Morphology, Development and Regeneration of *Thelethylax minutiflora*, a Madagascan River-weed (*Podostemaceae*)

By

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With 34 Figures

Summary

GROB V., PFEIFER E. & RUTISHAUSER R. 2007. Morphology, development and regeneration of *Thelethylax minutiflora*, a Madagascan river-weed (*Podostemaceae*). – Phyton (Horn, Austria) 47(1–2): 205–229, with 34 figures. – English with German summary.

Thelethylax (2 species) is one of three podostemoid genera, which are endemic to Madagascar. This paper deals with the structure and development of *T. minutiflora*. Characters typical for this species include: green ribbon-like roots have exogenous finger-like holdfasts; root tips are covered with prominent dorsiventral caps; shoots arise mainly from endogenous buds along roots; shoots are dimorphic: vegetative shoots have repeatedly forked leaves, reproductive shoots are reduced to 2–4 scales and a terminal flower. *Thelethylax* also shows endogenous flower formation along old stem portions, due to dedifferentiation of cortex tissue. Silica bodies as present in many *Podostemaceae* are absent or rare in *T. minutiflora*. *Thelethylax* and other Madagascan podostemoids (= *Thelethylax* & al.) appear to be basal to all podostemoids from continental Africa, whereas *Thelethylax* & al. are derived from Asian and/or American podostemoids (MOLINE & al. 2007). Thus, exact informations about architecture and development of *Thelethylax* will help to better understand character evolution from the American and Asian *Podostemaceae* towards the African taxa. Complete or partial floral inversion in bud stage is found in most African

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members (including *Thelethylax*), but lacking elsewhere. The flower buds of *Thelethylax minutiflora* are completely inverted within the sac-like spathella whereas those of *T. isalensis* are inclined only. Bilocular ovaries (as usual in America and Asia) are found in *Thelethylax* & al., whereas the ovaries are unilocular in most podostemoids from continental Africa. Pollen dyads (as found in Asian podostemoids and the American genus *Podostemum*) are observable in *Thelethylax* & al., but lost in many continental African taxa. The Madagascan podostemoids *Endocaulos* and *Paleodicraeia*, as well as *Stonesia* (tropical W Africa) show 5–7 ribs per capsule valve. Though indistinct, this pattern appears to be present in *Thelethylax minutiflora* too.

Zusammenfassung

GROB V., PFEIFER E. & RUTISHAUSER R. 2007. Morphologie, Entwicklung und Regeneration von *Thelethylax minutiflora*, einem madagassischen Blütentang (*Podostemaceae*). – Phyton (Horn, Austria) 47(1–2): 205–229, mit 34 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In madagassischen Wasserfällen und Stromschnellen kommen aus der mit *Hypericum* verwandten Blütenpflanzenfamilie der *Podostemaceae* drei Gattungen endemisch vor: *Endocaulos, Paleodicraeia* mit je einer Art und *Thelethylax* mit zwei Arten. Dieses hier als *Thelethylax* & Co. abgekürzte Gattungstrio zählt zur Unterfamilie der *Podostemoideae*. Die vorliegende Arbeit befasst sich mit dem Bau und der Entwicklung von *Thelethylax minutiflora*. Einige typische Merkmale werden mit Mikrotomschnitten und rasterelektronenmikrosopischen Bildern vorgestellt: Grüne bandförmige Wurzeln haften mit Klebhaaren und exogenen fingerförmigen Auswüchsen auf dem felsigen Substrat. Die Wurzelspitze wird mit einer asymmetrischen Haube geschützt. Wurzelbürtige Triebe entspringen paarweise aus endogenen Knospen. Die bei Hochwasser auftretenden vegetativen Triebe besitzen mehrfach verzweigte Blätter; die bei Niedrigwasser auftauchenden reproduktiven Sprösschen bestehen nur aus 2–4 Schuppenblättchen und einer Endblüte. Zusätzlich zeigt *Thelethylax* endogene Blütenbildung aus alten Stängelabschnitten, verbunden mit Dedifferenzierung von Rindengewebe. Kieselkörper (typisch für zahlreiche andere *Podostemaceae*) gibt es bei der als Salat und Gemüse essbaren *T. minutiflora* fast keine. – Die madagassischen *Podostemoideae* (= *Thelethylax* & Co.) erscheinen aufgrund molekularer Analysen (MOLINE & al. 2007) als Ausgangsgruppe für die kontinental-afrikanischen Vertreter dieser Unterfamilie; anderseits lassen sich *Thelethylax* & Co. von asiatischen und amerikanischen *Podostemoideae* ableiten. So erlaubt die hier bei *Thelethylax* gemachte morphologische Analyse auch ein besseres Verständnis der Merkmalsprogressionen, die von den Amerikanern und Asiaten zu den Vertretern des afrikanischen Kontinents geführt haben. Für viele Afrikaner und *T. minutiflora* charakteristisch ist eine hängende (inverse) Blütenknospenstellung im Innern der sackförmigen Schutzhülle (Spathella). Bei der Schwesterart *T. isalensis* und wenigen Afrikanern sind die Blütenknospen nur eingekrümmt (oder aufrecht), während sie bei den Asiaten und Amerikanern stets aufrecht in der Spathella stehen. Der Fruchtknoten besitzt bei *Thelethylax* & Co. und fast allen Nichtafrikanern zwei durch ein Septum getrennte Fächer. Durch Septumsverlust wird der Fruchtknoten bei vielen *Podostemoideae* des afrikanischen Kontinents einfächrig (unilokular). Für die amerikanische Gattung *Podostemum* und alle Asiaten typisch sind paarweise verbundene Pollenkörner (Pollendyaden). Diese gibt es auch bei *Thelethylax* & Co.

Mehrere afrikanische Gattungen haben die Pollendyaden wieder verloren. Innerhalb von *Thelethylax* & Co. besitzen nur *Endocaulos* und *Paleodicraeia* eine Erhöhung der Rippenzahl pro Kapselklappe von den üblichen drei auf 5–7. Dieses parallel auch bei *Stonesia* (im tropischen Westafrika) aufgetretene Rippenmuster kommt bei *Thelethylax* wohl auch vor, ist aber hier nur undeutlich zu erkennen.

1. Introduction

1.1.

The *Podostemaceae* (river-weeds; c. 49 genera and c. 280 spp.) are an aquatic family of the eudicots with a highly modified bauplan. Many of them resemble algae, liverworts or mosses. They occur on all continents except Antarctica and Europe, usually restricted to tropical streams, rivers, waterfalls and cataracts with distinct seasonality, where they grow attached to rocks or other solid substrata. Hence they are haptophytes and rheophytes. During the vegetative stage, the plants are completely submerged in swiftly running water during the rainy season. Emergent flowers and fruits are formed when the water recedes during the dry season (COOK & RUTISHAUSER 2007).

1.2. *Podostemaceae* on Madagascar

The family *Podostemaceae* is represented by six different species on Madagascar (for authors to all species names see LEBRUN & STORK 1991, also RUTISHAUSER & al. 2007 under www.systbot.uzh.ch/podostemaceae). Two of them (*Sphaerothylax abyssinica, Tristicha trifaria*) are more widespread occurring also elsewhere (COOK & RUTISHAUSER 2007). Three genera (all belonging to subfamily *Podostemoideae*) are endemic to Madagascar: *Endocaulos* (monotypic) with *E. mangorense* (PERR.) C. CUSSET, *Paleodicraeia* (monotypic) with *P. imbricata* (TUL.) C. CUSSET, and *Thelethylax* (with 2 spp.). These three genera were described by C. CUSSET 1972, segregating them from the rather large genus *Ledermanniella* (syn. *Inversodicraeia*; CUSSET 1983, 1984). The two *Thelethylax* species are *T. minutiflora* (TUL.) C. CUSSET and *T. isalensis* (PERR.) C. CUSSET. Both species were known before as *Inversodicraeia minutiflora* (TUL.) PERR. (syn. *Dicraea minutiflora* TUL.) and *I. isalensis* (PERR.) PERR. respectively (PERRIER 1952). *Thelethylax minutiflora* is called "Anandriana" (in French "légume des cascades") by the Malagasy people and eaten as cress-like salad or as cooked vegetable (GAFFIER 1932).

1.3. Molecular Systematics of *Podostemaceae*

Not until recently have the relationships of *Podostemaceae* within the angiosperms been resolved. Molecular data indicate that they belong to the *Malpighiales* clade in the eurosids I group, with the families most closely related being *Clusiaceae/Hypericaceae* and *Bonnetiaceae* (GUSTAFSSON &

al. 2002, DAVIS & al. 2005). Basal members of *Podostemaceae* are (in ascending order) *Tristichoideae, Weddellinoideae* and the New World genera *Mourera, Apinagia, Marathrum* and *Oserya* within *Podostemoideae* (KITA & KATO 2001, KATO & al. 2003, MOLINE & al. 2007). KITA & KATO 2001 also published molecular data on the two Madagascan genera *Endocaulos* and *Thelethylax*. These two genera are sister to all podostemoids studied so far from continental Africa (KITA & al. 2005, MOLINE & al. 2007). The African-Madagascan clade comprising both the Madagascan as well as the continental African podostemoids is related to all Asian (including Australian) *Podostemoideae* and the American genus *Podostemum* whereas all (or most) other New World *Podostemoideae* form a monophyletic sister group to the taxa mentioned above (MOLINE & al. 2006, 2007).

1.4. Developmental Morphology of *Podostemaceae* in General

The architecture of all *Podostemaceae* is peculiar compared to that of most other angiosperms (COOK & RUTISHAUSER 2007). Unique morphological features are, for example, green ribbon-like or crustose ("foliose") roots, exogenous root branching, endogenous shoot branching, terminal leaves with two sheaths, and a sac-like spathella in which the primordial flower develops (RUTISHAUSER & MOLINE 2005, COOK & RUTISHAUSER 2007). The modified morphologies of *Podostemaceae* are probably due to their haptophytic habit and their evolutionary history in river rapids. Despite progress made in studies on architecture and comparative morphology of several members of the *Podostemaceae,* some morphological and anatomical problems are yet to be resolved. – There is still disagreement among contemporary botanists over the interpretation of the vegetative body in the *Podostemaceae* (MOHAN RAM & SEHGAL 2007). In this study, we use the term 'root' in *Podostemaceae* for a creeping organ (with or without cap) adhering to the substratum and giving rise to endogenously formed 'shoots' whereas the 'shoot' is defined as a 'stem' that carries exogenously formed 'leaves' (MOLINE & al. 2007)

1.5. Developmental Morphology of African and Madagascan *Podostemoideae*

In contrast to most American and Asian members the various architectural rules of African *Podostemoideae* are less understood. Earlier morphological and anatomical studies on African and Madagascan *Podostemoideae* were done by WARMING 1891, 1899, GAFFIER 1932, JÄGER-ZÜRN 1967, and SCHNELL 1967. There is a renewed interest in the study of comparative and developmental morphology of African and Madagascan *Podostemoideae*. For example, JÄGER-ZÜRN 2000, 2002, AMEKA & al. 2002, 2003, RUTISHAUSER & MOLINE 2005, GHOGUE & al. 2007 and MOLINE & al. 2007, gave emphasis on members of *Djinga, Ledermanniella, Saxicolella* and *Sphaerothylax*.

1.6. Aim of the Paper

Preliminary morphological data on *Thelethylax minutiflora* were provided by TULASNE 1852, PERRIER 1929, 1952, C. CUSSET 1972, G. CUSSET 1974 and JÄGER-ZÜRN 2003. The aim of the present paper is to elucidate the architecture and developmental morphology of this polymorphic species that is basal to all podostemoids studied so far from the African continent (MOLINE & al. 2007). Emphasis is given on anatomical and morphological characters showing the intermediacy of *Thelethylax* between Asian *Podostemoideae* and the American genus *Podostemum* on one hand and the genera from continental Africa on the other hand. This study will also help to better understand the molecular and morphological evolution within the podostemoid clade from continental Africa which is much more species-rich than its Madagascan sister group (MOLINE & al. 2007).

2. Material and Methods

This work is based on the study of fixed and preserved plant material deposited in Königstein (Germany: JÄGER-ZÜRN'S wet collection taken over from W. RAUH, Heidelberg) and Zürich (Z/ZT). The following specimens were used:

Thelethylax minutiflora (TUL.) C. CUSSET, synonym: *Inversodicraea minutiflora* (TUL.) PERR.: collected by W. RAUH on Madagascar: Rio Mananjary. There are two specimens: No. 7205 (collected 28 July 1965) with vegetative material, and No. M586 (collected 12 November 1965) with red shoots, flowers and fruits. Specimen No. M586 was identified as *Thelethylax minutiflora* subsp. *orientialis* PERR. Diagnostic features of this subspecies are (1) the lack of the third tepal (present in the typical subsp. *minutiflora*), and (2) the occasional presence of flattened leaves (lacking in subsp. *minutiflora*) (C. CUSSET 1972).

The plant specimens were fixed in a mixture of formalin, propionic acid and ethanol (FPA; see JÄGER-ZÜRN 1967). Preserved material stored in 70% ethanol, was used for light and scanning electron microscopy (SEM). For scanning electron microscopy the dissected plant parts were critical point dried and sputter-coated with gold. The electron micrographs were taken with a JEOL scanning electron microscope at 20 kV. For microtome sections, specimens were embedded in Kulzer's Technovit (2-hydroethyl methacrylate), and sectioned with a MICROM HM 355 rotary microtome and conventional microtome knife types C and D. The mostly 7 µm thick sections were stained with ruthenium red and toluidine blue. The permanent slides of the microtome sections are deposited at the Institute of Systematic Botany of the University of Zurich (Z).

3. Results

3.1. Roots and Holdfasts

Root architecture of *T. minutiflora* is shown in Fig. 1–6. The root tips of *T. minutiflora* have prominent dorsiventral caps (Fig. 1–3). The roots are ribbon-like (diameter 1–2 mm). They are about twice as wide as thick, with

a somewhat crescent-shaped outline, which is concave towards the sub-
stratum and convex on the dorsal side. There is a strap-like central bundle
with prosenchymatous vascular cells, with xylem and phloem hardly dis-
tinguishable (Fig. 4).

The roots are attached to the substratum usually by adhesive hairs
which arise from the ventral root side (Fig. 4). Additionally, a sticky cya-
nobacterial slime has been suggested to enhance adhesion (G. CUSSET 1974,
JÄGER-ZÜRN & GRUBERT 2000). Furthermore, the roots cling to the sub-
stratum by holdfasts, which are often finger-like. They are produced exo-
genously from the root flanks and occur in opposite pairs (Fig. 5). The
shoot buds (including floriferous shootlets) originate endogenously from
the root flanks. There is usually one shoot bud found dorsal to each hold-
fast (Fig. 5, 6).

<center>3.2. Dimorphism of Shoots and Leaves</center>

During the wet season the completely submerged root-born shoots of
T. minutiflora are elongate and provided with forked leaves but no flowers.
In the dry season, the emerging shoots are usually short and sessile, with a
terminal flower after the formation of few scaly leaves. Intermediate
shoots were already described by CUSSET 1972. All leaves lack stipules
(Fig. 11, 14).

Elongate vegetative shoots: Stem and leaf features of *T. minuti-
flora* are shown in Fig. 7–13. Vegetative shoots are simple, not branched
and up to 25 cm in length including leaves (TULASNE 1852). The stems are
terete in transverse sections. The cross-sections (with diameters of 2 mm)
were taken from 5cm long stems (Fig. 11, 12). The vascular tissue is a
slightly flattened central strand with no obvious differentiation into xylem
and phloem elements (Fig. 13). A weak leaf trace derives from the central
vascular bundle and enters the leaf (Fig. 11, 12). There is a small number
(3–6) foliage leaves which are much longer than their stem. They are 10–25
(–100) cm long, with 10–20 subunits, each of them being 10–15 cm long and
repeatedly forked into thread-like or laciniate segments (Fig. 7, 8; CUSSET
1972; see her drawings in Internet: www.systbot.uzh.ch/podostemaceae).
Occasionally, it is difficult to clearly distinguish stems and leaves. How-
ever, cross-sections at the leaf insertion level and at the leaflet insertion
level help to distinguish stems and leaves. Leaves are transversally in-
serted at the stem with a broad and nearly symmetrical sheath (Fig. 11),
whereas leaflets as subunits of a compound leaf show a narrow and clearly
asymmetrical insertion at the rachis (Fig. 9). Only in specimen No. M586
(*Thelethylax minutiflora* subsp. *orientalis*) we observed a few leaves that
were cylindrical at the base but flattened towards their distal end. For
example, the flattened leaf portion shown in Fig. 18 is up to 8 mm broad
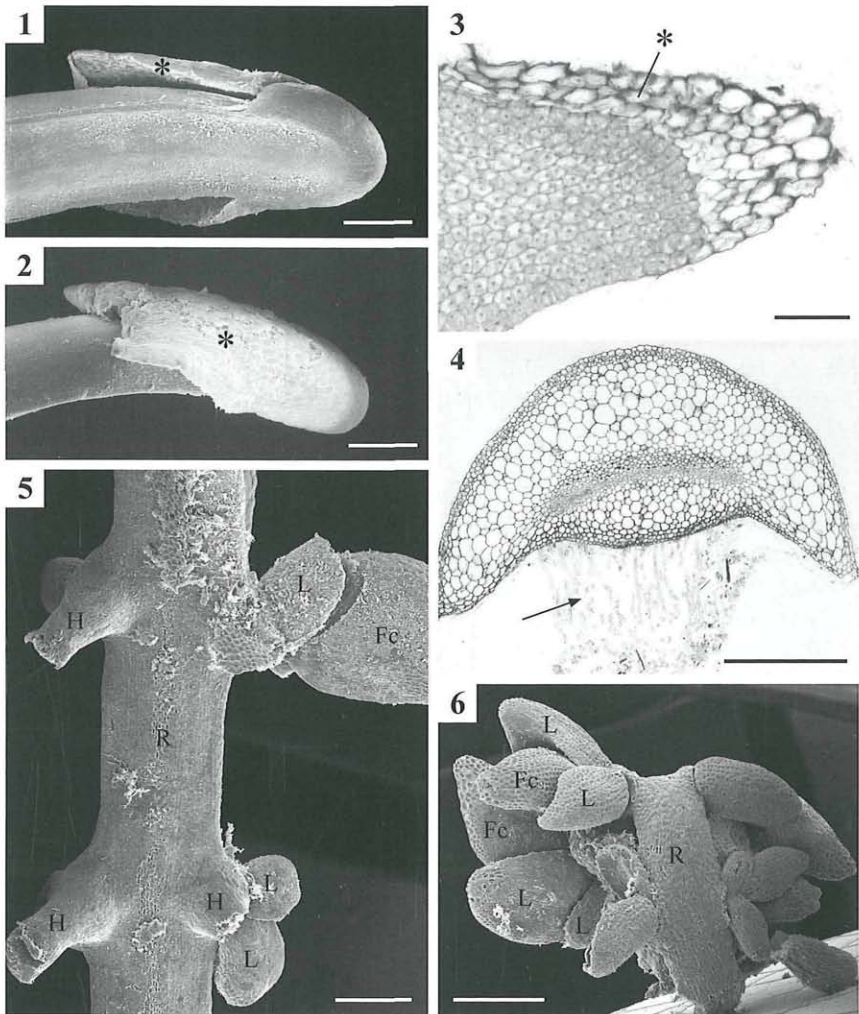and 2 cm long.

Fig. 1–6. *Thelethylax minutiflora*. Creeping roots and root–born shootlets. – Fig. 1, 2. Tip of ribbon–like root, with dorsiventral cap (asterisk), seen from below and above, respectively. Scale bar = 250 μm. – Fig. 3. Longitudinal section of root tip; dorsiventral cap (asterisk) with differentiated cells covering root apical meristem. Scale bar = 75 μm. – Fig. 4. Cross-section of nearly mature root. Note crescent-shaped outline, strap-like vascular tissue and adhesive hairs (arrow) arising from central portion of lower (ventral) side. Scale bar = 500 μm. – Fig. 5. Ribbon–like root (R), seen from below, with two pairs of endogenously formed buds, each first with few leaves (L), then with terminal flower in spathella (Fc). Note presence of finger–like holdfasts (H) arising exogenously along the root flanks (margins). Scale bar = 500 μm. – Fig. 6. Bow-shaped ribbon–like root (R), seen from above, with endogenously formed shootlets, each consisting of few (3 or 4) scale–like leaves (L) and flower bud in spathella (Fc). Scale bars = 1mm. – (Fig. 1–4 = RAUH 7205; Fig. 5 & 6 = RAUH M586).

Sessile reproductive shootlets: After floral induction (i.e. at the end of the rainy season) many reproductive shootlets arise from endogenous buds along the roots (Fig. 5, 6, 18). Each of them consists of a terminal flower and 2–4 boat-shaped scaly leaves arranged in one plane (Fig. 14, 15). Additional flowers also arise along fully mature stem portions as result of cell dedifferentiation in the stem cortex (Fig. 29–34, see below).

3.3. Spathellas and Flowers

All flowers of *Thelethylax* are solitary and located at the end of unbranched sessile shootlets. Each flower bud is totally covered by a membranous non-vascularized structure, the spathella (Fig. 14, 15), as typical for all members of the subfamily *Podostemoideae*. The spathellas of *T. minutiflora* are ovoid to ellipsoidal. Each spathella consists of 3–4 cell layers (Fig. 16). The flower buds of *T. minutiflora* are inverted or strongly inclined inside the spathella, with a slightly elongated flower stalk (pedicel) and an ovary in upside-down position (Fig. 16, 20). Anthesis occurs when the spathella is slightly above the water surface and exposed to air. The spathella splits irregularly releasing the flower with its elongating pedicel. The ovary and the androecium change into an oblique and finally a nearly upright position (Fig. 17, 21, 25). The flowers of *Thelethylax* are small, bisexual and zygomorphic. They consist of two stamens with a common stalk (andropodium). There are two inconspicuous, linear tepals on either side of the andropodium (Fig. 20, 21). A third linear tepal between the two filaments, as described for *T. minutiflora* subsp. *minutiflora* by PERRIER 1952 and C. CUSSET 1972 is lacking in the material studied (Fig. 23, 25). PERRIER 1952 described this form with only two tepals as *T. minutiflora* subsp. *orientalis* (see also Material & Methods). The introrse anthers consist of four pollen sacs which open longitudinally to release pollen. The pollen grains are tricolpate and arranged in dyads (Fig. 22).

3.4. Gynoecium, Capsule and Seeds

The gynoecium consists of a shortly stalked obovoid ovary and two linear stigmas. Inside the undehisced spathella the stigma lobes are appressed (next) to the ovary wall (Fig. 20, 21). Like in most non-African *Podostemoideae*, the ovary is bilocular. The ovary has two equal or subequal locules separated by a thin septum (Fig. 27, 28). Placentation is axile. The nearly mature capsules have the same shape and nearly the same size as the ovaries during anthesis. Capsule lengths are c. 1 mm. The capsule is narrowed towards the base, with a pedicel of 1–2 mm, but without gynophore (Fig. 24, 25). The capsules contain 10–20 seeds each. The seeds are ovoid, c. 0.2 mm long. The dehiscing capsule consists of two equal or subequal persistent valves which are smooth, except for a slightly papillate epidermis (Fig. 26–28). CUSSET 1972 described the capsule as being eight-
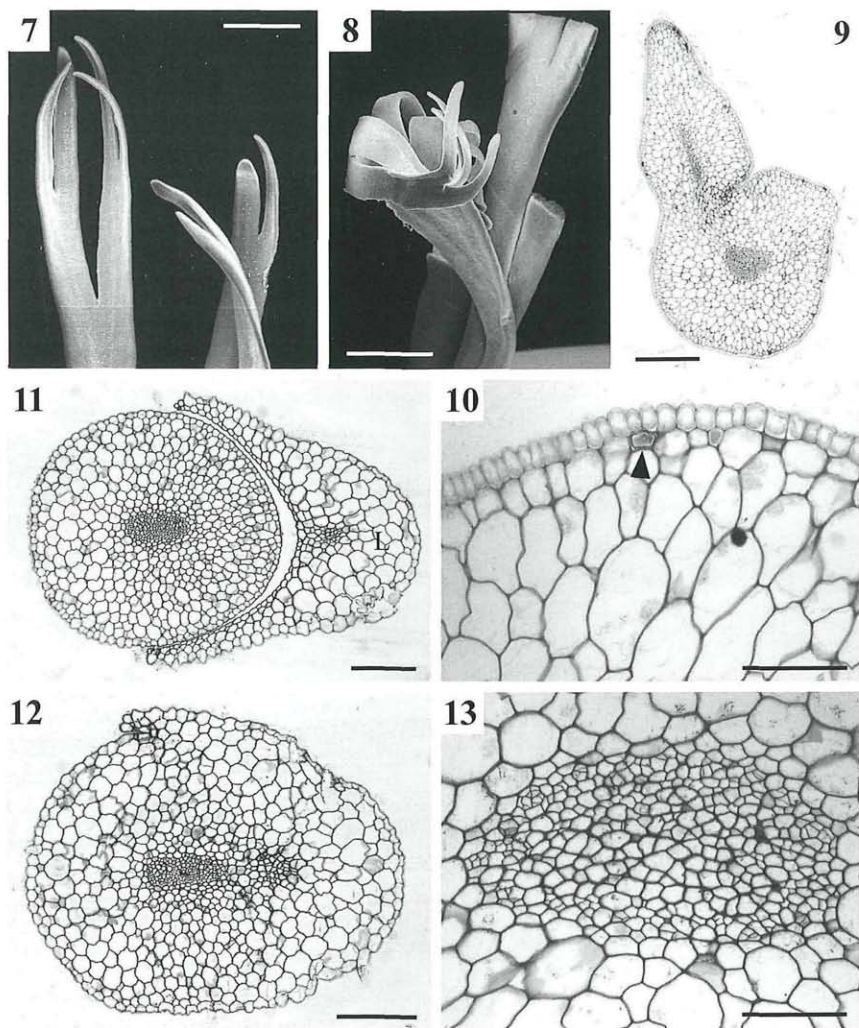
Fig. 7–13. *Thelethylax minutiflora*. Stems and forked leaves. – Fig. 7, 8. Distal portions of forked leaves with flattened segments. Scale bars = 500 μm and 1 mm, respectively. – Fig. 9. Insertion of distal leaf segment at rachis-like leaf portion. Note presence of vascular tissue. Scale bar = 500 μm. – Fig. 10. Peripheral area of leaf segment, showing large parenchyma cells, small-celled epidermis and few hypodermal cells with silica bodies (arrow). Scale bar = 100 μm. – Fig. 11, 12. Two stem cross-sections slightly above and slightly below insertion of sheathed leaf (L). Note presence of vascular tissue. Scale bar = 250 μm. – Fig. 13. Close-up of flattened vascular bundle in stem; differentiation into phloem and xylem not obvious. Scale bar = 100 μm. – (Fig. 7–13 = RAUH 7205).

ribbed, with the ribs being wider than the furrows in between. In the material observed for this study, these ribs were hard to identify. Even in cross-sections of nearly mature capsules obvious ribs or fibrous strands are inconspicuous (Fig. 28). In the image taken with a stereomicroscope (Fig. 25) only a slight tendency of a pattern of 8 (–10) darker stripes, alternating with the same number of brighter stripes can be observed. This pattern, however, is difficult to detect in SEM graphs (Fig. 26, 27). During and slightly after anthesis the ovary wall consists of four cell layers (Fig. 28). The inner epidermal cells are transversally elongated fibres whereas the inner hypodermal fibres are longitudinally elongated. Only the inner two cell layers (becoming filled with a dark content) persist at fruit maturity. The papillate outer epidermis and the small-celled outer hypodermis detach and drop during capsule maturation (Fig. 28).

3.5. Anatomical Aspects

3.5.1. Silica Bodies

The epidermis of the leaves and stems contains hardly any silica bodies. We found only few tiny silica bodies in small hypodermal cells of stems and roots (Fig. 4, 10). Large silica bodies, as typical for many *Podostemaceae*, seem to lack in *Thelethylax minutiflora*.
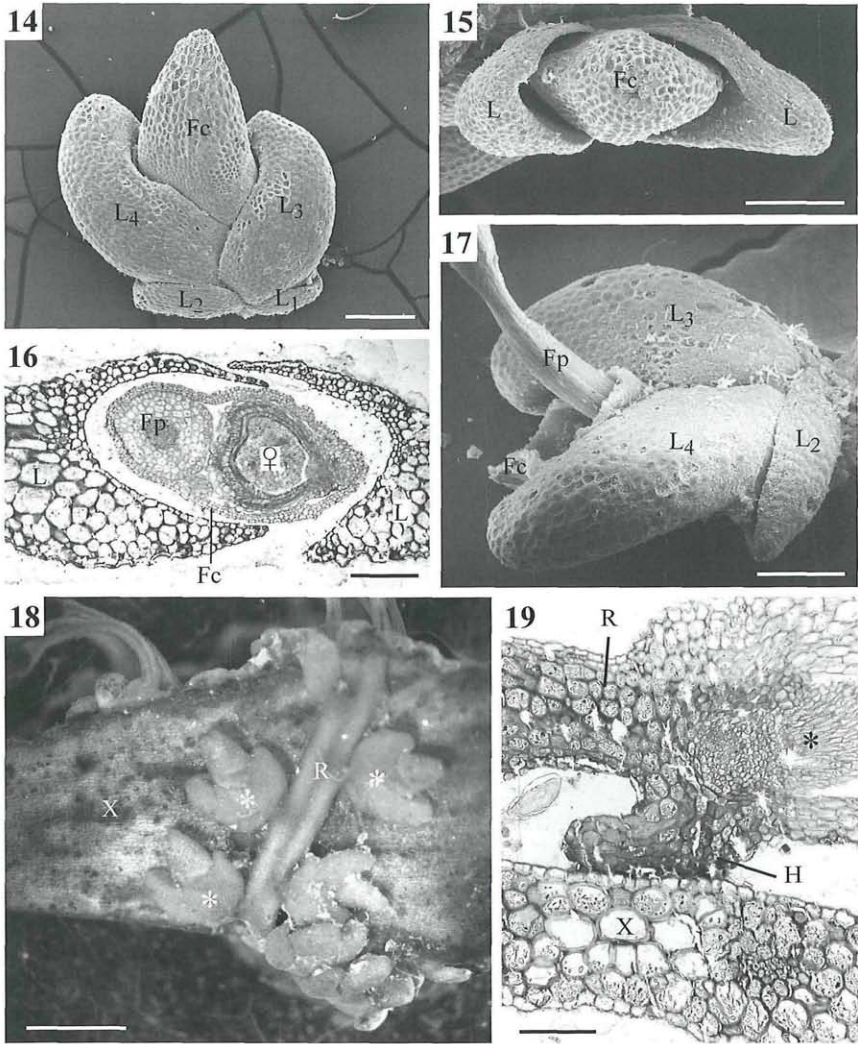
3.5.2. Vascular Tissue

Conspicuous fibrous bundles are found in stems and leaves (Fig. 9, 11–13, 30) and flower stalks (Fig. 16). All conductive tissue in *Thelethylax minutiflora* is not obviously differentiated into xylem and phloem. Most cells of vascular bundles are spindle-shaped and collenchymatous, not showing secondary wall thickening like rings or spirals (as typical for tracheids) or sieve plates (as typical for phloem).

3.6. Dedifferentiation of Stem Cortex

We observed adventitious and endogenous formation of reproductive shootlets along old stem portions which had completely dropped their foliage leaves (Fig. 29). This process is due to dedifferentiation of cortex cells in old stem portions. Parenchyma cells in one or two layers below the epidermis start to dedifferentiate and divide up into many young, i.e. meristematic, cells (Fig. 33, 34). These centres of mitotic activity finally give rise to reproductive shootlets with scaly leaves (Fig. 30–32).

Fig. 14–19. *Thelethylax minutiflora*. Reproductive root-born shootlets. – Fig. 14. Lateral view of shootlet, with terminal flower in spathella (Fc) and four scaly leaves (L1–L4) below. Scale bar = 500 μm. – Fig. 15. Top view of similar shootlet. Abbrevia-

tions as above. Scale bar = 500 µm. – Fig. 16. Cross-section of another reproductive shootlet, with two scales (L) and terminal flower in between. Note spathella (Fc) as covering tube, floral stalk (Fp) and inverted ovary (♀). Scale bar = 250 µm. – Fig. 17. Older stage of reproductive shootlet with spathella (Fc) ruptured and floral stalk (Fp) elongated (flower out of frame). Note horizontal orientation of the scaly leaves (L2–L4). Scale bar = 250 µm. – Fig. 18. Mature flattened leaf portion (X), as occasionally found in *T. minutiflora* subg. *orientalis*, seen with stereomicroscope. Note epiphytic young ribbon-like root (R) with lateral floral shootlets (asterisks). Scale bar = 3mm. – Fig. 19. Cross-section of flattened leaf portion (X) shown in Fig. 18. Young epiphytic root (R) with outgrowing shootlet (asterisk), fixed to "living substrate" (X) by hold-fast (H). Scale bar = 250 µm. – (Fig. 14–19 = RAUH M586).

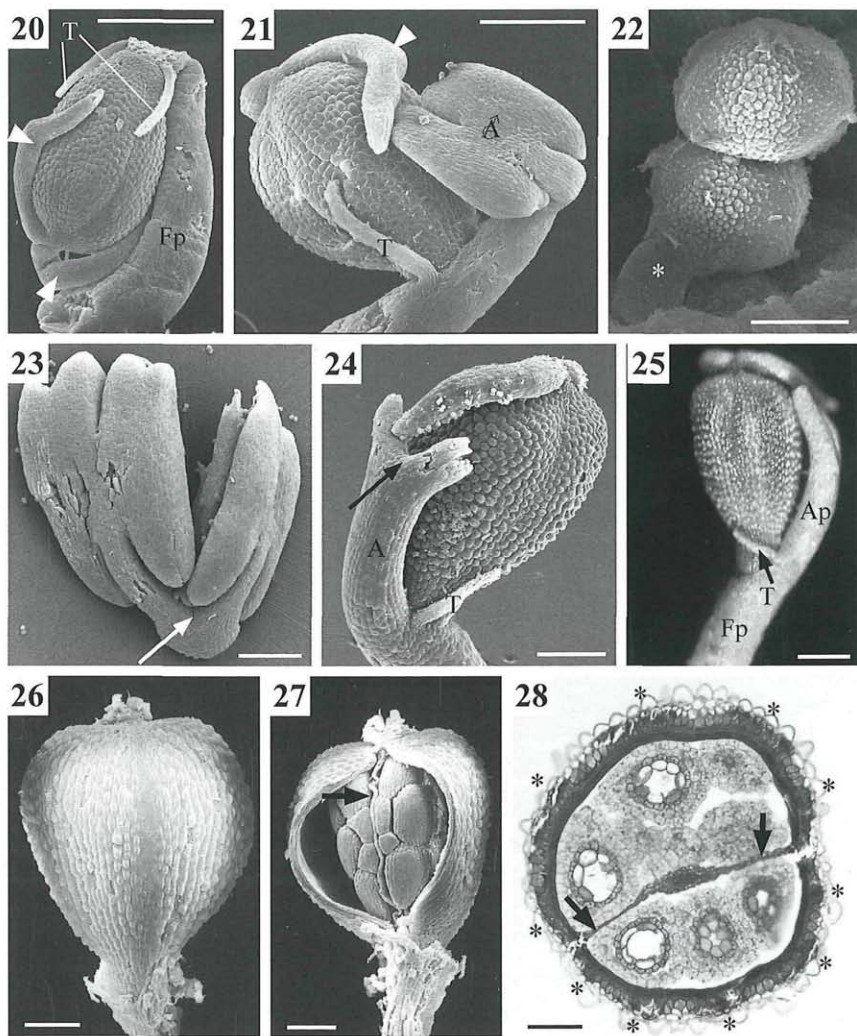# 4. Discussion

4.1. Morphological Characters in General

MOLINE & al. 2007 used the two Madagascan taxa *Thelethylax minutiflora* and *Endocaulos mangorense* as sister group in order to compare eight podostemoid species selected from continental Africa besides various Asian and American taxa. They included 39 morphological characters in a combined molecular and morphological study. Six morphological characters were selected and added to the cladogram that served as best hypothesis for phylogenetic reconstruction (mainly based on *matK* sequence data). MOLINE & al. 2007 presented character optimization for the following six characters: (A) tepal number, (B) septa in ovary/capsule, (C) flower orientation in bud (i.e. inside spathella), (D) root shape, (E) length of vegetative stem, and (F) double-sheathed leaves. These results will serve as basis for the paragraphs below.

4.2. Vegetative Structures

4.2.1. Roots and Holdfasts

Podostemaceae show an amazing diversity of root types. They all are dorsiventrally flattened and widened to some degree. This basic common bauplan can be varied from thread-like (nearly cylindrical) to ribbon-like and even crustose (i.e. foliose similar to foliose lichens). The variation in root tip and cap structure of various *Podostemaceae* has been demonstrated by RUTISHAUSER & MOLINE 2005 and KOI & al. 2006. For example, the root tip of *Podostemum ceratophyllum* (N America), and *Thelethylax* (Madagascar) have a prominent but clearly asymmetrical cap whereas in *Farmeria metzgerioides* (S India) and many African podostemoids only a rudimentary root cap, or no cap at all can be found. – C. CUSSET 1972 and G. CUSSET 1974, who studied *Thelethylax minutiflora*, used the terms "basal thalloid part" (in French "partie basale thalloïde") in order to avoid the term "root" (in French "racine"). According to them the creeping ribbons giving rise to endogenous shoot buds in *Thelethylax* and other podostemads are not equivalent (homologous) to the roots of other (terrestrial) angiosperms. This disagreement over interpretation of the vegetative body

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Fig. 20–28. *Thelethylax minutiflora*. Flowers and fruits. – Fig. 20. Flower in bud stage, after removal of spathella and androecium. Scale bar = 500 μm. Note inverted (hanging) position of ovary; thread-like tepals (T), flower stalk (Fp); arrow-heads point to cylindrical stigma lobes. Scale bar = 300 μm. – Fig. 21. Flower shortly before anthesis, with ovary in oblique (inclined) position. Androecium with one of two anthers (A) observable. Other abbreviations as above. Scale bar = 300 μm. – Fig. 22. Dyad of tricolpate pollens on stigma, pollen tube is marked with asterisk. Scale bar = 10 μm. – Fig. 23. Two introrse anthers removed from anthetic flower, seen from

abaxial side. Arrow points to fork at distal end of andropodium devoid of third tepal. Scale bar = 300 μm. – Fig. 24, 25. SEM graph and stereomicroscope view of flower in anthesis. The two anthers removed in order to better observe andropodium (Ap) with distal fork and ovary with indistinct ribs. Other abbreviations as above. Scale bar = 250 μm. – Fig. 26, 27. Two lateral views of mature capsule, dehisced septicidally on one side. Note presence of several (10 or more) seeds. Arrow points to septum separating the two locules. Scale bars = 200 μm. – Fig. 28. Cross-section of nearly mature capsule, cut below inflated placenta. The 4 or 5 inconspicuous ribs per valve are marked with asterisks. Capsule wall with horizontal fibers in inner epidermis and longitudinal fibers in hypodermis. Note papillate outer epidermis. Arrows point to septum . Scale bars = 100 μm. – (Fig. 20–28 = RAUH M586).
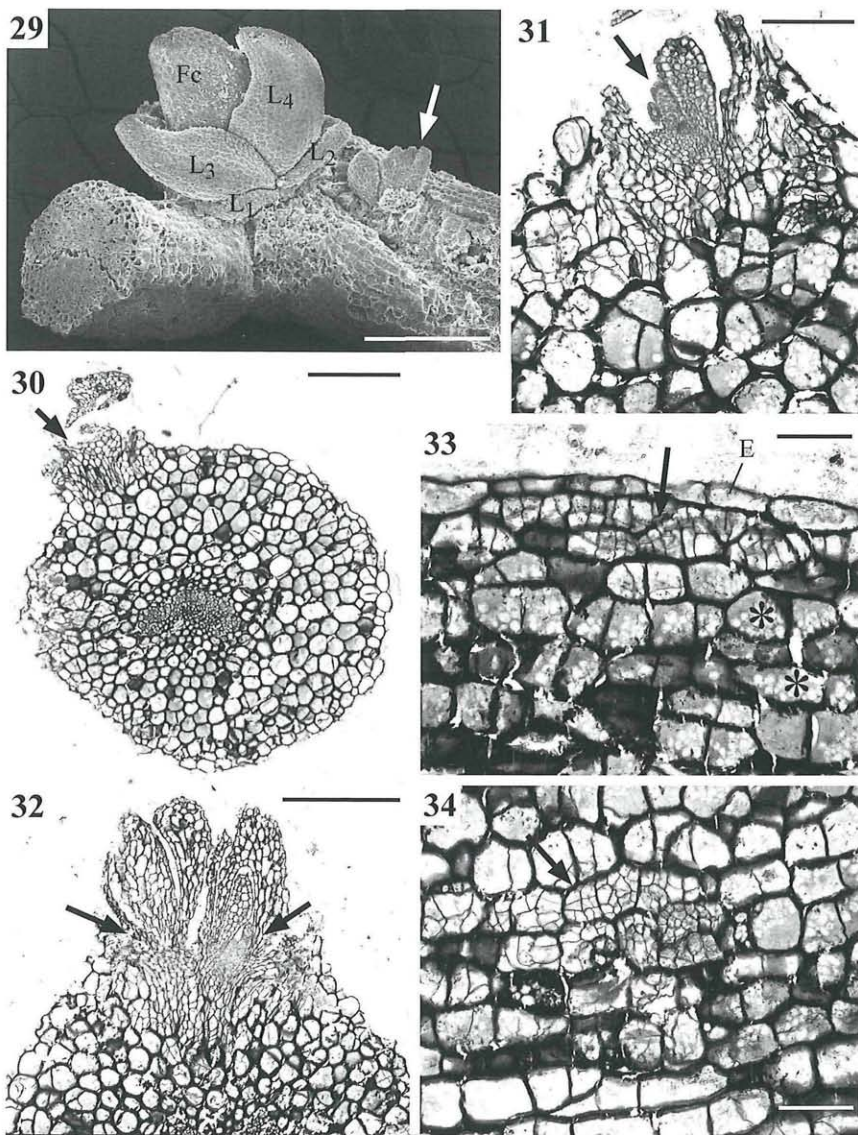
in the *Podostemaceae* goes on among contemporary botanists (MOHAN RAM & SEHGAL 2007). However, structural terms such as 'root' and 'shoot' in *Podostemaceae* do not necessarily imply a total correspondence (total homology) with organ categories of typical angiosperms (RUTISHAUSER & al. 2008). As done in earlier papers on *Podostemaceae* (JÄGER-ZÜRN 2003, RUTISHAUSER & MOLINE 2005, COOK & RUTISHAUSER 2007, MOLINE & al. 2006, 2007), we have used in the present paper a pragmatic approach by defining the 'root' of *Thelethylax* and other podostemads as an organ giving rise to endogenously formed 'shoots', whereas the 'shoot' is defined as a 'stem' that carries exogenous 'leaves'. We prefer the term 'root' in *Podostemaceae* although we have to admit that these roots deviate often very much from typical roots found in other angiosperms, including loss of root-cap, exogenous root branching, pronounced dorsiventrality and flattening (RUTISHAUSER & al. 2008).

Podostemaceae roots are fixed to the hard substratum (usually rock) by adhesive hairs and – occasionally – by multicellular holdfasts (RUTISHAUSER 1997). Besides *Thelethylax minutiflora*, also the ribbon-like roots of e.g., *Podostemum* spp., *Saxicolella submersa* are provided with finger-like holdfasts as exogenous outgrowths (Fig. 5; AMEKA & al. 2002, RUTISHAUSER & al. 2003, MOLINE & al. 2006). In many other *Podostemaceae* the exogenous holdfasts are disk-like (RUTISHAUSER 1997). Holdfasts arising from endogenous buds inside the root cortex, however, were observed in *Indotristicha ramosissima* (RUTISHAUSER & HUBER 1991).

4.2.2. Shoots and their Position Along Roots

The endogenous origin of paired shoots from ribbon-like roots, as observable in *Thelethylax minutiflora*, is found in many *Podostemaceae* (AMEKA & al. 2002, JÄGER-ZÜRN 2007). *Thelethylax minutiflora* and *Sphaerothylax abyssinica* are the only Madagascan podostemoids showing shoot dimorphism. During the rainy season there are elongate stems and large, usually compound foliage leaves, which are completely submerged. In the dry season reproductive short shoots ("shootlets") with imbricate scaly leaves, arising from the roots and stems can be found when the water-level has dropped sufficiently. *Endocaulos, Paleodicraeia* and *Thelethylax isalensis*, however, lack elongate shoots. They have short shoots (shootlets) only, provided with densely arranged and entire leaves. The scaly leaves of *Paleodicraeia* and *T. minutiflora* lack a filamentous tip (blade), whereas the shootlets of *Endocaulos* and *T. isalensis* have im-

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Fig. 29–34. *Thelethylax minutiflora*. Regenerative outgrowths of reproductive shootlets along stems, due to dedifferentiation of cortex cells. – Fig. 29. Old stem portion with reproductive shootlets, large one with four scaly leaves (L1–L4) and terminal flower (Fc). Arrow points to additional small shootlet. Scale bar = 1mm. – Fig. 30.

Cross-section of old stem portion. Arrow points to outgrowth of new shoot bud. Scale bar = 500 μm. – Fig. 31, 32. Two close-ups of stem cross-sections, showing different stages of shootlet regeneration (arrows). Scale bars = 150 μm and 500 μm, respectively. – Fig. 33, 34. Longitudinal sections through cortical stem region, one section radial, the other one parallel to stem surface. The parenchyma cells below the epidermis (E) started to dedifferentiate, dividing up into many meristematic cells (arrows). Most parenchyma cells are filled with starch grains (see asterisks in Fig. 33). Scale bars = 100 μm. – (Fig. 29-34 = RAUH M586).

bricate leaves with a widened ("scaly") base and a thread-like entire blade up to 5 cm long. In *T. minutiflora* we found neither axillary stem branching (as typical for the Ghanaian podostemoid *Saxicolella submersa*) nor stem bifurcation associated with double-sheathed leaves (as typical for several African and American *Podostemoideae*, see RUTISHAUSER & GRUBERT 2000, MOLINE & al. 2006, 2007, COOK & RUTISHAUSER 2007). – Certain varieties of *T. minutiflora* do not have dissected leaves at all; instead they show entire elliptical to oblong leaf blades, 3–4 cm long and 7–17mm wide, containing several parallel nerves (PERRIER 1952: his Fig. IV/8-9, JÄGER-ZÜRN 2003: her Fig. 4, as shown in www.systbot.uzh.ch/podostemaceae).

4.2.3. Anatomical Aspects: Silica Bodies and Vascular Tissue

Silica bodies in peripheral cells of long-lived parts such as roots and stems are frequently found in *Podostemaceae* (AMEKA & al. 2002, PHILBRICK & NOVELO 2004). Among African *Podostemaceae*, silica bodies occur in *Saxicolella* spp., *Ledermanniella* subg. *Phyllosoma*, and *Tristicha trifaria* but seem to be (nearly) absent in *Thelethylax minutiflora* and *Ledermanniella bowlingii* (AMEKA & al. 2003). The silica bodies may have a protective function against mechanical injury and also prevent the plant from collapse during short periods of desiccation. They may also protect the plant against grazing animals (COOK & RUTISHAUSER 2007).

The vascular tissue in the organs of *T. minutiflora* is not well developed. The prosenchymatous cells often lack a clear differentiation into xylem and phloem. OTA & al. 2001 called similar strands lacking obvious xylem and phloem elements in crustose *Hydrobryum* roots "nonvascular strands". G. CUSSET 1974 described phloem elements with sieve plates and tracheids in roots of *T. minutiflora*. We could not confirm these findings in this study. Typical phloem elements, however, were only rarely identified in *Podostemaceae* (ROMANO & DWYER 1971, UNIYAL & MOHAN RAM 2001). Xylem elements, especially tracheids with annular and helical thickenings, can be found more often in large *Podostemaceae* (e.g., *Apinagia*, *Mourera* and *Rhyncholacis*), especially in mature stems, leaf midribs, and flower stalks (RUTISHAUSER & GRUBERT 2000, COOK & RUTISHAUSER 2007).

4.2.4. Dedifferentiation and Organ Regeneration

Water plants such as *Podostemaceae* exhibit a great capacity for regeneration from wounded or ruptured portions of the plant body. WARMING 1881 and HAMMOND 1937 illustrated roots and holdfasts of *Podostemum ceratophyllum* which had regenerated their tips. In this paper we show for *Thelethylax minutiflora* that new roots and floral shoots may also be regenerated from injured stems. Detached shoots with outgrowing roots were observed in *Apinagia multibranchiata* by GRUBERT 1974. Also leaves (detached or not) of *P. ceratophyllum* and *Ledermanniella letouzeyi* can re-

generate new roots and shoots (HAMMOND 1936, RUTISHAUSER & MOLINE 2005). It seems that many *Podostemaceae* are able to produce new plants from detached subunits as result of dedifferentiation and regeneration (HAMMOND 1937). We showed that cortical cells of old stem portions of *Thelethylax minutiflora* may dedifferentiate and divide up into many small and meristematic cells prior to the endogenous formation of floral buds. Tissue dedifferentiation and regeneration of new parts (mainly flowers) from old roots, stems and even leaves seems to be a mean of various *Podostemaceae* (e.g., *Ledermanniella letouzeyi* in Cameroon) to increase the reproductive success at the end of the growing season when the water level is receding (RUTISHAUSER & MOLINE 2005, MOLINE & al. 2007, RUTISHAUSER & al. 2008).

4.3. Reproductive Structures

4.3.1. Anthesis

In *Thelethylax minutiflora* anthesis above the water surface and wind pollination seem to occur. All non-American *Podostemoideae* (and various American members as well) have inconspicuous flowers with one or two (or rarely three) stamens (CUSSET 1987, COOK & RUTISHAUSER 2007). These taxa are adapted to wind pollination. They are (according to molecular data given by KITA & KATO 2001, MOLINE & al. 2007) derived from neotropical genera such as *Apinagia, Marathrum* and *Mourera*. Several species of the latter three genera have showy, multistaminate flowers adapted to insect pollination (RUTISHAUSER & GRUBERT 2000). Further studies need to show if in *T. minutiflora* and other oligostemonous *Podostemoideae* self-pollination and even pre-anthesis cleistogamy within the unruptured spathella is more important than allogamy (as shown in *Podostemum ceratophyllum* by PHILBRICK 1984).

4.3.2. Flower Position Along Shoots and Presence of Double-sheathed Leaves

Flowers in non-American *Podostemoideae* are either solitary in terminal position of short shoots, which do not contain double-sheathed leaves (e.g., *Saxicolella amicorum, Thelethylax* spp., and most Asian members). Or they occur in clusters, positionally associated with double-sheathed leaves, besides leaves (bracts) with one sheath, as observable in species of the African genera *Dicraeanthus, Ledermanniella, Sphaerothylax*, besides the Asian *Zeylanidium subulatum* (JÄGER-ZÜRN 1999, 2000, AMEKA & al. 2002, 2003, MOLINE & al. 2007). Double-sheathed leaves (bracts) as parts of long shoots and/or short shoots are already found in American podostemoids which are accepted as basal to all Asian, Madagascan and African members of this subfamily.

4.3.3. Ovary Position of Flower Buds within the Spathella

Flower position (or more exactly ovary position) inside the spathella is an important character to distinguish the four Madagascan podostemoid genera and species. *Paleodicraeia* has erect flowers. *Sphaerothylax* has completely inverted (i.e. hanging) flower buds. *Endocaulos* is somewhat intermediate having its flower buds slightly to strongly inclined (oblique) in the spathella, i.e. the longitudinal axis of the ovary forms a right or obtuse angle as compared to the longitudinal axis of the spathella (PERRIER 1952, CUSSET 1972). The two *Thelethylax* spp. differ with respect to flower position inside the spathella. Flower buds (ovaries) of *Thelethylax minutiflora* are inverted in the spathella whereas they are inclined only in *T. isalensis*, its sister species. However, just prior to anthesis also the ovary of *T. minutiflora* is oblique (Fig. 21), as a transitory stage between completely inverted in bud (Fig. 20) and nearly erect when flowering (Fig. 25).

Flower bud position inside the spathella is again useful for the distinction of the *Podostemoideae* from continental Africa (ENGLER 1928, CUSSET 1987, COOK & RUTISHAUSER 2007). All podostemoid members in America, Asia and Australia have erect (or only slightly inclined) flower buds inside their spathellas. This pattern is found only in a few podostemoid species from Africa and Madagascar, i.e. in *Saxicolella, Djinga* and *Letestuella* (AMEKA & al. 2002, COOK & RUTISHAUSER 2007, GHOGUE & al., in press). Most African *Podostemoideae,* including the large genus *Ledermanniella*, have inverted flower buds inside the spathellas. Thus, completely or partially inverted flower buds are a unique feature (synapomorphy) of several African and Madagascan *Podostemoideae*. It seems that podostemoids with inverted flowers evolved only once from podostemoids with erect flowers (as typical for all non-African *Podostemaceae*) (see MOLINE & al. 2007). Then, *Djinga* (in Cameroon) and *Thelethylax isalensis* (in Madagascar) returned to the ancestral floral bud position. For the African genus *Saxicolella* (with upright flower buds) molecular data needs to be gathered prior to a well-founded hypothesis about character evolution (AMEKA & al. 2002).

4.3.4. Pedicel and Gynophore

A pedicel (i.e. a stalk below the insertion level of androecium and tepals) is present in all podostemoids with inverted (i.e. hanging) flower buds. In *Thelethylax minutiflora* the pedicel elongates up to 5 mm after spathella opening, during and especially after anthesis. Much longer pedicels (> 5 cm) are common in New World *Podostemoideae* with prostrate stems and pedicel insertion close to the substratum (e.g., *Marathrum* spp., RUTISHAUSER & al. 1999). – Various *Podostemoideae*, especially African taxa with inverted flower buds have a stalked ovary (gynophore). The gynophore is a stalk which separates the ovary from the insertion level of

androecium and perianth, as observable in, e.g., *Zehnderia* with gyno-phores of up 8 mm (CUSSET 1987, COOK & RUTISHAUSER 2007). Short gyno-phores of up to 1 mm, are found in African taxa with erect flower buds, e.g., *Saxicolella* (AMEKA & al. 2002). The flowers and fruits of all Mada-gascan podostemoids, including *T. minutiflora*, lack a gynophore com-pletely.

4.3.5. Tepals and Stamens

Most podostemoid genera with two fused stamens (i.e. with andropo-dium as common foot for the two stalked anthers) have two tepals, i.e. one tepal on each side of the andropodium. This character state is typical for *Thelethylax minutiflora* subsp. *orientalis* (Fig. 20, 23, 24; PERRIER 1952, COOK & RUTISHAUSER 2007). Few podostemoids with two stamens on a common andropodium have added a third tepal, occupying the fork be-tween the filaments. For example, most members of *Podostemum* (New World), *Stonesia* (tropical W Africa) and *Thelethylax* (with *T. isalensis* and *T. minutiflora* subsp. *minutiflora*) show three (subulate) tepals per flower (CUSSET 1972, PHILBRICK & NOVELO 2004, MOLINE & al. 2006, COOK & RUTISHAUSER 2007). – The flowers of the three podostemoid genera re-stricted to Madagascar, i.e. *Endocaulos*, *Paleodicraeia* and *Thelethylax*, have two stamens (with andropodium). Reduction of stamen number from two to one seems to have happened several times in podostemoids (MOLINE & al. 2007). Various African genera such as *Djinga* and *Saxicolella* have flowers with a single stamen (CUSSET 1987, AMEKA & al. 2002, COOK & RUTISHAUSER 2007). Stamen number is variable in African genera such as *Letestuella* and *Sphaerothylax* (with 1 or 2 stamens per flower), *Le-dermanniella* (with 1, 2 or even 3 stamens), *Macropodiella* and *Winklerella* (with 2 or occasionally 3 stamens) (WARMING 1899, TAYLOR 1953, CUSSET 1987).

4.3.6. Pollen

BEZUIDENHOUT 1964 and LOBREAU-CALLEN & al. 1998 compared several African/Madagascan genera to non-African genera with respect to pollen type and pollen wall ultrastructure. They recognised three main types: tricolpate pollen in monads or dyads in *Podostemoideae*, pantoporate (= forate, periporate) monads in *Tristichoideae*. Within the *Podostemoi-deae* most genera have either monads or dyads. *Ledermanniella* and *Po-dostemum* seem to be the only podostemoid genera showing both monads and dyads (PHILBRICK & NOVELO 2004, COOK & RUTISHAUSER 2007, MOLINE & al. 2006, 2007). *Thelethylax* as well as all other Madagascan podoste-moids have pollen dyads (Fig. 22; JÄGER-ZÜRN 1967). Monads appear to be the plesiomorphic character state for all podostemoids because the basal American members of this subfamily such as *Apinagia*, *Marathrum*, and

Mourera have them (JÄGER-ZÜRN & al. 2006). Dyads have arisen in the American genus *Podostemum*, also in all podostemoid genera from Asia, Australia and Madagascar. Molecular data indicate that these taxa showing dyads are basal to all podostemoids from continental Africa where some but not all genera have – as reversals – switched back to monads (KITA & KATO 2001; MOLINE & al. 2007).

4.3.7. Number of Locules in Ovary and Capsule

Ovary and capsule of most *Podostemoideae* outside continental Africa are bilocular, with a prominent central placenta and a thin septum (COOK & RUTISHAUSER 2007). This septum is still observable in the mature and dehiscing capsule of *Thelethylax minutiflora* (Fig. 27, 28) and the three other podostemoid genera (*Endocaulos, Paleodicraeia, Sphaerothylax*) occurring on Madagascar. TULASNE 1852 and CUSSET, e.g., 1972, 1987 gave emphasis on the presence vs. absence of the septum as an important taxonomic character for distinguishing mainly podostemoid genera from continental Africa. For example, *Saxicolella* pro parte and *Sphaerothylax* have bilocular ovaries whereas the majority of African genera (including all members of the large genus *Ledermanniella*) show unilocular ovaries, lacking the septum, except for rudiments in the basal ovary zone (JÄGER-ZÜRN 2000, AMEKA & al. 2002, 2003). The two non-African podostemoid genera with unilocular ovaries are *Castelnavia* (Brazil) and *Hydrobryum* pro parte (COOK & RUTISHAUSER 2007, MOLINE & al. 2007).

4.3.8. Capsule Shape and Number of Capsule Ribs

Capsule morphology was traditionally used to classify the subfamily *Podostemoideae* (ENGLER 1928, C. CUSSET 1987, 1992). The mature capsules have nearly the same size as the ovaries during anthesis. Fusiform (i.e., spindle-shaped) capsules are observable in several genera (*Ledermanniella, Saxicolella*) of the African continent, but are lacking on Madagascar. Capsules of most Madagascan podostemoids are globose to ovoid, with circular or slightly flattened cross-section (CUSSET 1972, COOK & RUTISHAUSER 2007). The ovaries and capsules of *Thelethylax minutiflora* are obovoid rather than ovoid, with the largest diameter in the upper half (Fig. 25, 26). The capsules of *T. minutiflora* and many other podostemoid taxa usually dehisce with two equal or subequal valves. Incomplete dehiscence, such as only along one suture (Fig. 27) seems to be an artifact of critical-point drying prior to scanning electron microscopy.

Presence vs. absence of capsule ribs and number of capsule ribs are used as important characters for the distinction of podostemoid genera (COOK & RUTISHAUSER 2007). In many podostemoids (e.g., *Cladopus, Saxicolella*) capsule ribs appear during fruit maturation when the outer cortical layers of the ovary wall are shed (RUTISHAUSER & PFEIFER 2002). This

decay pattern is found in many *Podostemaceae* with ribbed mature capsules (AMEKA & al. 2002, 2003).

Many *Podostemoideae*, including most members of the African continent, have three ribs per valve, each extending from base to apex. If the two sutures (dehiscence lines) are also conspicuous, there will be a total of eight ribs per capsule (CUSSET 1972, COOK & RUTISHAUSER 2007). There are exceptions to this rule, especially on Madagascar (*Endocaulos, Paleodicraeia*) and in West tropical Africa (*Stonesia*). An increased rib number per capsule valve (i.e. more than 3 ribs per valve), with the ribs nearest the sutures shorter than the others and not reaching the ends of the valves is found in: *Endocaulos mangorense* [Madagascar] with capsules asymmetrically ovoid and slightly flattened, each valve with 7 ribs; *Paleodicraeia imbricata* [Madagascar] with capsules ovoid and slightly flattened, each valve with 5 ribs; and *Stonesia* (c. 4 spp.) [Guinea, Sierra Leone and Cameroon] with capsules broadly ellipsoidal, each valve with 5 or 7 ribs (i.e. with 12 or 16 per capsule). A newly discovered Cameroonian podostemoid species was recognized as new member of the genus *Stonesia*, mainly based on the presence of the peculiar *Endocaulos-Paleodicraeia-Stonesia* pattern of capsule ribs (PFEIFER & al., submitted).

Thelethylax minutiflora is usually described as having three wide ribs per capsule valve (CUSSET 1972, COOK & RUTISHAUSER 2007). However, we did not always observe three ribs per valve. The capsules shown by SEM graphs and stereomicroscope images (Fig. 24–26) show only very indistinct ribs. In a cross-section of a nearly mature capsule there are about five rib-like sectors per valve (marked with asterisks in Fig. 28) containing a few additional fibres as compared to the sectors in between. However, it is not clear if the *Thelethylax* ribs when present are always extending from base to apex. The figure given by CUSSET 1972 (her Fig. 3–4), leaves us with the impression that the two lateral ribs of the sister species *T. isalensis* do not reach the ends of the valves. Thus, the *Endocaulos-Paleodicraeia-Stonesia* pattern of capsule ribs seems to be present in *Thelethylax*, but being indistinct in *T. minutiflora*.

4.3.9. Shape of Stigma Lobes

Various characters distinguishing the podostemoids from Madagascar are observable in the flowers and fruits. Most podostemoids have two linear stigmas such as *Thelethylax minutiflora* (Fig. 20, 21). Its sister species *T. isalensis* seems to be the only Madagascan species having short club-shaped rather than long linear stigmas (CUSSET 1972).

4.3.10. Ovules and Seeds

Ovule development of *Thelethylax minutiflora* was already studied by JÄGER-ZÜRN 1967, including the presence of a 4-celled embryo sac and a

pseudo-embryo sac (nucellar plasmodium) below (as typical for all *Podostemaceae*). Seed numbers of 10–20 per capsule as observed in *T. minutiflora* are found in many podostemoid genera (PHILBRICK & NOVELO 1997). The number of seeds in *Podostemaceae* capsules varies from 2,000–2,400 in *Mourera fluviatilis* from S America, to one (or rarely two) in *Farmeria metzgerioides* from S Asia (COOK & RUTISHAUSER 2007). The seeds of most *Podostemaceae* have nearly the same size as the ovules; they are small (0.1–0.3 mm long) and predictably dispersed by wind or water.

6. References

AMEKA G. K., CLERK C. G., PFEIFER E. & RUTISHAUSER R. 2003. Developmental morphology of *Ledermanniella bowlingii* (*Podostemaceae*) from Ghana. – Plant Syst. Evol. 237: 165–183.

— , PFEIFER E. & RUTISHAUSER R. 2002. Developmental morphology of *Saxicolella amicorum* and *S. submersa* (*Podostemaceae: Podostemoideae*) from Ghana. – Bot. J. linn. Soc. 139: 255–273.

BEZUIDENHOUT A. 1964. The pollen of the African *Podostemaceae*. – Pollen et Spores, 6: 463–478.

COOK C. D. K. & RUTISHAUSER R. 2007. *Podostemaceae*. – In: KUBITZKI K. (ed.), The families and genera of vascular plants. Vol. 9: 304–344. – Springer, Berlin.

CUSSET C. 1972 (published 1973). Les *Podostemaceae* de Madagascar. – Adansonia Sér. 2, 12(4): 557–568.

— 1983, published 1984. Contribution à l'étude des *Podostemaceae*. 7. *Ledermanniella* ENGL. sous-genre *Phyllosoma* C. CUSSET. – Bull. Mus. nation. Hist. natur. Paris, Sér. 5, (Sect. B, Adansonia) 4: 361–390.

— 1984. Contribution à l'étude des *Podostemaceae*. 8. *Ledermanniella* ENGL. sous-genre *Ledermanniella*. – Bull. Mus. nation. Hist. natur. Paris, Sér. 6, (Sect. B, Adansonia) 3: 249–278.

— 1987. *Podostemaceae* and *Tristichaceae*. – In: SATABIÉ B. & MORAT Ph. (eds.), Flore du Cameroun 30: 51–99.

— 1992. Contribution à l'étude des *Podostemaceae*. 12. Les genres asiatiques. – Bull. Mus. nation. Hist. natur. Paris, Sect. B, Adansonia 14: 13–54.

CUSSET G. 1974. Quelques traits remarquables de l'organisation du *Thelethylax minutiflora* C. CUSSET (Podostémacée). – Actes du 99ᵉ Congrès national des sociétés savantes, Besançon, 1974, Sciences, fasc. 2: 177–188.

DAVIS C. C., WEBB C. O., WURDACK K. J., JARAMILLO C. A. & DONOGHUE M. J. 2005. Explosive radiation of *Malpighiales* supports a mid-Cretaceous origin of modern tropical rain forests. – American Naturalist 165: E36-E65.

ENGLER A. 1928. Reihe *Podostemales*. – In: ENGLER A. & PRANTL K. (eds.), Die natürlichen Pflanzenfamilien, 2. Aufl. 18a: 1–68, 483–484. [published as volume in 1930]

GAFFIER L. 1932. Étude morphologique et anatomique des Podostémacées de Madagascar. – Thèse, Marseille, 140p.

GHOGUE J.-P., AMEKA G. K., GROB V., PFEIFER E. & RUTISHAUSER R. 2007. Comparative and developmental morphology of *Djinga felicis* (*Podostemaceae*) from Western Cameroon. – Bot. J. linn. Soc.: in press.

GRUBERT M. 1974. Podostemaceen-Studien. Teil 1. Zur Ökologie einiger venezolanischer Podostemaceen. – Beitr. Biol. Pflanzen, 50: 321–391.

GUSTAFSSON M. H. G., BITTRICH V. & STEVENS P. F. 2002. Phylogeny of *Clusiaceae* based on rbcL sequences. – Int. J. Plant Sci. 163: 1045–1054.

HAMMOND B. L. 1936. Regeneration of *Podostemon ceratophyllum* MICHX. – Bot. Gaz. 97: 834–845.

— 1937. Development of *Podostemon ceratophyllum*. – Bull. Torrey bot. Club 64: 17–36.

JÄGER-ZÜRN I. 1967. Embryologische Untersuchungen an vier Podostemaceen. – Österr. bot. Zeitschr. 114: 20–45.

— 1999. Developmental morphology of the shoot system of *Podostemum subulatum* (*Podostemaceae – Podostemoideae*). – Beitr. Biol. Pflanzen 71: 281–334.

— 2000. The unusual ramification mode of *Sphaerothylax abyssinica* (WEDD.) WARM. (*Podostemaceae – Podostemoideae*). – Flora 195: 200–227.

— 2002. Comparative studies in the morphology of *Crenias weddelliana* and *Maferria indica* with reference to *Sphaerothylax abyssinica* (*Podostemaceae: Podostemoideae*). – Bot. J. linn. Soc. 138: 63–84.

— 2003. Comparative morphology as an approach to reveal the intricate structures of the aquatic flowering plant family *Podostemaceae*. – Recent Res. Devel. Plant Sci. (Trivandrum) 1: 147–172.

— 2007. The shoot apex of *Podostemaceae*: De novo structure or reduction of the conventional type? – Flora 202: 383–394.

— & GRUBERT M. 2000. *Podostemaceae* depend on sticky biofilms with respect to attachment to rocks in waterfalls. – Int. J. Plant Sci. 161: 599–607.

— , NOVELO A. R., PHILBRICK C. T. 2006. Microspore development in *Podostemaceae-Podostemoideae*, with implications on the characterization of the subfamilies. – Plant Syst. Evol. 256: 209–216.

KATO M., KITA Y. & KOI S. 2003. Molecular phylogeny, taxonomy and biogeography of *Malaccotristicha australis* comb. nov. (syn. *Tristicha australis*) (*Podostemaceae*). – Austr. syst. Bot. 16: 177–183.

KITA Y. & KATO M. 2001. Infrafamilial phylogeny of the aquatic angiosperm *Podostemaceae* inferred from the nucleotide sequence of the matK gene. – Plant Biol. 3: 156–163.

— , IMAICHI R., GHOGUE J.-P., AMEKA G. K. & KATO M. 2005. Molecular phylogeny of Cameroonian and Ghanaian *Podostemaceae*. – Poster Abstract P 1215. – In: XVII International Botanical Congress 2005, Vienna. Abstracts p. 430. – Vienna.

KOI S., FUJINAMI R., KUBO N., TSUKAMOTO I., INAGAWA R., IMAICHI R. & KATO M. 2006. Comparative anatomy of root meristem and root cap in some species of *Po-*

dostemaceae and the evolution of root dorsiventrality. – Am. J. Bot. 93: 682–692.

LEBRUN J.-P., STORK A. L. 1991. Enumération des plantes à fleurs d'Afrique tropicale. Vol. I. Généralités et *Annonaceae* à *Euphorbiaceae* et *Pandanaceae*. – Genève: Editions des Conservatoire et Jardin Botaniques.

LOBREAU-CALLEN D., LeTHOMAS A. & SUAREZ-CERVERA M. 1998. Ultrastructural characters of the pollen of some *Podostemales*. Affinities with advanced *Rosidae* [in French]. – Comptes Rendues de l'Academie des Sciences, Sér. III, 321: 335–345.

MOHAN RAM H. Y. & SEHGAL A. 2007. *Podostemaceae* – an evolutionary enigma. – Zoological Survey of India. Ernst May Commemoration Volume 2007: 37–66.

MOLINE P. M., DON L., PHILBRICK C. T., NOVELO R. A., PFEIFER E. & RUTISHAUSER R. 2006. Comparative morphology and molecular systematics of *Podostemum* (including *Crenias*) – American River-weeds (*Podostemaceae*). – Bot. Jahrb. Syst. 126: 427–476.

— , THIV M., AMEKA G. K., GHOGUE J.-P., PFEIFER E. & RUTISHAUSER R. 2007. Comparative morphology and molecular systematics of African *Podostemaceae* – *Podostemoideae*, with emphasis on *Dicraeanthus* and *Ledermanniella* from Cameroon. – Int. J. Plant Sci. 168(2): 159–180.

OTA M., IMAICHI R. & KATO M. 2001. Developmental morphology of the thalloid *Hydrobryum japonicum* (*Podostemaceae*). – Am. J. Bot. 88: 382–390.

PERRIER DE LA BÂTHIE H. 1929. Les Podostémacées de Madagascar. – Archives Bot., Bull. mens. 3: 17–25.

— 1952. 88e Famille. Podostémonacées. Flore de Madagascar et des Comores. – Paris: Firmin-Didot.

PHILBRICK C. T. 1984. Aspects of floral biology, breeding system, and seed and seedling biology in *Podostemum ceratophyllum* (*Podostemaceae*). – Syst. Bot. 9: 166–174.

— & NOVELO R. A. 1997. Ovule number, seed number and seed size in Mexican and North American species of *Podostemaceae*. – Aquat. Bot. 57: 183–200.

— & — 2004. Monograph of *Podostemum* (*Podostemaceae*). – Syst. Bot. Monogr. 70: 1–106.

ROMANO G. R. & DWYER J. D. 1971. A demonstration of phloem in the *Podostemaceae*. – Bull. Torrey bot. Club, 98: 46–53.

RUTISHAUSER R. 1997. Structural and developmental diversity in *Podostemaceae* (river-weeds). – Aquat. Bot. 57: 29–70.

— , GROB V. & PFEIFER E. 2008. Plants are used to having identity crises. – In: MINELLI A. & FUSCO G. (eds.), Evolving pathways. Key themes in evolutionary developmental biology, pp. 194–213. – Cambridge University Press, Cambridge.

— , — , PFEIFER E. & BERNHARD A. 2007. *Podostemaceae* of Africa and Madagascar: Keys to gernera and species, including genera description, illustrations to all species known, synomyms, and literature list. – Revised version (first version 2004). Internet: http://www.systbot.uzh.ch/podostemaceae

— & GRUBERT M. 2000. Developmental morphology of *Apinagia multibranchiata* (*Podostemaceae*) from Venezuela. – Bot. J. linn. Soc. 132: 299–323.

— & HUBER K. A. 1991. The developmental morphology of *Indotristicha ramosissima* (*Podostemaceae, Tristichoideae*). – Plant Syst. Evol. 178: 195–223.

— & MOLINE P. M. 2005. Evo-devo and the search for "sameness" in biological systems. – Theor. Biosci. 124: 213–241.

— , NOVELO R. A. & PHILBRICK C. T. 1999. Developmental morphology of New World *Podostemaceae: Marathrum* and *Vanroyenella*. – Int. J. Plant Sci. 160(1): 29–45.

— & PFEIFER E. 2002. Comparative morphology of *Cladopus* (including *Torrenticola, Podostemaceae*) from East Asia to north-eastern Australia. – Austr. J. Bot. 50: 725–739.

— , — , MOLINE P. & PHILBRICK C. T. 2003. Developmental morphology of roots and shoots of *Podostemum ceratophyllum* (*Podostemaceae – Podostemoideae*). – Rhodora 105: 337–353.

SCHNELL R. 1967. Etudes sur l'anatomie et la morphologie des Podostémacées. – Candollea 22: 157–225.

TAYLOR G. 1953. Notes on *Podostemaceae* for the revision of the Flora of West Tropical Africa. – Bull. brit. Mus. (natur. Hist.), Bot. 1: 53–79.

TULASNE L.-R. 1852. Monographia Podostemacearum. – Arch. Mus. Hist. natur. 6: 1–208.

UNIYAL P. L. & MOHAM RAM H. Y. 2001. Studies on the morphology and in vitro seed germination in *Willisia selaginoides* (BEDD.) WARM. ex WILLIS (*Podostemaceae*). – Flora 196: 370–380.

WARMING E. 1881. Familien *Podostemaceae* I. – Kongel. danske Vidensk. Selsk. naturv. math. Afhandl. 2: 1–34.

— 1891. Familien *Podostemaceae*. IV. – Kongel. danske Vidensk. Selsk. naturv. math. Afhandl. 7: 133–179.

— 1899. Familien *Podostemaceae*. V. – Kongel. danske Vidensk. Selsk. naturv. math. Afhandl. 9: 105–154.

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