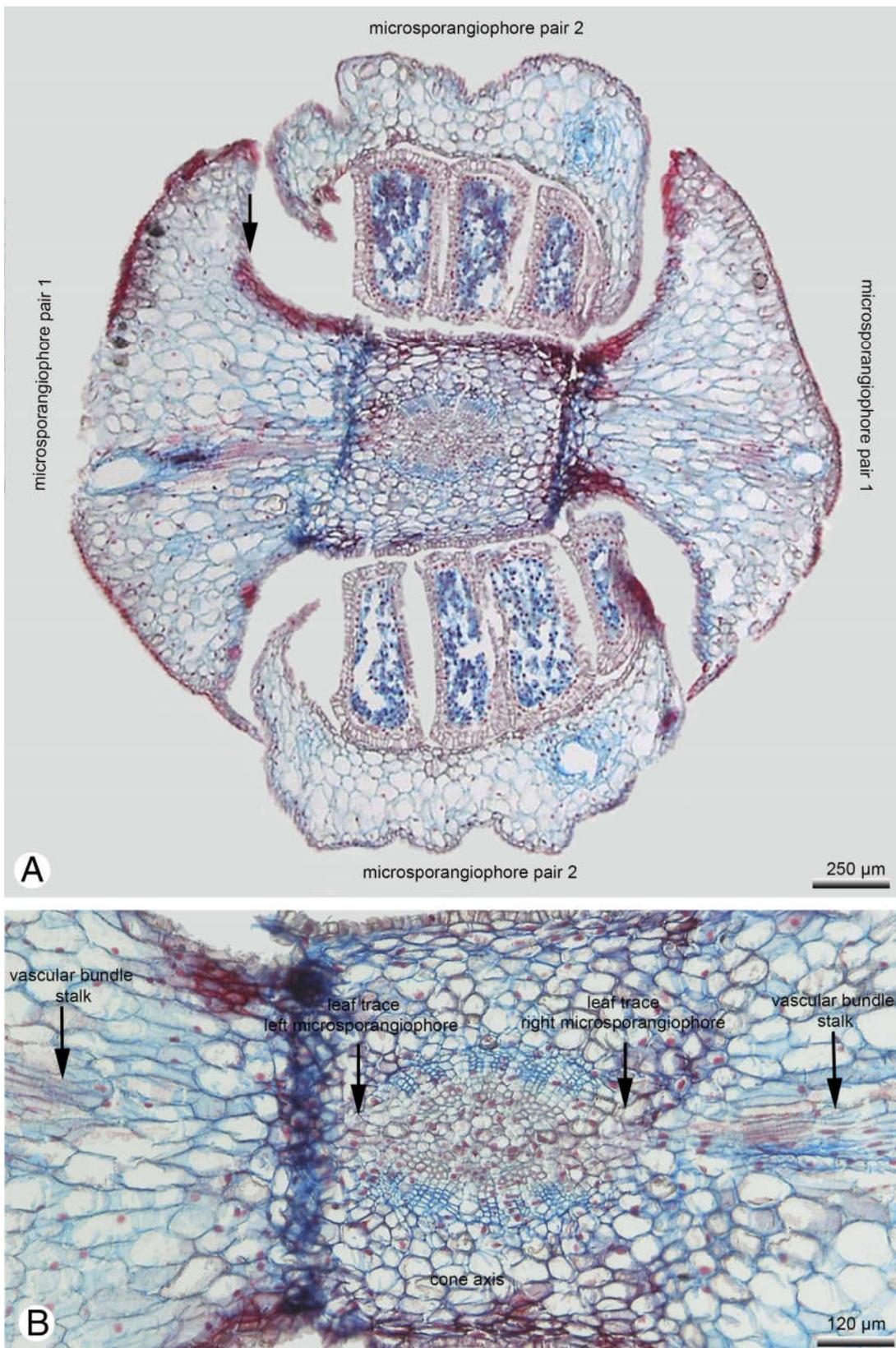


Fig. 4: *Papuacedrus arfakensis*, cross section of a pollen cone.

A: Overview showing the decussate microsporangiophores. **B:** Detail of the pollen cone axis (cross section) showing the two leaf traces of the decussate microsporangiophores leaving the concentric stem bundle at the same level.

Images: V.M. DÖRKEN.

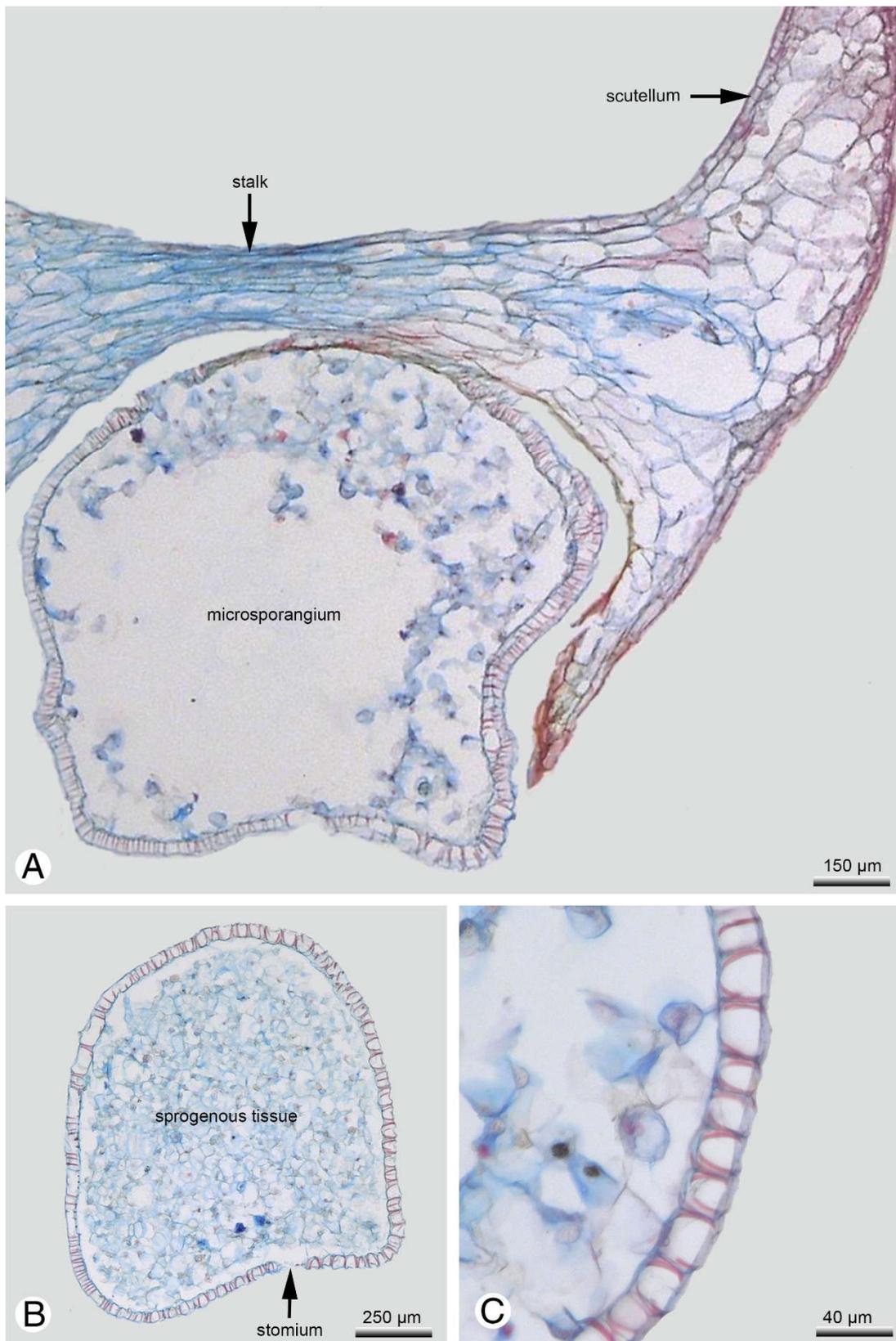


Fig. 5: *Papuacedrus arfakensis*, anatomy of microsporangia.

A: Scutellum and microsporangia are always free and never fused to each other. **B:** Cross section of a juvenile microsporangium; thin walled cells mark the later line of dehiscence (arrow). **C:** Detail of the microsporangium wall; cells with U-shaped wall thickenings.

Images: V.M. DÖRKEN.

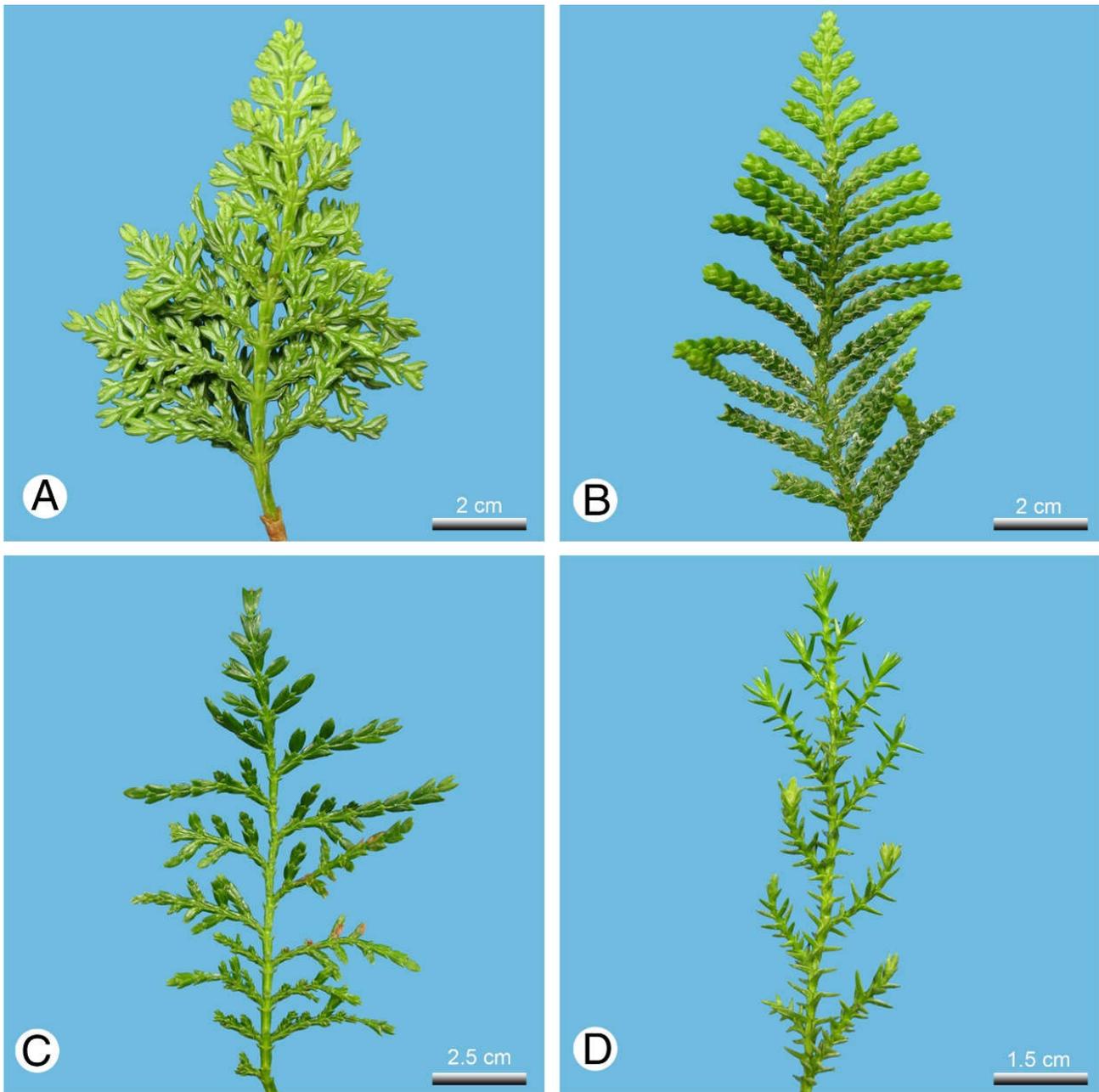


Fig. 6: Lateral shoots morphology of the *Libocedrus s.l.* group.

A: *Austrocedrus chilensis*. **B:** *Libocedrus plumosa*. **C:** *Papuacedrus arfakensis*. **D:** *Pilgerodendron uviferum*.

Lateral shoots of the genera *Austrocedrus* (A), *Libocedrus* (C) and *Papuacedrus* (D) two-dimensional flattened; in *Pilgerodendron* (G) three-dimensional spreading.

Images: V.M. DÖRKEN.

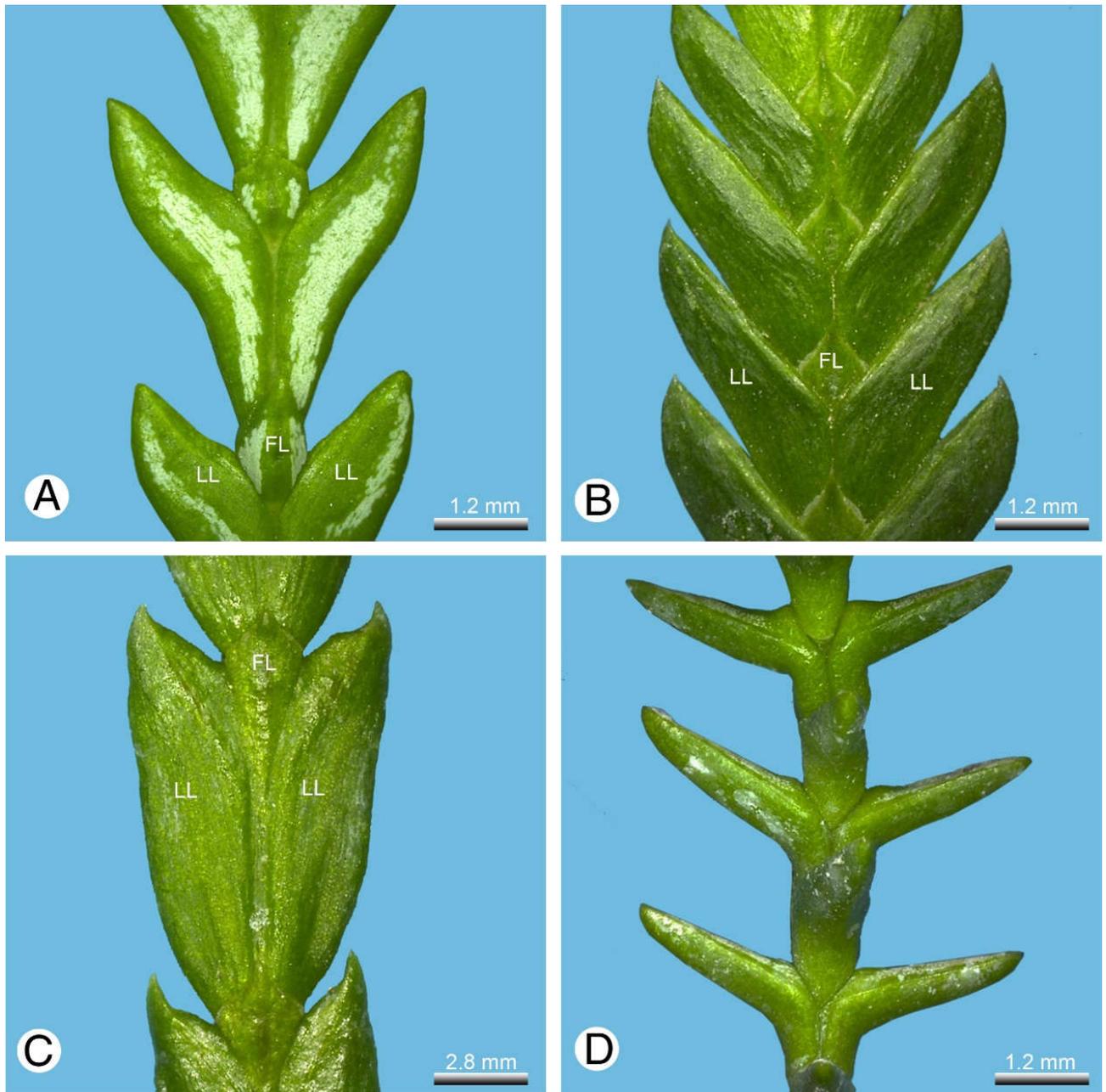


Fig. 7: Leaf morphology of the *Libocedrus* s.l. group.

A: *Austrocedrus chilensis*. **B:** *Libocedrus plumosa*. **C:** *Papuacedrus arfakensis*. **D:** *Pilgerodendron uviferum*.

Austrocedrus (B), *Libocedrus* (B) and *Papuacedrus* (C) with dimorphic scale leaves, that can be distinguished in facial (FL) and lateral leaves (LL); *Pilgerodendron* (H) monomorphic, small spreading needle leaves (FL = facial leaves; LL = lateral leaves).

Images: V.M. DÖRKEN.

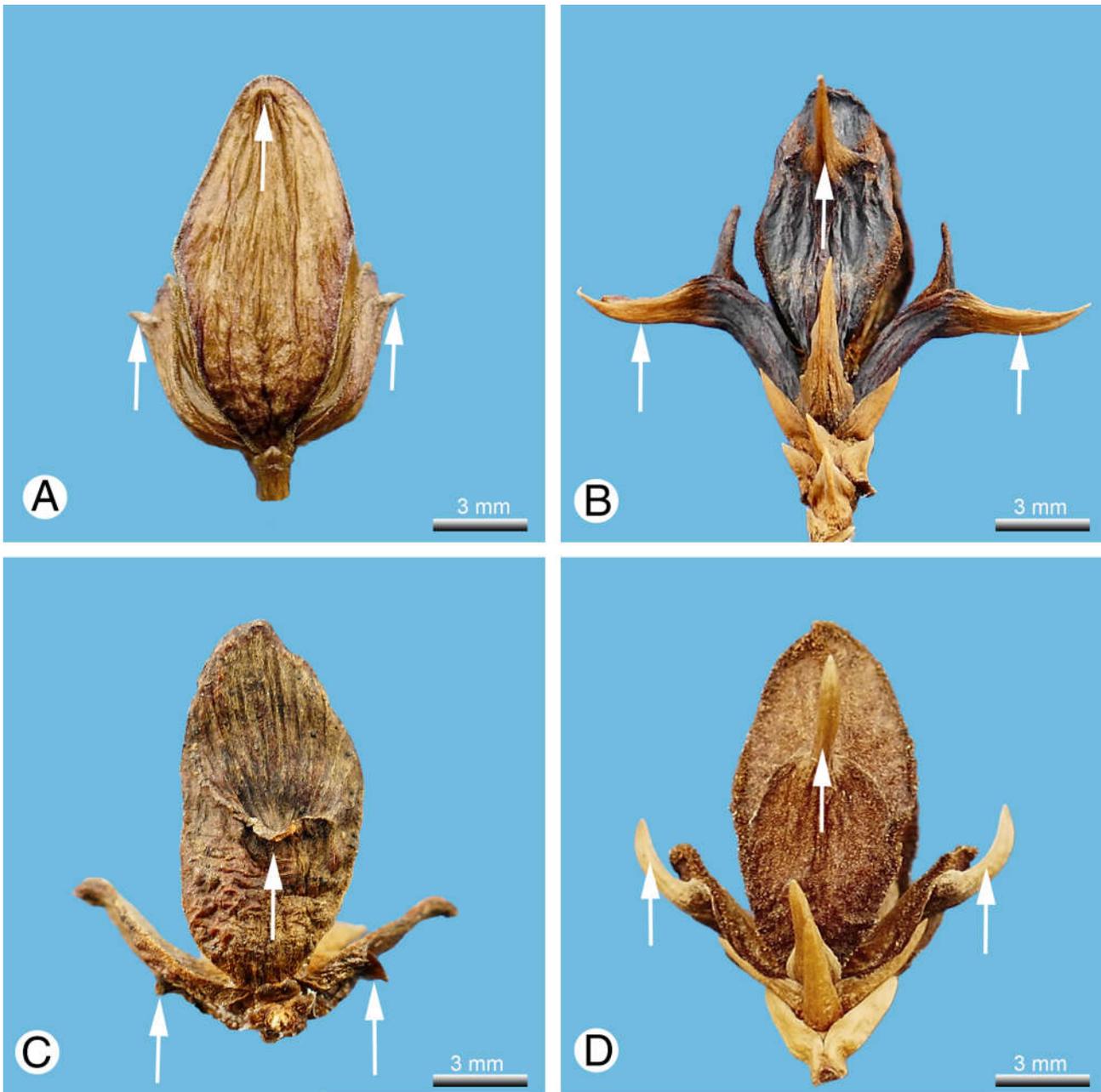


Fig. 8: Seed cones of the *Libocedrus s.l.* group; seed cones more or less uniformly shaped; they mainly differ by the formation of the dorsal umbo developed on the back of the cone scales (arrows). See discussion p. 6.

A: *Austrocedrus chilensis*; **B:** *Libocedrus plumosa*; **C:** *Papuacedrus papuana*; **D:** *Pilgerodendron uviferum*.

Images: V.M. DÖRKEN.



Fig. 9: *Libocedrus yateensis*, seed cone displaying its long umbos. © A. LESPES, [Parc provincial de la Rivière Bleue](#), New Caledonia.

All photos on this page were obtained thanks to [endemia.nc](#) and their photographers.

Fig. 10: *Libocedrus chevalieri*, seed cone with shorter umbos. © R. AMICE, New Caledonia.



Fig. 11: *Libocedrus austrocaledonica*, seed cones. © D. & I. LÉTOCART, New Caledonia.

