COMPARATIVE RHIZOME ANATOMY OF SOME SPECIES OF *CERADENIA* L.E. BISHOP AND *ZYGOPHLEBIA* L.E. BISHOP (POLYPODIACEAE, FORMERLY GRAMMITIDACEAE) FROM MADAGASCAR

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Abstract. Rhizome anatomy of 2 species of *Ceradenia* and 2 species of *Zygophlebia*, mainly Malagasy endemics, was studied in detail, and compared with preliminary results previously achieved in 2 other *Zygophlebia* species from Madagascar. Stele architecture was reconstructed in each species, with two sketches (a cross section and a splitting-out diagram), thus providing anatomical features and allowing a discussion about their relevance for distinguishing the two genera. Especially, we emphasize that *Ceradenia* differs from *Zygophlebia* by lacking any accessory ventral gap, but that character has a value only in combination with the occurrence of whitish waxy hairs in the sori. On the other hand, histology provides few correlated results, due to a narrow sampling, but tracheids lumen appears in transverse section sinuate in *Ceradenia*, rounded in *Zygophlebia*. Rhizotaxis was analyzed, revealing a more or less helical insertion of roots, along meridians in definite number, mainly crowded at the ventral rhizome side, and whose divergence angle seems to be little altered by environmental constraints. Phyllopodial divergence angles are constant too. All these geometrical data may be useful for characterizing species, or species groups, but not at a generic level. They appear however not tightly correlated to rhizome size and might have an adaptive significance.

Key words: *Ceradenia, Zygophlebia,* Grammitidaceae, Polypodiaceae, Madagascar, accessory stele gaps, comparative anatomy of rhizome, rhizotaxis

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Introduction

Rhizome anatomy, especially vasculature, was proved to be of the greatest taxonomic and phyletic importance in the former Grammitidaceae by Bishop (1988, 1989), as moreover in all homosporous ferns as recently restated by Srivastava & Chandra (2009). In this context the generic distinction between *Zygophlebia* and *Ceradenia* was discussed by Rakotondrainibe & Deroin in 2006, by considering the occurrence of accessory gaps in the stele, as well as rhizo- and phyllotaxis. The present study extends this approach to 4 other species growing in Madagascar and previously included in the genus *Ctenopteris* Blume ex Kunze.

Material and methods

The following specimens were examined in this study: *Ceradenia alboglandulosa* (Bonap.) Parris:

MADAGASCAR, Province d'Antsiranana, Parc National de Marojejy, 13 km SE de Doany, alt. 1150-1300 m, 25.X.2001, *Rakotondrainibe 6402* (P [P00244972]); *C. deltodon* (Baker) Parris: MADAGASCAR, Province d'Antsiranana, Andapa, forêt d'Analabe (massif d'Anjanaharibe-Sud, versant W), 13 km SW du village de Befingotra, alt. 1660-1680 m, 6.XI.1999, *Rakotondrainibe 5170* (P [P00181162]); *Zygophlebia humbertii* (C. Chr.) Parris: MADAGASCAR, Province de Fianarantsoa, Parc National de l'Andringitra, forêt d'Anjavidilava, alt. 1950-2000 m, 19.XI.2004, *Rakotondrainibe 6954* (P [P00411830]); *Z. torulosa* (Baker) Parris: MADAGASCAR, same place and date, *Rakotondrainibe 6937* (P [P00411843]).

Rhizome fragments (Fig. 1) were removed from herbarium sheets and processed as previously described (Rakotondrainibe & Deroin 2006), i.e. restored by a 10% aqueous
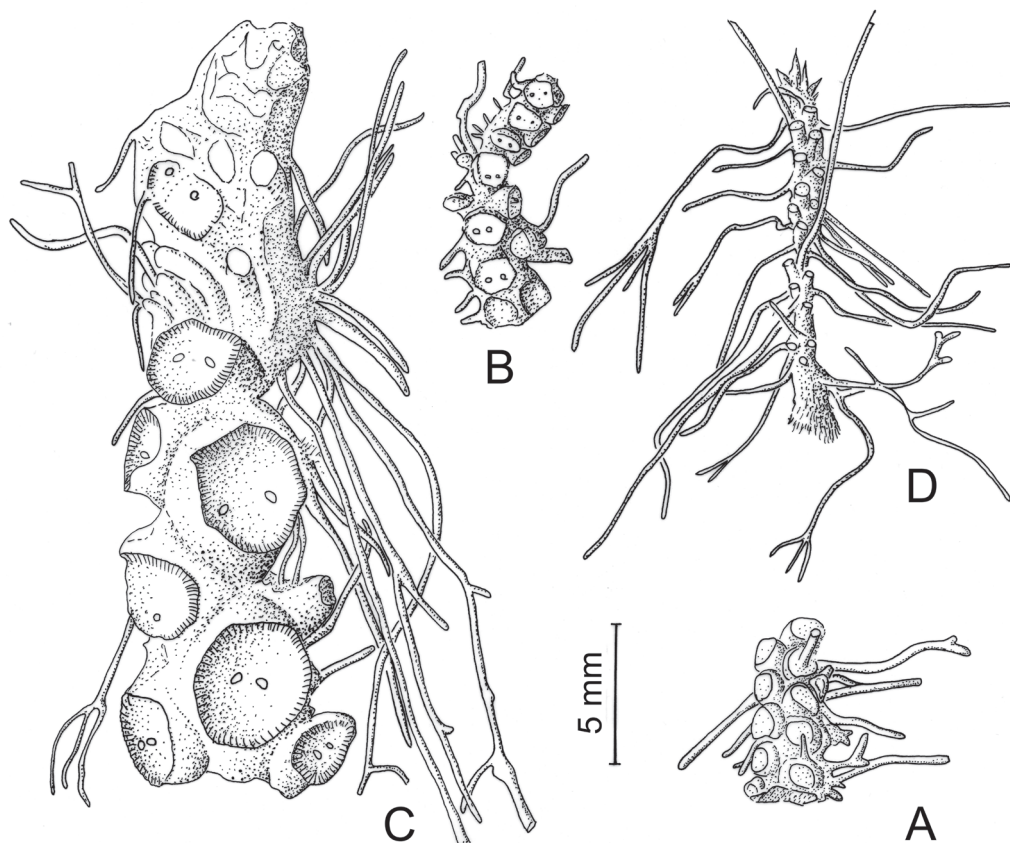
Fig. 1. External rhizome morphology (dorsal view): **A** – *Ceradenia alboglandulosa*; **B** – *C. deltodon*; **C** – *Zygophlebia humbertii*; **D** – *Z. torulosa*.

solution of NH_4OH at 60° C, then paraffin embedded (Gerlach 1984) and cut at a thickness of 15 (sometimes 25 or 50) µm. Some slides were stained by aqueous Astrablue 0.5% and Ziehl's Fuchsine 10%, a major part was left unstained as far as conducting tissues are well distinguishable. Mountings are kept in the plant histological collection of the Museum (Dept. Systematics and Evolution) under the numbers *Deroin 180, 181, 182* and *183* respectively. Reconstruction of the steles was made by superimposing tracing papers of cross sections drawn by a camera lucida.

### Results

Comparative rhizome anatomy

Tissues are described in cross section, from outside to the centre.

Ceradenia alboglandulosa (Fig. 2 A):

Epidermal cells rounded not very papillose, with an ornamented cuticle; cortex with two zones: an outer one of 2-3 rows of quadratical cells, wholly cutinized, with a resinous content; an inner zone of ca. 7 rows of somewhat wider rounded-polygonal cells, sclerified and including mucilage; endodermis composed of narrow quadrate cells, without oxalate crystals and with rather inconspicuous Casparian stripes; pericycle of 2-3 rows of cells including large nuclei; phloem narrow of 1-3 rows, continuous all around the siphonostele; xylem with a concentric structure, showing a peripheral xylem parenchyma (named "tissu conjonctif" in Lachmann 1889) of 1-2 rows of somewhat oval cells, and tracheids equally lignified, with deeply sinuate lumen outline, with outer protoxylem poles; pith like the inner cortical zone, but with some scattered pitted sclereids.
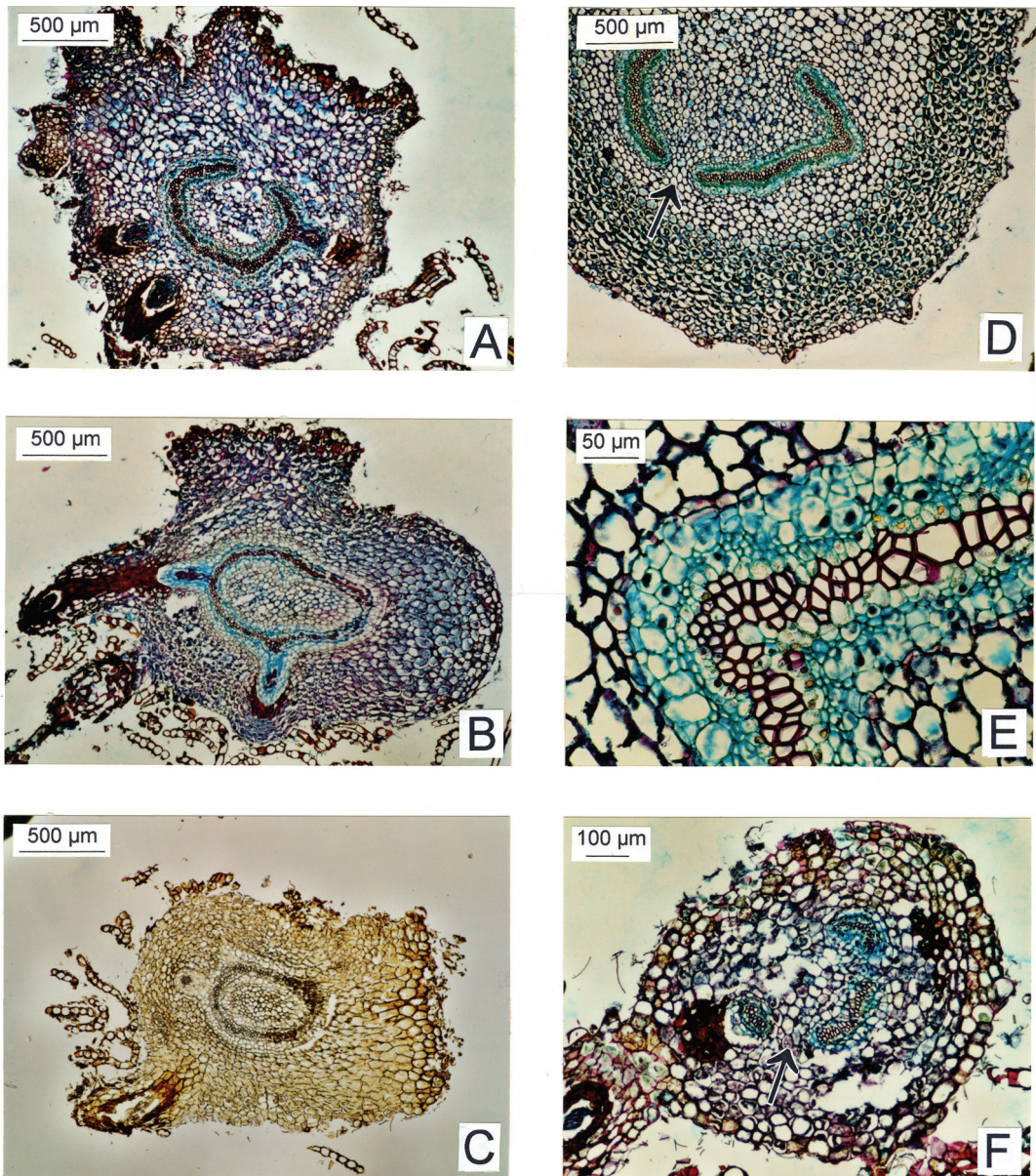
Fig. 2. Rhizome anatomical features: **A** – *Ceradenia alboglandulosa* (*Rakotondrainibe 6402*); **B, C** – *C. deltodon* (*Rakotondrainibe 5170*); **D, E** – *Zygophlebia humbertii* (*Rakotondrainibe 6954*); **F** – *Z. torulosa* (*Rakotondrainibe 6937*). **A, B, C, F** – cross section (dorsal side at the top); **D** – portion of rhizome cross section; **E** – detail of the stele. Accessory ventral gap is arrowed. Stained by the combination of aqueous Astrablue 0.5% and Ziehl's Fuchsine 10%, except for cross section **C** left unstained.

Ceradenia deltodon (Fig. 2 B, C):

Epidermal cells quadratical, with cutinized walls; cortex composed of wholly lignified, oval or rounded, cells, divided into two zones: the outer 2-3 rows with a clear content, the inner 6-8 rows almost wholly mucilaginous; endodermis as in *Ctenopteris alboglandulosa*; pericycle composed of 2 rows outside, but until 8 rows thick in the inner side of the stele; phloem narrow of 1-2 rows, discontinuous

inside; peripheral xylem parenchyma uniseriate outside, discontinuous inside the siphonostele; tracheids arranged in 1-3 rows, equally lignified with sinuate lumen outline; pith like the inner cortical zone, but mucilaginous cells scarcer.

Zygophlebia humbertii (Fig. 2 D, E):
Epidermal cells rounded and wholly cutinized; cortex with lignified walls showing two zones: an outer one with ca. 16 rows of rounded, both mucilaginous and tanniniferous, cells, an inner zone of ca. 4 rows of somewhat smaller cells, most frequently without any stained content; endodermis well visible outside the stele, discontinuous inside; pericycle composed of 1-2 rows of mucilaginous cells including large nuclei; phloem of 1-3 rows discontinuous by places, especially in the inner side of the stele; peripheral xylem parenchyma uniseriate and continuous, composed of quadratical cells with large nuclei; tracheids polygonal, sometimes very wide, equally lignified with a rounded lumen outline, with outer protoxylem poles; pith similar to the inner cortical zone.

Zygophlebia torulosa (Fig. 2 F):
Epidermal cells rounded, very large, wholly cutinized; cortex homogeneous composed of 7-8 rows of wholly lignified oval or quadrate cells with a resinous or mucilaginous content; endodermis not differentiated (merged in cortex); pericycle uniseriate and including large nuclei; phloem of 1-2 rows but lacking by places especially inside the stele; peripheral xylem parenchyma as in *Z. humbertii*; xylem very thin composed of 1-2 rows of tracheids equally lignified with a rounded lumen outline; pith like the cortex.

Comparative stele anatomy
The rhizome dorsiventrality is obvious in external morphology (Fig. 1), and clearly expressed in the stele structure. Accordingly, phyllopodes are alternately inserted on the upper (dorsal) side (Fig. 3 A-D) and basically supplied by two traces from the borders of gaps, somewhat narrow in *Ceradenia alboglandulosa* and *C. deltodon* (Fig. 3 A, B), larger and broader in *Zygophlebia humbertii* (Fig. 3 C), and spread

to a maximum in *Z. torulosa* (Fig. 3 D). It is noticeable that in this last species, overlapping gaps result in a discontinuous stele in cross section (Fig. 2 F), with the both phyllopode traces inserted in the upper margin, and frequently wholly fused. Lateral buds are not constantly present, and fed by a single bundle from the posterior (upper in *Z. torulosa*) margin of the gap, and which does not appear double in origin as in *Zygophlebia goodmanii* and *Z. anjanaharibensis* (Rakotondrainibe & Deroin 2006). These buds should be here interpreted as extraxillary, as previously reported in polypodiaceous ferns *sensu stricto* by Hovenkamp (1990).

On the lower (ventral) side of the rhizome, the roots are randomly arranged along four (*Ceradenia deltodon*, Fig. 3 B, B') or five (*C. alboglandulosa, C. humbertii,* and *Zygophlebia torulosa*: Fig. 3 A, A', C, C', D and D') meridians. Accessory ventral perforations are lacking in *Ceradenia alboglandulosa* and *C. deltodon*, while they are recognized upon two patterns, *i.e.* in *Zygophlebia humbertii* as small rounded perforations between the lowest meridians (Fig. 3 C) like in *Zygophlebia goodmanii* (Rakotondrainibe & Deroin 2006), or in *Z. torulosa*, as somewhat stretched oblique perforations interrupting two meridians (Fig. 3 D).

Divergence angles were measured between root ($α_r$) and phyllopodial ($α_p$) meridians (Fig. 3 A'-D'), and may be compared to those already established in *Zygophlebia anjanaharibensis* Rakotondrainibe and *Z. goodmanii* Rakotondrainibe (Fig. 3 E, F).

Discussion

We focus here on two questions:

Ventral gaps
As previously seen in the grammitid ferns of Polypodiaceae s.l. (Bishop 1988, 1989; Rakotondrainibe & Deroin 2006), accessory gaps may occur or not at the ventral side of the rhizome stele. When present (*Zygophlebia goodmanii, Z. humbertii* and *Z. torulosa*), they appear neither wide nor
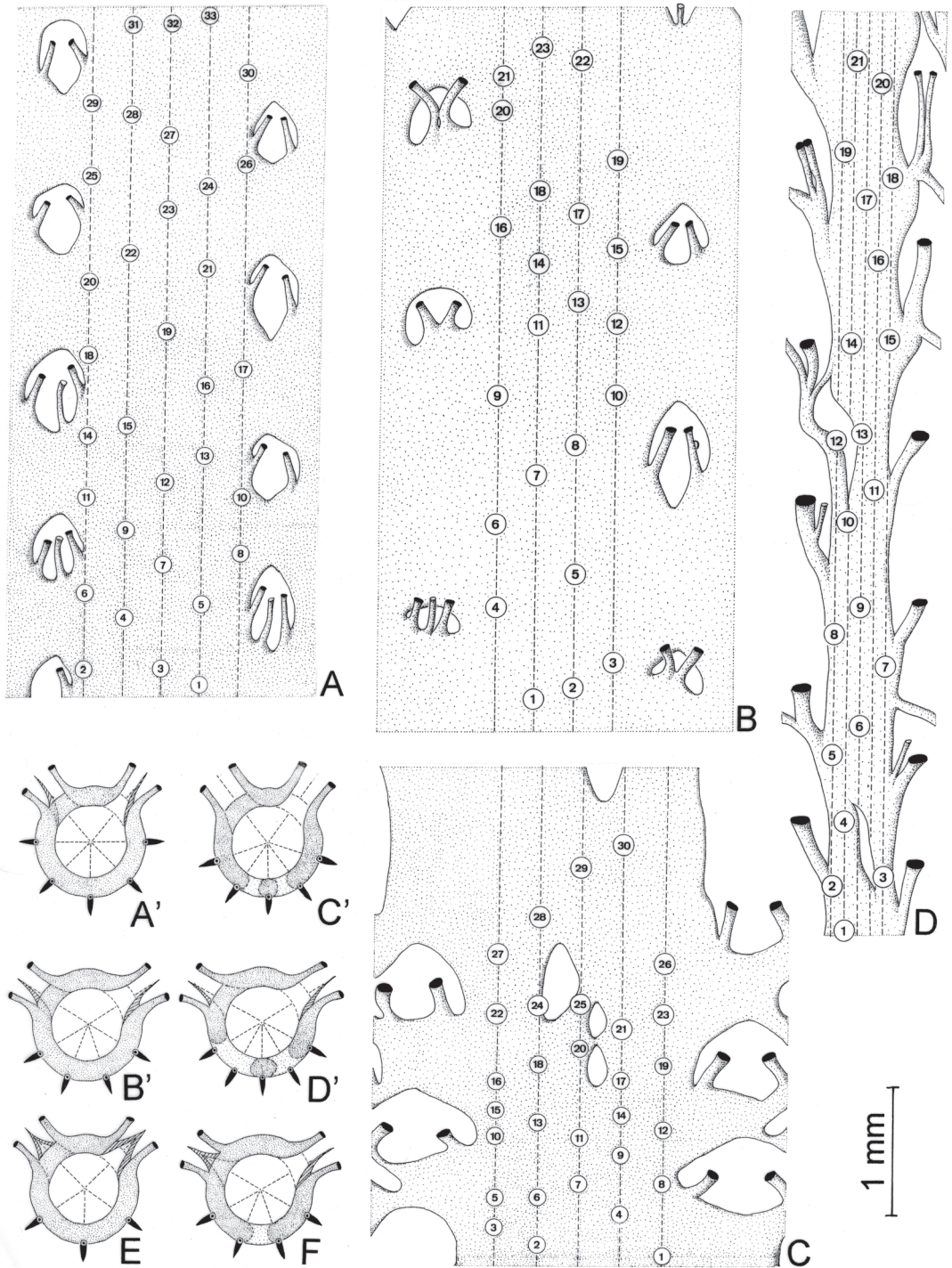
Fig. 3. Rhizome vascular skeletons: **A-D** – developed diagrams of the stele (ventral side in the middle, root insertions numbered) for *Ceradenia alboglandulosa, C. deltodon, Zygophlebia humbertii* and *Z. torulosa* respectively; **A'-D'** – corresponding diagrammatic cross sections of the stele (roots in black, phyllopodial traces with black tips, bud vasculature hatched); **E-F** – same for *Zygophlebia anjanaharibensis* and *Z. goodmanii,* after Rakotondrainibe & Deroin (2006).

regularly alternate, although more or less lying between the same root meridians (Fig. 3 C, D). These perforations are not so spread as in *Polypodium lasiostipes* Mett. (= *Ctenopteris lasiostipes* (Mett.) Brownlie; Ogura 1938: 59), in which we observe two files of alternates oblong gaps, spreading along two internodes. Interestingly Ogura's illustrations of *Polypodium* also show other grammitid species, where the rhizome fails to exhibit any ventral perforation such as *Polypodium setigerum* Bl. (= *Radiogrammitis setigera* (Blume) Parris), or *P. zeylanicum* (Fée) Mett. (= *Oreogrammitis zeylanica* (Mett.) Parris).

The meaning of these accessory gaps was recently discussed in detail by Sundue (2010: 720), who suggested that *"Plants lacking perforations generally had small rhizomes"*. Such a hypothesis should be moreover checked by a developmental study of the vasculature in some selected species, in order to bring to the fore any correlation between occurrence of ventral gaps, stele diameter and rhizome diameter. Whatever their right significance and possible role, these gaps seem too much variable for distinguishing the genera, but they might be of interest for defining some species groups, or clades. In fact several states are to be described for this character in grammitids: absent in *Ceradenia alboglandulosa*, *C. deltodon*, and *Zygophlebia anjanaharibensis* (doubtful as far as the available rhizome segment was very short), few spreading in *Zygophlebia humbertii*, *Z. torulosa*, and *Z. goodmanii*, strongly perforated in *Ctenopteris lasiostipes*, or even wholly dissected – *i.e.* modified in a meshwork of thin bundles – (as in *Platycerium alcicorne* Desv. cited by Tansley 1907: 189, much more references to this highly dissected dictyostele might be given indeed for Polypodiaceae in recent time). Our sampling is not enough to find correlations with other features, especially histological ones. For example we observed here that ventral perforations seem to be correlated with a rounded – not sinuate – lumen outline in tracheids, most likely due to absence of a wall thickening, However, cell wall ornamentation cannot be fully understood without an examination of longitudinal sections, as demonstrated by Leroux *et al.* (2011) for the cortical root cells of Aspleniaceae, which exhibit a similar problem, even if helical thickenings are not lignified there. Probably other relations are to be looked for with details of the xylem and phloem arrangement. Conversely, the rhizome cortex is rather thick and lignified in all studied species, and provides no other distinctive feature.

Root insertion on the stele

Fern rhizotaxis was rather overlooked in the past and our knowledge about it remains patchy. It was considered when vascular skeletons were studied after a careful removal of cortical and medullary tissues (Lachmann 1889), but a pulling out of some weak root insertions could not be avoided. In many cases, only outer rhizome morphology was described, with the shortcoming that only visible root insertions are quoted. Although this character is easy to code (Sundue 2010: 727), it appears unsatisfactory, as fern roots go through the cortex along complex – and almost never straight – pathes. However, the outer rhizome morphology provides a first and coarse approach of the rhizotaxis. Thus, crowding of the root insertions at the ventral side and reduction of their number were properly quoted by Wetter (1952: 56), *e.g.* for the genus *Stenochlaena*, in accordance with a creeping or climbing habit, the same brought to the fore in our study (Fig. 1). Frequently, roots were wholly forgotten, and authors focused on phyllopodial insertions and branching pattern, outer features which may be more closely linked to the habit (Hovenkamp 1990). Strikingly rhizotaxis was still neglected in a recent analysis of the comparative rhizome anatomy of four *Polypodium* species (Srivastava & Chandra 2009), and consequently no root insertion was drawn in the stele sketches.

For our studies, we benefited from long and well-preserved rhizome fragments, and the use of paraffin cross sections allowed a detailed reconstruction of the stele both in sizes and the true anatomical insertion of roots. Moreover, we chose to split up the stele dorsally so that

we got a full view of the ventral side with root meridians and possible accessory gaps. Thus roots are obviously arranged along meridians, and demonstrate in some places a kind of helical arrangement (Fig. 3, *e.g.* in A: 6-9-12-13, and 20-22-23-24-26; B: 6-7-8-10; C: 3-6-7-9-12, and 16-18-20-21-23; more chaotic in D), modified by dorsiventrality. The most useful character is probably the divergence angle of root meridians (Tab. 1: α_r), which might be less altered by environmental constraints. The diagrams showing the relation between phyllopodial angles, accessory gaps and buds (Fig. 3 A'-D', E, F) may be very useful for an extensive comparative anatomy of fern rhizome, and its interpretation at the phylogenetical and adaptive levels.

Conclusion

After the Tab. 1, the genera *Ceradenia* and *Zygophlebia* appear to be anatomically distinguished in Madagascar by the lack/ presence of accessory stelar perforations, a very weak distinction within the grammitids, as shown by Sundue (2010: 720), but confirmed by the presence/lack of whitish waxy hairs in the sori (Bishop 1988, 1989), and possibly the sinuate/rounded lumen outline of the tracheids (this study). As we suggested (Rakotondrainibe & Deroin 2006), additional gaps are not recognized in *Zygophlebia anjahanaribensis* most likely because it was not possible to study a sufficient length of rhizome in such a rare species (thus the absence of gap is not wholly demonstrated in this *Zygophlebia* species, as claimed by Sundue (2010: 720)). These genera are rather similar by their rhizome histology, and even by the stele habit (see *Ceradenia deltodon* and *Zygophlebia goodmanii*, Fig. 3 B' and F), thus strengthening the cladistic analysis of Sundue (2010: 721-2), mainly based on neotropical taxa, in which *Zygophlebia* appears moreover closest to a third genus: *Enterosora* Baker. This last result was confirmed by Sundue *et al.* (2014: 201), a phylogenetical study encompassing much more palaeotropical taxa.

Tab. 1. Comparative rhizo- and phyllotaxis data in the studied species of *Ceradenia* and *Zygophlebia*: * after Rakotondrainibe & Deroin (2006); ** available rhizome piece too short for any definitive conclusion.

Features	*Zygophlebia anjanaharibensis**	*Z. goodmanii**	*Z. humbertii*	*Z. torulosa*	*Ceradenia alboglandulosa*	*C. deltodon*
Accessory stelar perforations	-**	+	+	+	-	-
Root meridians						
Number	3	4	5	5	5	4
α_r	55°	40°	36°	30°	45°	40°
α_p	90°	120°	72°	120°	80°	120°
Traces number of lateral bud (when any)	2	1-2	?	1	1	1

No simple correlation appears for quantitative features such as those concerning rhizotaxis (especially root meridians number), especially with the stele diameter (compare *e.g. Zygophlebia humbertii* and *Z. torulosa*, Fig. 3 C and D), with the same meridians number (5).

Conversely, the range of phyllopode divergence angles (α_p) spreads around ca. 50°, and might be a rather good measure of the rhizome dorsiventrality. So the most dorsiventral rhizome should be *Zygophlebia humbertii* with the lowest angle (Tab. 1, α_p=72°). Traces number of lateral bud is too variable and cannot be used (Tab. 1).

The root divergence angle: α_r is in major part linked both to the dorsiventrality and the meridians number (M). Interestingly (Tab. 1), the whole root insertion zone covers approximately the same angle: (M-1) α_r, like phyllopodes (α_p), in *Zygophlebia goodmanii*, *Z. torulosa*, and *Ceradenia deltodon* (120°), or even *Z. anjanaharibensis* (110° instead of 90°), but is much more larger in the most dorsiventral rhizomes of *Zygophlebia humbertii* and *Ceradenia alboglandulosa* (144° *vs.* 72°; 180° *vs.* 80°, respectively).

However the right value of anatomical characters needs to be assessed on a wider study, involving much more taxa, especially the genus *Enterosora*.

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