Morphology and anatomy of pollen cones and pollen in *Podocarpus gnidioides* Carrière (Podocarpaceae, Coniferales)

Abstract

*Podocarpus gnidioides* is one of the rarest *Podocarpus* species in the world, and can rarely be found in collections; fertile material especially is not readily available. Until now no studies about its reproductive structures do exist. By chance a 10-years-old individual cultivated as a potted plant in the living collection of the second author produced 2014 pollen cones for the first time. Pollen cones of *Podocarpus gnidioides* have been investigated with microtome technique and SEM. Despite the isolated systematic position of *Podocarpus gnidioides* among the other New Caledonian Podocarps, it shows no unique features in morphology and anatomy of its hyposporangiate pollen cones and pollen. Both the pollen cones and the pollen are quite small and belong to the smallest ones among recent *Podocarpus*-species. The majority of pollen cones are unbranched but also a few branched ones are found, with one or two lateral units each of them developed from different buds, so that the base of each lateral cone-axis is also surrounded by bud scales. This is a great difference to other coniferous taxa with branched pollen cones e.g. *Cephalotaxus* (Taxaceae), where the whole “inflorescence” is developed from a single bud. It could be shown, that the pollen presentation in the erect pollen cones of *Podocarpus gnidioides* is secondary. However, further investigations with more specimens collected in the wild will be necessary.

Key words: Podocarpaceae, *Podocarpus*, morphology, pollen, cone

1 Introduction

*Podocarpus gnidioides* is an evergreen New Caledonian shrub, reaching up to 2 m in height (DE LAUBENFELS 1972; FARJON 2010). In older literature it is described as extinct in nature and only recorded from the original collection. Thus, *Podocarpus gnidioides* was regarded as a doubtful taxon (KRÜSSMANN 1983) and its systematic position among recent Podocarpaceae was therefore unclear for a long time. However, today some stable populations (figs 6 & 7) are well known in the south of the Baie de St. Vincent in South New Caledonia (ECKENWALDER 2009). Therefore the present conservation state of *Podocarpus gnidioides* is described as “near threatened” (THOMAS 2010). Thus, enough vegetative material was available to solve the systematic position of *Podocarpus gnidioides* based on molecular and also on foliar data (internal and external microscopic leaf characters). Following current cladistic analyses of Podocarpaceae *Podocarpus gnidioides* is placed within the Australis clade. Thus, *Podocarpus gnidioides* is closely related to the New Zealand taxa *Podocarpus acutifolius*, *Podocarpus cunninghamii*, *Podocarpus hallii*, *Podocarpus totara* (subclade Australis I) and to *Podocarpus nivalis* from New Zealand, *Podocarpus alpinus* and *Podocarpus lawrencei* from Australia (subclade Australis II) (KNOPF et al. 2011, fig. 4). *Podocarpus gnidioides* has no close affinities to the other taxa native in New Caledonian as e.g. *Podocarpus decumbens*, *Podocarpus longifoliolatus*, *Podocarpus lucienii*, *Podocarpus novae-caledoniae*, *Podocarpus polyspermus* and *Podocarpus sylvestris* (ECHENWALDER 2009). Within the Australis clade *Podocarpus gnidioides* is a sister taxon to the Australis I and Australis II subclades.

*Podocarpus gnidioides* is rare in cultivation and material (vegetative and fertile) is hardly available. Thus, currently only limited data about morphology and anatomy especially about its cones are available. *Podocarpus gnidioides* is dioecious as is also the case for nearly all other *Podocarpus*
species (Dallimore & Jackson 1966; Del Fuego 1996; Eckenwalder 2009; Farjon 2010). A line drawing in De Laubenfels (1972) is one of the rare illustrations of the pollen cones of Podocarpus gnidioides that exist. A 10-years-old male individual cultivated as a potted plant and overwintered in a temperate house in the private living collection of Hubertus Nimsch, Bollschweil, St. Ulrich (Germany), started forming pollen cones for the first time in spring 2014. This was taken as an opportunity to investigate the morphology and anatomy of pollen cones and pollen of Podocarpus gnidioides in detail.

2 Material & Methods

2.1 Material

21 pollen cones were collected on 5.5.2014 shortly before anthesis. As typical for conifers the pollen cone development is simultaneous within an individual and all material that was available has been collected in a more or less the same late ontogenetic stage. Thus, ontogenetic studies about the pollen cone development could not be done.

2.2 Methods

Freshly collected material was photographed and then fixed in FAA (100 ml FAA = 90 ml 70% ethanol + 5 ml acetic acid 96% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The cone-anatomy was studied from serial sections using the classical paraffin technique and subsequent astrablue/safranin staining (Gerlach 1984). For SEM-analysis the FAA-material was dehydrated in formaldehyde dimethyl acetal (FDA) for at least 24 hours (Gerstberger & Leins 1978) and critical point dried. Sputter coating was done with a Sputter Coater SCD 50 Bal-tec (Balzers). The specimens were examined with an Auriga Zeiss TM. Macrophotography was accomplished using 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).

2.3 Special terms

The term “sporophyll” or “microsporophyll” is avoided for the sporangia bearing structure in conifers. Otherwise this would introduce a priori a homology with the terminology applied. They are termed here as “sporangiophore” or “microsporangiophore”. The small green scale developed adaxial at the central stalk of hyposporangiate sporangiophores is called “scutellum”.

3 Results

3.1 Morphology and anatomy of pollen cones

On lateral shoots pollen cones are developed in distal parts of the last year’s growth (figs 1A, 1C, 2A). They are inserted solitary in the axial of a typical needle-leaf (figs 1A, 1C). The pollen cone is surrounded by 13-17 small persisting bud scales (fig. 2C, 2 E). The majority of the bud scales are small triangular, green and about 1 mm long and 1 mm wide (figs 2C, 2E ). Only the inner bud scales can sometimes show the appearance of a typical needle-leaf (figs 1E, 1F), however they are strongly reduced in size. Ripe cones are erect and between 10-25 mm long and 2-2.5 mm wide (fig. 2A). The stalk is between 5-8 mm long and 1-1.5 mm in diameter (figs 2C, 2D). The cones consist of 83-126 densely spirally set hyposporangiophore microsporangiophores (fig. 2B). Perisporangiophore microsporangiophores were not found. Microsporangiophores are developed even shortly below the apex and the apex can still be recognised as a small tip (figs 4A, 4B, 4C, 4D). Even the most distal microsporangiophores are fertile (fig. 3D). In one of the investigated cones the cone-axis terminates with a microsporangiophore (figs 4E, 4F). The scutellum of this terminal microsporangiophore is quite short and roundish, peltate-like (figs 4E, 4F). The vascular bundle strand of the cone-axis terminates blindly in the distal part of the scutellum of the terminal microsporangiophore (figs 4E, 4F).
A typical microsporangiophore consists of a central stalk about 1 mm long, 2 abaxial sporangia each about 0.4-0.5 mm in diameter and an adaxial green more or less triangular, slightly serrate, upright scutellum 0.5-0.7 mm long and 0.8-1 mm wide (fig. 3A, 3B, 3C). The ripe sporangia are parallel to the central stalk of the microsporangiophore (fig. 3A). The scutellum of the most distal microsporangiophores is strongly elongated, and about 1.1-1.8 mm long and 0.8-1 mm wide (figs 3D, E). Especially in the scutellum of the most distal sporangiophores a huge resin duct is developed (fig. 4E). The microsporangia and the scutellum are strongly fused with each other (figs 3C, 3E). The scutellum is attached in an angle of nearly 80° to the stalk and also fused with the sporangia (fig. 3C). In young cones, the scutellum of the microsporangiophores are covering the sporangia of the more distal microsporangiophores. Before anthesis nearly only the phylloid part is visible externally. The cone-axis elongates strongly at anthesis and the microsporangia are freely exposed to the airflow. Within the sporangiophore no intercellular spaces are developed (figs 3C, 3E).

For better pollen release microsporangiophores diverge distally from each other at anthesis. At pollination time each microsporangium opens along a median, longitudinal preformed line (fig. 3A left) and releases the pollen grains over a period of 7-13 days. The release of the pollen starts even when the cone is not already completely open. The majority of pollen grains are collected first on the upper surface of the lower microsporangiophores especially on the scutellum. From here the pollen is taken secondary by the airflow, when the cone-axis has reached its final length. After releasing the pollen the cones dry out and are abscised as a unit.

The majority of cones are unbranched. Only some pollen cones are branched structures, showing 1-2 lateral axillary units in the basal part (fig. 2B). Each of these lateral units is inserted in the axil of a green triangular bud scale of the terminal cone. The lateral units are originated from a separate bud (figs 1E, 1F). Thus also at the base of the lateral cones persisting bud scales develop (figs 2E, 2F). In the investigated cones only the most basal bud scales were fertile – the distal ones were always sterile. The vascular bundle strand of the lateral cone and the fertile bud scale enter the vascular bundle strand of the axis of the terminal cone in separate strands (fig. 2F). They do not fuse. In the anomalous branched pollen cones the development of the terminal pollen cone is hurrying ahead to the lateral cones.

3.2 Morphology of pollen
The pollen grains are bisaccate (figs 5A, 5B, 5C). Their overall length including the sacci varies between 40-50 µm. The corpus is elliptic and varies between 27-36 µm x 20-26 µm (fig. 5C). The sacci are broadly attached at the corpus (figs 5A, 5B). They are between 15-20 µm in diameter and between 10-12 µm in height. The two sacci are attached at an obtuse angle to the corpus, ranging between 110°-130° (fig. 5A). The outer surface of the sacci is covered with several tiny papillae and has several perforations (figs 5A, 5B, 5E). The corpus has a strongly rugulate, thick sculpturing without perforations (figs 5C, 5D). The leptoma is 18-24 µm long and 8-13 µm wide. Its surface is fossulate. Perforations are absent (figs 5B, 5F).

4 Discussions
In its vegetative parts *Podocarpus gnidioides* differs significantly from the other taxa of the subclade Australis especially in some morpho-anatomical characters of the leaf, e.g. by forming a double layered hypodermis and lacking of hypodermal fibres between the abaxial stomata rows (KNOFF et al. 2011). When regarding the morpho-anatomical data of the pollen cones *Podocarpus gnidioides* complies with those of the other taxa of subclade Australis. Despite the relative isolated systematic position of *Podocarpus gnidioides* among the other New Caledonian *Podocarpus* species, its pollen cones do not have special features that are exclusively presented in this taxon. They show all features typical for pollen cones among *Podocarpus*. 

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Typical pollen cones of *Podocarpus gnidioides* are unbranched structures limited in growth and carrying several hyposporangiate sporangiophores, which are inserted directly at the cone-axis as is also the case in all other *Podocarpus* species (e.g. KRÜSSMANN 1983, DALLIMORE & JACKSON 1966, MUNDRY 2000, MUNDRY & MUNDRY 2001, FARJON 2010, DÖRKEN et al. 2011) and in nearly all other coniferous taxa. Most authors regard the coniferous microsporangiophores as microsporophylls and thus, unbranched pollen cones fulfill the definition of “flowers”. However, also some of the investigated pollen cones of *Podocarpus gnidioides* had a branched structure developing 1 or 2 lateral units in the basal part, each of them developed from different buds, so that the base of each lateral cone-axis is also surrounded by bud scales. This is a great difference to other coniferous taxa showing branched pollen cones e.g. *Cephalotaxus* (Taxaceae). In *Cephalotaxus* the whole “inflorescence” is developed from a single bud. Thus, bud scales are only developed at the base of the stalk of the complete cone.

The microsporangiophores of *Podocarpus gnidioides* differ from other coniferous microsporangiophores by lacking intercellular spaces, which are distinctly developed for example in microsporangiophores of *Pinus* (MUNDRY 2000). The change in the orientation of maturing microsporangia and their final position is quite different among recent conifers, sometimes even within a genus. MUNDRY (2000) has shown that in *Podocarpus macrophyllus* the young developing microsporangia are first orientated parallel to the stalk, perhaps due to a lack of space within the young, developing cone. In late ontogenetic states, when the cone-axis elongates, the sporangia turn in a more or less vertical position to the stalk. The orientation of ripe microsporangia of *Podocarpus gnidioides* differs strongly from *Podocarpus macrophyllus*. In *Podocarpus gnidioides* even the ripe sporangia are orientated parallel to the central stalk of the microsporangiophore. In this respect *Podocarpus gnidioides* is quite similar to *Pinus*. In *Pinus* young sporangia are developed parallel to the central stalk and keep this position also at maturity. It seems that in *Podocarpus gnidioides* this position is caused due to the lack of space even within ripe cones. For a successful release of the airborne pollen a vertical position of sporangia as developed in *Podocarpus macrophyllus* is more favourable. The microsporangia open along a preformed longitudinal line which is also more or less vertical to the central stalk and thus well exposed to the airflow. Thus a huge amount of pollen can be released by the microsporangia. In this respect the parallel orientation of microsporangia as developed in *Podocarpus gnidioides* (fig. 3A) or *Pinus* is not so favourable. The sporangia also open along a longitudinal preformed line, which is developed, however, parallel to the central stalk of the sporangiophore (fig. 3A) and therefore in some parts deeply placed within the cone. Thus, only a small amount of pollen can be released from the sporangia by the wind. In taxa with parallel orientated microsporangiophores the pollen is presented secondary. The released pollen is first collected especially on the adaxial side of the scutellum of the lower microsporangiophores. From here the pollen is taken by the wind.

The number of microsporangiophores in pollen cones of *Podocarpus gnidioides* (83-126) is slightly lower compared to closely related taxa. In this respect, pollen cones, e.g. of *Podocarpus totara* with 100-120 microsporangiophores, are quite similar to *Podocarpus gnidioides* (WILSON & OWENS 1999). In other *Podocarpus* species with large pollen cones the number of inserted microsporangiophores can reach up to 284 (SCHULZ et al. 2014). Thus, with only 83-126 microsporangiophores per pollen cone *Podocarpus gnidioides* has one of the smallest pollen cones among recent *Podocarpus* species.

Each microsporangium produces several bisaccate pollen grains as in all other *Podocarpus*-species (e.g. SPORNE 1965; TOMLINSON et al. 1991; OWENS et al 1998; GELBART & VON ADERKAS 2002; FERNANDO et al. 2010; LESLIE 2010). The bisaccate pollen grains correlate well with the downward facing micropyles and secretion of a pollination drops. The sacci help the pollen grains floating upward in the pollination drops. Thus the pollination mechanism in *Podocarpus* is similar to several Pinaceae, e.g. *Cedrus*, *Pinus* or *Picea*, albeit with a different seed cone morphology (e.g. SPORNE 1965; TOMLINSON et al. 1991; OWENS et al. 1998; GELBART & VON ADERKAS 2002; FERNANDO 2010; LESLIE 2010).
Podocarpus pollen grains are quite variable especially concerning the overall length, size, shape and sculpturing of the corpus and the width of the leptoma. Compared to pollen grains of other taxa among the subclade Australis the pollen of Podocarpus gnidioides with an overall length of 45 µm (average), a corpus length of 23 µm (average) and a leptoma 10.5 µm (average) in width are significantly smaller. Only the pollen grains of the New Zealand species Podocarpus acutifolius are quite similar to Podocarpus gnidioides in their dimensions (overall length 54 µm, corpus length 26 µm; width of the leptoma 13 µm) (POCKNALL 1981). The feature of a strongly rugulate sculpturing of the corpus is quite common among the taxa of subclade Australis, e.g. the New Zealand species Podocarpus acutifolius, Podocarpus hallii and Podocarpus totara (POCKNALL 1981). However this feature is also developed in not closely related Podocarpus-species such as the south American Podocarpus nubigena and Podocarpus parlatorei (DEL FUEYO 1996). Also the perforations in the surface of the sacci are not a specific feature of Podocarpus gnidioides or exclusively developed among taxa of the subclade Australis. Such perforations are also developed in other not closely related taxa as e.g. Podocarpus nerifolius, Podocarpus gracilior or Podocarpus macrophyllus (e.g. VASIL & ALDRICH 1970; TIWARI et al. 2012). Thus, the pollen of Podocarpus gnidioides does not show features that are exclusively developed in this taxon.

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6 References

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References


Fig. 1: *Podocarpus gnidioides*, young pollen cones and foliar details.

**A:** Habitus of a 2-years-old individual produced from cuttings collected from a 10 years old individual. **B:** Detail of a lateral shoot with the typical densely arranged needle-leaves. **C-F:** Young pollen cones developed at the individual illustrated in A. **C & D:** Unbranched pollen cone; the terminal pollen cone is surrounded by bud scales. **D:** Detail of C. **E & F:** Compound pollen cone; the terminal and the lateral pollen cone originate from different buds. **F:** Detail of E.
Fig. 2: *Podocarpus gnidioides*, morphology and anatomy of ripe pollen cones.

**A:** Pollen cones are erect at anthesis. **B:** Detail of a branched pollen cone. **C:** Base of an unbranched pollen cone with several persisting bud scales. **D:** Cross section of C. **E:** Base of a branched pollen cone; in one of the bud scales a lateral cone is inserted. **F:** Cross section of E.
Fig. 3: *Podocarpus gnidioides*, morphology and anatomy of ripe pollen cones and ripe microsporangiophores.

A: Microsporangiophores in abaxial (left), adaxial (middle) and lateral view (right); sporangia are parallel to the central stalk. B: Microsporangiophores in the middle of the cone have a small scutellum. C: Cross section of a microsporangiophore in the middle part of the cone. D: In the distal part of the cone the scutella are strongly elongated. E: Cross section of a distal microsporangiophore.
Fig. 4: *Podocarpus gnidioides*, details of the cone tip.

A-D: Typical pollen cones; the apex of the cone-axis is still recognizable; microsporangiophores are developed even shortly below the apex. A & B: Details with SEM. C & D: Longitudinal microtom sections of the distal part. E & F: Anomalous pollen cone terminating with a microsporangiophore; the scutellum rest is more or less peltate-like and carries two microsporangia (MS); the vascular bundle of the cone-axis (marked with arrows) ends blindly in the distal part of the scutellum. F: Detail of E; the sporangia of the terminal microsporangiophore are both fertile.
Fig. 5: *Podocarpus gnidioides*, pollen-morphology.

**A-C**: Overview of a bisaccate pollen grain. **A**: Equatorial view. **B**: Distal view. **C**: Proximal view. **D**: Detail of the corpus; surface strongly rugulate. **E**: Detail of the saccus; surface with several tiny papillae and perforations (some marked with arrows). **F**: Detail of the leptoma; surface fossulate.
Fig. 6: *Podocarpus gnidioides*, details of pollen cones and leaves.  
A: Ripe pollen cones at anthesis. B: Young leaves are bright green.  
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Fig. 7: *Podocarpus gnidioides* at its natural New Caledonian habitat on Mont-Dore, SE of Nouméa, at about 770 m above sea level; all photos taken in December 1995 by HN.

A: Mature shrub about 1.5 m high. B: *Araucaria muelleri* is also native in the same habitat. C: Foliar details of *Podocarpus gnidioides*. D: Massive erosion of the soil caused by human activities is one of the main problems threatening *Podocarpus gnidioides*. E: Ripe seed cone carrying one seed. F: Young maturing seed cones, each with two seeds and an intensively red colored receptaculum.