

Comparative morphology and molecular systematics of
Podostemum (including *Crenias*) — American river-weeds
(Podostemaceae)

By

Philip M. Moline, Don Les, C. Thomas Philbrick, Alejandro Novelo R.,
Evelin Pfeifer, and Rolf Rutishauser

With 82 figures and 2 tables

Abstract

Moline, P.M., Les, D., Philbrick, C.T., Novelo R., A., Pfeifer, E. & Rutishauser, R.: Comparative morphology and molecular systematics of *Podostemum* (including *Crenias*) — American river-weeds (Podostemaceae). — Bot. Jahrb. Syst. 126: 427-476. 2006. — ISSN 0006-8152.

Podostemaceae live as haptophytes in swift-running rivers with stony beds and seasonally changing water levels, mainly in the Tropics. The present study provides a comparative morphological study of eight *Podostemum* spp. including the former genus *Crenias* (*P. comatum*, *P. distichum*, *P. irgangii*, *P. muelleri*, *P. ovatum*, *P. rutifolium*, *P. scaturiginum* and *P. weddellianum*), as well as a phylogenetic analysis including *P. ceratophyllum* of nrDNA (ITS) and *trnK* intron sequences to infer the infrageneric classification of *Podostemum*. Twenty-seven morphological characters were coded in a data matrix and used for a cladistic analysis in combination with molecular data. Emphasis is given to the stipule types and how they are attached to the obliquely inserted leaf sheaths. A monophyletic group is formed by *P. ceratophyllum*, *P. comatum*, *P. muelleri*, *P. rutifolium*, and species of the former genus *Crenias* (i.e. *P. ovatum*, *P. scaturiginum* and *P. weddellianum*). This suggests that *Podostemum* (with ribbed capsules, two linear stigmas, short stigmatic papillae) is paraphyletic without the inclusion of "*Crenias*" (characterized by smooth capsules, mainly two palmately branched stigmas, and hairlike stigmatic papillae). The "*Crenias*" species investigated appear as sister clade to *Podostemum* except *Podostemum distichum* and *P. irgangii* which are sister to the remainder of *Podostemum* and "*Crenias*". The phylogenetic analysis confirms a close relationship of *P. distichum* and *P. irgangii*. These species share the tendencies to increase the number of stipular lobes (axillary teeth) per leaf and to produce three-dimensional compound leaves with whorls or pseudo-whorls of scales along the rachides.

Keywords: comparative morphology, *Crenias*, leaf polymorphism, ITS, *trnK* intron, phylogenetic analysis, Podostemaceae, *Podostemum*, stipules, taxonomy, water plants.

DOI: 10.1127/0006-8152/2006/0126-0427

0006-8152/06/0126-0427 \$ 12.50

© 2006 E. Schweizerbart'sche Verlagsbuchhandlung, D-70176 Stuttgart

1. Introduction

Podostemaceae are an exclusively aquatic family of angiosperms. They are haptophytes and rheophytes, growing attached to rocks in rapids of seasonal rivers and in the swift currents near waterfalls (Cook 1996). Podostemaceae seem to be the only family in angiosperms that underwent an extreme radiation above the genus level after going back into the water (Cook 1999). The adaptation of Podostemaceae (49 genera, 280 species) to life in lotic currents has resulted in a radically altered morphology compared with other angiosperms (Rutishauser 1997, Cook & Rutishauser, in press). The combination of unusual morphology, and unusual embryology has led to different opinions regarding the systematic relationships of Podostemaceae within angiosperms (summarized in Les et al. 1997, see also Warming 1888, Engler 1928, Royen 1954, Philbrick & Novelo 1995, 1997, Ueda et al. 1997). Recent results based on analysis of combined *rbcL* and 18S rDNA data and *matK* sequences indicate that Podostemaceae belong to the Malpighiales clade in the eurosids I group (Soltis et al. 1999, Savolainen et al. 2000, Kita & Kato 2001, Gustafsson et al. 2002). An enormous range of structural variability makes Podostemaceae challenging for the taxonomist. Conventional demarcation into stem, leaf and root are not obvious in several Podostemaceae, but seem clearer in the genus *Podostemum*.

Taxonomy of *Podostemum* and related genera

There is good evidence that *Podostemum* is restricted to the New World (Philbrick & Novelo 2004). The three Asian species of *Podostemum* (*P. barberi*, *P. munnarensense*, *P. subulatum*) were transferred to the genera *Zeylanidium* and *Polypleurum* (Cusset 1992, Philbrick & Novelo 2004). In spite of several taxonomic contributions by e.g., Warming (1881, 1882, 1891, 1899), Chodat & Vischer (1917), Royen (1954), the structural variability and developmental morphology of most species of *Podostemum* is not really understood. *Podostemum ceratophyllum* (Eastern North America to Central America, Honduras and Dominican Republic) is the only member whose developmental morphology is already well known (Warming 1881, 1882, Hammond 1936, 1937, Rutishauser et al. 2003). The closely related genus *Crenias* (syn. *Mniopsis*, c. 5 species) has been sunk into *Podostemum* by Philbrick & Novelo (2004). *Crenias* was included in *Podostemum*, as it is quite similar vegetatively to *P. muelleri*. The characters used by the previous authors to distinguish the two genera were the smooth capsules and two palmately branched stigma lobes in most species of "*Crenias*", whereas *Podostemum* sensu stricto is characterised by ribbed capsules and two linear stigma lobes.

Species demarcation in *Podostemum* is difficult because of enormous plasticity found between individuals of the same species (Tur 1997). Morphological diversity within *Podostemum* allowed Royen (1954) to distinguish seventeen spe-



cies, but his conclusions were based mainly on herbarium specimens. Based on extensively recollected specimens of American *Podostemum* species Philbrick & Novelo (2004) suggest that several of Royen's taxa are environmental forms (ecotypes) of a considerably smaller number of species. Consequently, they have amended the taxonomic circumscription of *Podostemum* s. str. to consist of not more than six species: *Podostemum ceratophyllum*, *P. comatum*, *P. distichum*, *P. irgangii*, *P. muelleri* and *P. rutifolium*. In addition, they sank the species in "Crenias" (4 spp.: *P. ovatum*, *P. saldanhanum*, *P. scaturiginum*, *P. weddellianum*) and *Devillea* (1 sp.: *P. flagelliforme*) into *Podostemum* s. l. which thus comprises a total of now 11 species (Philbrick & Novelo 2004). In the present paper we use the name "Crenias" (with quotation marks) when referring to the *Podostemum* species formerly in the genus *Crenias*. Similar to Royen (1954) but unlike Philbrick & Novelo (2004) we still consider *Devillea* as a monotypic genus on its own. Molecular data of *Devillea* is lacking, and morphological evidence is not satisfactory (*Devillea* with only one stamen per flower, pollen in monads — as in *Oserya*) and is based only on the type collection collected 160 years ago.

Morphological diagnosis of the genus *Podostemum* sensu lato (including "Crenias")

The genus is described by the following characters (Warming 1881, 1888; Engler 1928, Royen 1954, Cook 1996, Tur 1997, Rutishauser et al. 2003 and Philbrick & Novelo 2004): Aquatic perennial herbs, attached to rocks in river-rapids and waterfalls. Roots thread- to ribbon-like, laterally flattened (Fig. 1), branched, with adhesive hairs and exogenous finger-like holdfasts (Fig. 3). Stems distinct (up to 70 cm long) or indistinct, arising laterally from the margins of the root (Figs. 3, 8), often in opposite pairs, simple or branched; vegetative shoots turn into reproductive ones when the water recedes, or (more rarely) there are flowering stems separate from the vegetative ones along the same root. Leaf blades simple, or repeatedly forked into linear segments (Figs. 11, 12). Leaf sheaths simple or double (Figs. 35, 36). Stipules of various shape, in some species two or even more intrapetiolar stipules (Figs. 16, 24). Spatheas, as sac-like shaped membranous covers of the immature flowers (Fig. 8), splitting irregularly from the top (Fig. 66) consisting of 3–5 cell layers (Figs. 37, 67, 68). Flowers solitary and positionally associated with a double-sheathed leaf each; pedicel 1–5 (rarely up to 8) mm long. Tepals linear (Fig. 18), one each side of the andropodium and usually one on the top of the andropodium in the fork between the two basally fused filaments (Fig. 20). Stamens usually two, born on an andropodium; anthers dehiscing introrsely or latrorsely (Fig. 19); pollen in dyads. Capsules ovoid, opening by two slightly unequal valves, the larger valve persistent (Figs. 22, 51); each valve smooth ("Crenias") or with 3 distinct or indistinct ribs (Figs. 21, 56); style short; stigmas 2, linear or palmately branched (Figs. 18, 66) with long stigma papillae in "Crenias." Seeds per capsule up to 200.

Morphological diversity of Podostemaceae in general

Podostemaceae show various morphological peculiarities (developmental idiosyncrasies) that are difficult to interpret. Structural categories such as 'root', 'stem' and 'leaf' are best accepted as convenient descriptive terms in Podostemaceae. The creeping axis in e.g. *Podostemum* is called the 'root' although it is typically green and photosynthetic (Rutishauser et al. 2003, Rutishauser & Moline 2005). The root is the main prostrate axis fixing the plants to the rocks. It is often dorsiventrally flattened. The range of shapes that the root can develop in different members of the Podostemaceae is stunning. Long and creeping ribbons (e.g. *Podostemum* including "Crenias") to crustose and lichen-like modified roots (e.g. *Hydrobryum*, *Zeylanidium*) can be observed (Ota et al. 2001, Kita & Kato 2004). The rhizodermal layer and other long-lived plant parts of several Podostemaceae (including *Podostemum*) contain silica bodies protecting them from mechanical damage (Ancibor 1990). Many Podostemaceae roots develop finger- or disc-like lateral outgrowths — so-called holdfasts — that serve as anchoring for the plant in the tormentous habitat. These multicellular holdfasts are exogenous outgrowths of the root in *Podostemum* and positionally associated with endogenous root-born shoots (Fig. 3). Root attachment to the substratum is due to adhesive, unicellular hairs which grow into the slimy matrix of the extracellular pectic substances of the cyanobacterial biofilm (Figs. 52; Jäger-Zürn & Grubert 2000).

Leaf size and morphology in Podostemaceae range from tiny (less than 1 cm long) to big (more than 1 m long) and from entire to complex 3-dimensionally structured leaves (e.g., *Marathrum* and *Mourea* spp.). Unlike typical angiosperms many Podostemaceae lack axillary shoot branching. Some of the leaves are double-sheathed (also called dithecaous) having an inner (adaxial) and an outer (abaxial) sheath. They give rise to a peculiar type of shoot branching with vegetative daughter shoots arising in both the inner and outer sheath of such a double-sheathed leaf. The sheaths may also contain single flowers in one or both sheaths (Rutishauser et al. 2003). Each young flower is enclosed and protected by a spathella, i.e. a thin non-vascular tubular or saccate membrane. Growth of the flower bud and pedicel elongation leads to spathella rupturing. The presence of this spathella is characteristic for all Podostemoideae whereas the members of the small subfamilies Tristichoideae and Weddellinoideae lack it (Cook 1996, Cook & Rutishauser in press). Anthesis in most Podostemaceae takes place when the water recedes, i.e. during seasons of low water levels. Wind or (more rarely) insects act as pollen vectors although autogamy (including cleistogamy) is common in several taxa (Rutishauser & Grubert 2000, Okada & Kato 2002, Philbrick & Novelo 2004). The flowers of most Podostemaceae are bisexual. Many Podostemoideae taxa (including *Podostemum*) show a uniform dorsiventral (zygomorphic) morphology of the flower, with a 2-locular ovary containing many ovules. The perianth of *Podostemum* and allies is inconspicuous and consisting of 2 (or 3) linear or subulate tepals. The androecium of *Podostemum* and allies is a Y-shaped

structure consisting of two basally fused filaments forming a common stalk (andropodium) and two distally free arms.

Ecology and distribution

Podostemaceae are restricted to rivers with a rocky bed, a seasonal water level change and swift current. *Podostemum* has a rather disjunct distribution. The center of diversity for this genus lies in SE Brazil (here all species of "Crenias"), NE Argentina, Paraguay and Uruguay (Royen 1954, Royen & Reitz 1971, Tur 1997, 1999). *Podostemum rutifolium* subsp. *ricciiforme* occurs in Mexico, Belize, Costa Rica and Colombia, whereas *P. rutifolium* subsp. *rutifolium* and *P. comatum* are restricted to southeastern Paraguay, northeastern Argentina, western Uruguay and southern Brazil (Novelo & Philbrick 1997, Philbrick & Novelo 2004). The range of *P. ceratophyllum* extends from the Dominican Republic to NE Canada (Royen 1954, Philbrick & Crow 1983, Philbrick & Novelo 2004). There are no known localities of *Podostemum* (including "Crenias") for the Amazon Basin.

Aim

The aim of this paper is a comparison of morphological and developmental characters of selected species of *Podostemum* (including "Crenias"). The comparative morphology of these taxa is linked with molecular data, especially ITS and *trnK* sequence data. This study has three goals: (I) We aim to elucidate the infrageneric relationship of *Podostemum* (including "Crenias") using the wide species definitions as proposed by Philbrick & Novelo (2004). (II) We evaluate the morphological distinctness of three species of "Crenias" (i.e. *Podostemum ovatum*, *P. scaturiginum* and *P. weddellianum*) because they share morphological peculiarities with *Podostemum muelleri*. (III) We compare the morphology of *Podostemum* (including "Crenias") with results obtained from the phylogenetic analysis of molecular data.

2. Material and methods

Sampling

We included 9 *Podostemum* species sensu Philbrick & Novelo (2004). The species investigated are restricted to America and most of the studied material was collected from Brazil by C. T. Philbrick (Western Connecticut State University, Danbury, USA) and A. Novelo R. (Universidad Nacional Autónoma de México, Mexico City) during field trips between January 1998 and May 2000 (Table 1). Due to difficulties in the sampling various collection numbers of specimens differ between the morphological and molecular

ntal
ot',
ma-
pi-
line
ften
rent
e.g.
ots
ato
eral
iem
lop
an-
are
ted
n is
the
örn

cm
ally
gio-
ives
iter
eta-
ou-
oth
l by
the
e of
the
ok
the
in-
on
&
ste-
or-
les.
r 3)
ped

Table 1: List of species with accession numbers and their collection sites (CTP = collections of C.T. Philbrick et al., LM = collection of D. Les and P. Moline, Novelo = collection of A. Novelo). Collections used in both comparative morphological and molecular studies are indicated by an asterisk (*). For more information on collections see Philbrick & Novelo (2004).

SPECIES (NEW)	AUTHOR	ACCESSION	LOCATION
Ingroup			
<i>Podostemum ceratophyllum</i>	Michx.	CTP 4615	USA, Connecticut: Chaplin, Natchaug River
<i>Podostemum ceratophyllum</i>	Michx.	LM 0001	USA, Connecticut: Chaplin, Natchaug River
<i>Podostemum comatum</i>	Hicken	CTP 5022	Brazil, Rio Grande do Sul: Arroyo do Lajeado
<i>Podostemum comatum</i>	Hicken	CTP 5027	Brazil, Rio Grande do Sul: Arroyo do Lajeado
<i>Podostemum comatum</i>	Hicken	CTP 5045	Brazil, Rio Grande do Sul: Rio Taquarembo
<i>Podostemum comatum</i>	Hicken	CTP 5048*	Brazil, Rio Grande do Sul: Rio Taquarembo
<i>Podostemum comatum</i>	Hicken	CTP 5633*	Paraguay, Guairá: Rio Tebicuary-mi
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5003*	Brazil, Rio Grande do Sul: Rio Francesco Alves
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5004*	Brazil, Rio Grande do Sul: Rio Francesco Alves
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5005	Brazil, Rio Grande do Sul: Rio Francesco Alves
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5006	Brazil, Rio Grande do Sul: Rio Francesco Alves
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5019	Brazil, Rio Grande do Sul: Rio Francesco Alves
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5020	Brazil, Rio Grande do Sul: small river at BR-392
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5063*	Brazil, Rio Grande do Sul: small river at BR-392
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5078*	Brazil, Rio Grande do Sul: Rio Bitia
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5079	Brazil, Rio Grande do Sul: Rio Tijela
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5195	Brazil, Rio Grande do Sul: Rio Tijela
<i>Podostemum distichum</i>	(Cham.) Wedd.	CTP 5598*	Arroyo Chuvisquiro
<i>Podostemum irgangii</i>	C.T.Philbrick & Novelo	CTP 5441*	Brazil, Goias: Rio Bonito
<i>Podostemum muelleri</i>	Warm.	CTP 5008	Brazil, Santa Catarina: Rio Chapecozinho
<i>Podostemum muelleri</i>	Warm.	CTP 5010	Brazil, Rio Grande do Sul: Rio de Vacacai
<i>Podostemum muelleri</i>	Warm.	CTP 5023	Brazil, Rio Grande do Sul: Rio de Vacacai
<i>Podostemum muelleri</i>	Warm.	CTP 5039*	Brazil, Rio Grande do Sul: Rio Jaguari
<i>Podostemum muelleri</i>	Warm.	CTP 5496*	Brazil, Rio Grande do Sul: Rio Jaguari
<i>Podostemum ovatum</i>	C.T.Philbrick & Novelo	CTP 5001*	Brazil, Santa Catarina: Rio Itajai-Acu
<i>Podostemum rutifolium</i>	(Liebm.) Novelo	Novelo 1426	Brazil, Rio de Janeiro: Rio da Cidade
subsp. <i>ricciiforme</i>	& C.T.Philbrick		Mexico, Oaxaca: Rio Usila
<i>Podostemum rutifolium</i>	(Liebm.) Novelo	Novelo 3979*	Mexico, Oaxaca: Rio Usila
subsp. <i>ricciiforme</i>	& C.T.Philbrick		

Pod
sub:
Pod
sub
Pod
sub
Pod
sub
Pod
sub
Poc
Poc
Ou
Ap1
Ap1
Ma
Ma
Os

rick et 1 com- collec-	<i>Podostemum rutifolium</i> subsp. <i>riccifforme</i>	(Liebm.) Novelo & C.T.Philbrick	Novelo 3985*	Mexico, Veracruz: Rio Rancho Viejo
—	<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	Warm.	CTP 5018	Brazil, Rio Grande do Sul: small river at BR-392
—	<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	Warm.	CTP 5021	Brazil, Rio Grande do Su: Lagoa dos Patos
—	<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	Warm.	CTP 5025	Brazil, Rio Grande do Sul: Arroyo do Lajeado
—	<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	Warm.	CTP 5040*	Brazil, Rio Grande do Sul: Rio Jaguari
—	<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	Warm.	CTP 5406*	Brazil, Santa Catarina: Rio Irani
—	<i>Podostemum scaturiginum</i>	(Mart.) C.T.Philbrick & Novelo	CTP 5602*	Brazil, Goias: Cachaeira do Pantano
—	<i>Podostemum weddellianum</i>	(Tul.) C.T.Philbrick & Novelo	CTP 5000*	Brazil, Rio de Janiero: Rio da Cidade
	Outgroup			
	<i>Apinagia</i> cf. <i>rangiferina</i>	(Engler) v.Royen	CTP 5579	Brazil, Mato Grosso: Rio Araguaia
	<i>Apinagia yguazuensis</i>	(Engler) v.Royen	TPN 2122	Argentina, Misiones: Cataratas Iguazú
	<i>Marathrum minutiflorum</i>	(Cham.) Tul.	Novelo 3978*	Mexico, Tabasco: Rio Pedregal
-mi	<i>Marathrum schiedeanum</i>	(Cham.) Tul.	Novelo 3983	Mexico, Veracruz: Balneario, Carrizal
	<i>Oserya coulteriana</i>	Tul.	Novelo 3235	Mexico

studies. For six taxa (*P. distichum*, *P. irgangii*, *P. ovatum*, *P. rutifolium* subsp. *riccifforme*, *P. scaturiginum*, *P. weddellianum*) specimens with the same collection numbers (marked with * in Table 1) were used in both morphological and molecular studies. Where we had to use specimens with different collection numbers for morphological than for molecular studies, we tried to select specimens from the same river or even the same collection site. We included two accessions per species from different collection sites to estimate the variation in the species, and to test species delimitation. Species delimitation was particular interesting in the polymorphic species *P. distichum*; therefore we included four accessions that represent a broad range of morphological variability. For *P. irgangii* only one accession could be included due to sampling constraints. Due to a lack of fixed and silica-dried material, *Podostemum flagelliforme* (Tulasne & Weddell) Philbrick & Novelo (syn. *Devillea flagelliformis*) could not be studied.

Morphological data

The morphological data presented in this study are mainly based on material that was fixed and preserved in 70 % ethanol. Pictures presented in this study are based on scanning electron microscopy (SEM). The dissected plant material was critical-point dried and sputter-coated (Au). The 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), as described by Igersheim & Cichocki (1996), and sectioned with a MICROM HM 355 rotary microtome and conventional microtome knife types C and D. The mostly 7 mm thick sections were stained with ruthenium red and toluidine blue (Weber & Igersheim 1994). The permanent slides of the microtome sections are deposited at the Institute of Systematic Botany, University of Zurich (Z).

bitia
ijela
ijela

iguari
Acu

In addition, 30 morphological characters from habit, leaves, flowers, and fruits were coded in a matrix for phylogenetic analysis (appendices 1 and 2). Character states were compiled using ethanol-fixed and herbarium specimens (Table 1). All characters were equally weighted and treated as unordered.

Molecular data

For the molecular analysis each species was represented by up to four accessions from different populations. Our data matrix comprised 24 OTU's, 9 species out of the 11 species in *Podostemum* (Philbrick and Novelo 2004) comprised the ingroup, and five species from the Podostemaceae comprised the outgroup (Table 2). We chose *Apinagia*, *Marathrum*, and *Oserya* as outgroups as these genera have been shown to be more basal than *Podostemum* within the subfamily Podostemoideae (Kita & Kato 2001). Total (genomic) DNA of silica-dried tissue was obtained following a modified CTAB protocol after Doyle (1987). We amplified the complete ITS region, including ITS-1, ITS-2 and the 5.8S rDNA and the *trnK* 5'-intron, including only the beginning of the *matK* gene.

Table 2. Accessions of *Podostemum* (including "*Crenias*") included in the molecular phylogenetic study. The species only represented by partial *trnK* intron sequences are indicated by an asterisk (*).

SPECIES	ACCESSION	ITS	<i>trnK</i> intron
Ingroup			
<i>Podostemum ceratophyllum</i>	CTP 4615	DQ397959	DQ397980
<i>Podostemum ceratophyllum</i>	LM 0001	DQ397960	n.a.
<i>Podostemum comatum</i>	CTP 5048	DQ397961	DQ397981
<i>Podostemum comatum</i>	CTP 5633	DQ397962	n.a.
<i>Podostemum distichum</i>	CTP 5003	DQ397963	DQ397982
<i>Podostemum distichum</i>	CTP 5004	DQ397964	DQ397983
<i>Podostemum distichum</i>	CTP 5063	DQ397965	DQ397984
<i>Podostemum distichum</i>	CTP 5598	DQ397966	n.a.
<i>Podostemum irgangii</i>	CTP 5441	DQ397967	DQ397985
<i>Podostemum muelleri</i>	CTP 5039	DQ397968	DQ397986
<i>Podostemum muelleri</i>	CTP 5496	DQ397969	DQ397987
<i>Podostemum ovatum</i>	CTP 5001	DQ397953	DQ397976
<i>Podostemum rutifolium</i> subsp. <i>ricciiforme</i>	Novelo 3979	DQ397970	DQ397988
<i>Podostemum rutifolium</i> subsp. <i>ricciiforme</i>	Novelo 3985	DQ397971	DQ397989
<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	CTP 5040	DQ397972	DQ397990*
<i>Podostemum rutifolium</i> subsp. <i>rutifolium</i>	CTP 5406	DQ397973	n.a.
<i>Podostemum scaturiginum</i>	CTP 5602	DQ397954	n.a.
<i>Podostemum weddellianum</i>	CTP 5000	DQ397952	DQ397975*
Outgroup			
<i>Apinagia</i> cf. <i>rangiferina</i>	CTP 5579	DQ397958	DQ397979*
<i>Apinagia yguazuensis</i>	TPN 2122	DQ397951	DQ397974*
<i>Marathrum minutiflorum</i>	Novelo 3978	DQ397955	DQ397977*
<i>Marathrum schiedeanum</i>	Novelo 3983	DQ397956	DQ397978*
<i>Oserya coulteriana</i>	Novelo 3235	DQ397957	n.a.

were
were
were

from
ecies
from
rum,
than
mic)
loyle
DNA

ular
e in-

ron

80

81

82

83

84

85

86

87

76

88

89

90*

75*

79*

74*

77*

78*

The ITS region was amplified using the primers ITS-5 and ITS-4 of White et al. (1990). The PCR profile was as follows: an initial denaturation step of 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 45 sec, and a final extension step of 72 °C for 5 min. We then sequenced the ITS region of cleaned PCR products using the same amplification primers plus the internal primers ITS-2 and ITS-3 (White et al. 1990).

The *trnK* 5'-intron was amplified using the primers *trnK*-3914F and *trnK*-2R by Johnson & Soltis (1994). The PCR profile was as follows: an initial denaturation step of 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 53 °C for 1 min and 72 °C for 3 min, and a final extension step of 72 °C for 5 min.

We then sequenced the ITS region using the same amplification primers plus the internal primers ITS-2 and ITS-3 (White et al. 1990). The *trnK* intron was sequenced using the same primers as for the amplification plus *trnK*-253F (Johnson & Soltis 1994). Sequencing was done on an ABI 377 Sequencer, using a cycle sequencing procedure (PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit, Applied Biosystems Inc.). Sequences were aligned visually with gaps treated as missing data. The ITS data contained two highly variable regions, one in ITS-1, and one in ITS-2. The pattern of divergence made it impossible to align the outgroup sequences against the ingroup sequences in the highly variable regions. Consequently, the outgroup sequences were represented by "?"-characters in the variable regions in the matrix. We were unsuccessful in acquiring the *trnK* intron sequences for the following specimens: *Podostemum scaturiginum* (CPT 5602), *Oserya coulteriana* (CPT 3235), *Podostemum comatum* (CPT 5633), *P. ceratophyllum* (LM 0001), *P. distichum* (CPT 5598), and *P. rutifolium* (CPT 5406). The following species are represented by partial *trnK* intron sequences only in the data matrix (due to a failing primer): *Apinagia cf. rangiferina* (CPT 5579), *A. yguazuensis* (TPN 2122), *Marathrum minutiflorum* (Novelo 3978), *M. schiedeanum* (Novelo 3983), *Podostemum rutifolium* subsp. *rutifolium* (CPT 5040), and *Podostemum weddellianum* (CPT 5000).

Phylogenetic analysis

The data set was analysed with PAUP* 4.0b10 (Swofford 2000) using the branch-and-bound algorithm (furthest addition, Maxtrees set to 100,000). Morphological and molecular data were analysed separately to test congruence, and combined in a total evidence approach. The criterion of congruence was arbitrarily defined as conflicting nodes that have at least 70 % bootstrap support (Mason-Gamer & Kellogg 1996). The trees from the individual data sets were found to be equivalent when they contained no robust incongruent groupings, thus suggesting that they could be combined (Johnson & Soltis 1998, Wiens 1998, Eldenäs & Linder 2000).

The support for each node was calculated using bootstrap analysis (Felsenstein 1985). Bootstrap values were obtained using PAUP* with 1000 bootstrap replicates, each replicate comprising 10 random addition sequence replicates (nchuck=10, chuckscore=1) with TBR branch-swapping and MULPARS in effect.

3. Results

Comparative morphology

To illustrate essential features of *Podostemum* (including "Crenias") we have chosen *P. distichum* (Figs. 1–23) as it is morphologically the most variable species in the genus. In the description of the remaining species groups (Figs. 24–77)

we give emphasis on anatomical data and focus on characters deviating from *P. distichum*. We identified three major groups in *Podostemum* (including "Crenias") based on their morphological similarity and the results of the cladistic analysis (see Fig. 79). These are the *P. distichum* clade, the *P. ceratophyllum* clade and the *P. muelleri* grade, comprising *P. muelleri* and all "Crenias" species (e.g. *P. ovatum*, *P. scaturiginum*, and *P. weddellianum*). The three groups and additional subgroupings are described below.

Podostemum distichum clade

The species in the *P. distichum* clade have a suite of morphological characters in common (see description below) that allow to distinguish them clearly from other species in the genus. Philbrick & Novelo (2004) came to the conclusion that six closely related species, i.e.: *P. aguirense* (syn. *P. warmingii*), *P. atrichum*, *P. distichum* sensu stricto, *P. glaziovianum*, and *P. schenckii* as recognized by Royen (1954), have to be lumped into one species, *P. distichum*. This species together with the recently described new species *P. irgangii* (Philbrick & Novelo 2001) form the *P. distichum* group.

1. *Podostemum distichum* (Figs. 1–23)

Roots: Green, elongated and ribbon-like, cross section narrow elliptical (Fig. 1), provided with an asymmetrical root cap (Fig. 2), attached to the substratum via fingerlike holdfasts (Fig. 3) and adhesive hairs (Fig. 4); roots endogenously branched or (rarely) exogenously branched (Fig. 6), growing intertwined; silica-bodies present in peripheral root cells (Fig. 5).

Shoot position on root: Shoots arising endogenously (Figs. 4, 10) and often opposite along the margin of the root; positionally associated with exogenous holdfasts (Figs. 3, 8).

Shoots: Up to 8 cm long in the material studied here (up to 60 cm according to Philbrick & Novelo 2004), flexible (upright when young), larger stems often twisted and with longitudinal furrows (resembling *P. comatum*), stem 1–3 mm in diameter at base, internodes short, distichous phyllotaxis, branched or unbranched (Fig. 7). Branching is correlated with presence of double-sheathed leaves (Figs. 8, 17). Proximal stem portions show leaf sheaths only (upper leaf portions fallen away), complete leaves are still present towards the shoot tip.

Leaves: Repeatedly forked or pinnate, 1–4 cm long (Figs. 11, 12), petiolate, cross section elliptical at base to flattened near tip with narrow groove along midrib, with unicellular hairs in groove (Fig. 13). The leaf divisions are often arranged in a 3-dimensional manner, with consecutive forks more or less perpendicular to the plane of previous ones along the growth axis of the leaf (Figs. 11, 14). Depending on the population the forked leaves are provided with additional verticillate or semi-verticillate scales along the rachides, as found in *P. distichum* s. str. (Figs. 7, 15); or the forked leaves lack such additional scales along the elongated leaf segments totally. This is observable in what was formerly known as *P. atrichum*, now conspecific with *P. distichum* (Figs. 11, 12).

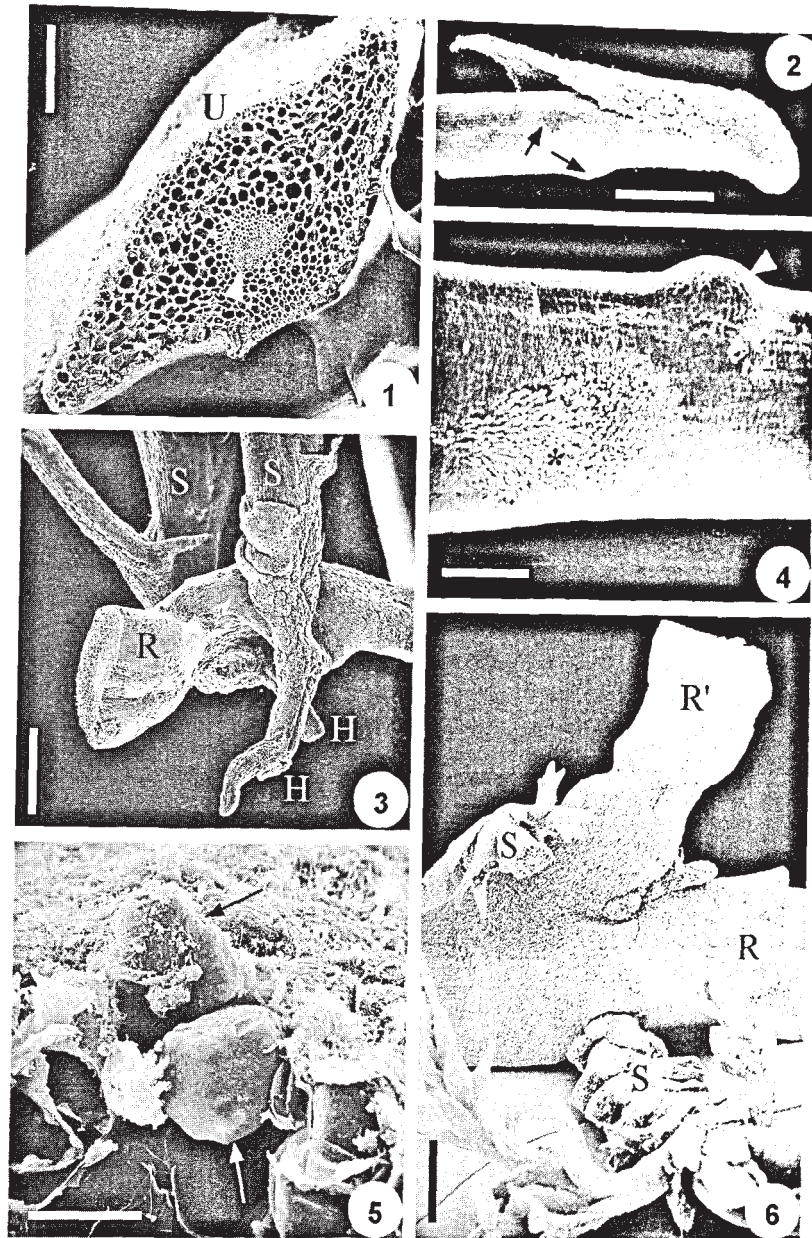
g from
cluding
ladistic
n clade
es (e.g.
l addi-

racters
7 from
on that
chum,
ed by
ies to-
lovelo

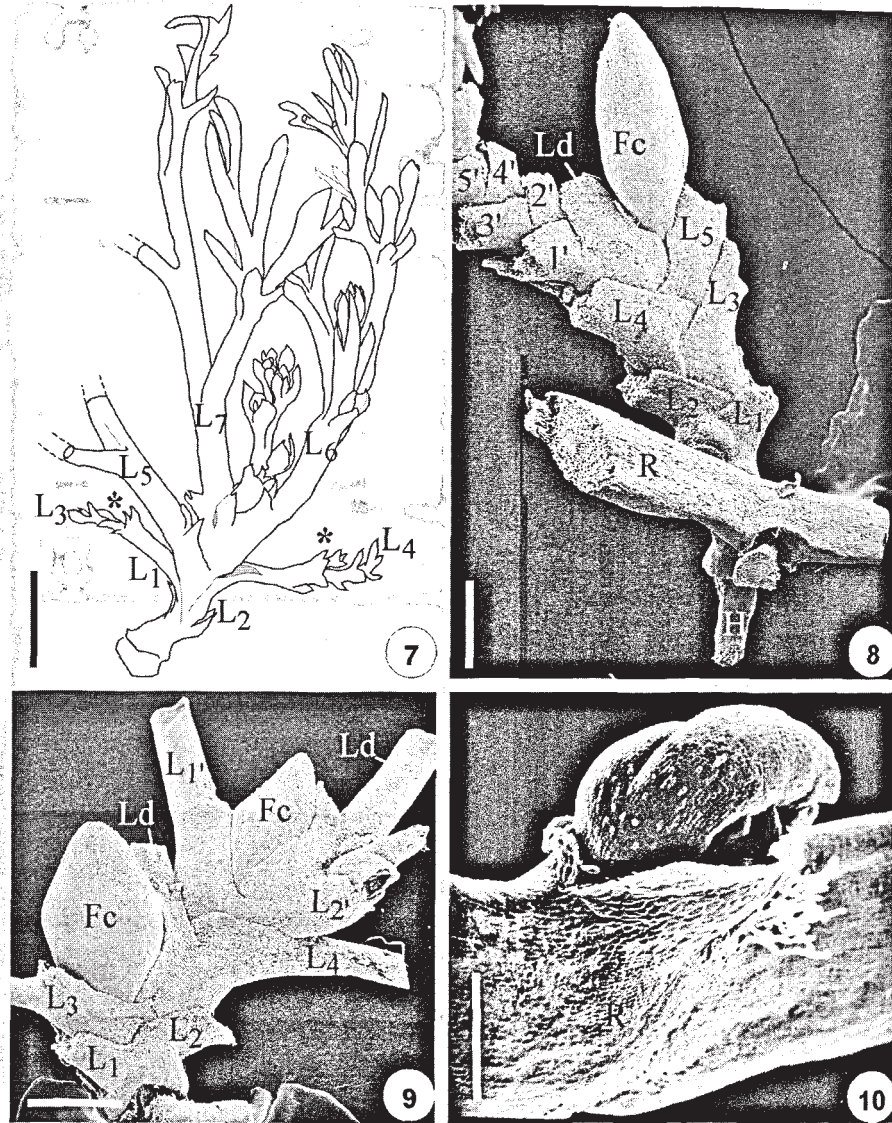
ptical
bstra-
dogen-
ined;

id of-
nous

rding
often
mm
d or
thred
leaf
tip.
plate,
long
n ar-
pen-
. 11,
onal
bum
lon-
n as



Figs. 1-6. Roots and adjacent structures of *Podostemum distichum*. — 1 (CTP 5003) Cross section of ribbon-like root, U= its upper side; arrowhead indicates central vascular tissue; bar = 250 μ m. 2 (CTP 5078) Root tip with cap and pair of bumps = sites of endogenous shoot buds (arrows); bar = 500 μ m. 3 (CTP 5195) Root portion (R) with opposite root-born shoots (S) associated with finger-like holdfasts (H); bar = 1 mm. 4 (CTP 5019) Patch of adhesive hairs (asterisk) on ventral root surface and endogenous shoot bud (arrowhead) at root margin; bar = 250 μ m. 5 (CTP 5003) Silica bodies (arrows) in rhizodermal cells; bar = 25 μ m. 6 (CTP 5006) Root portion (R) with exogenous root branching (R') and endogenous shoots (S); bar = 1 mm.



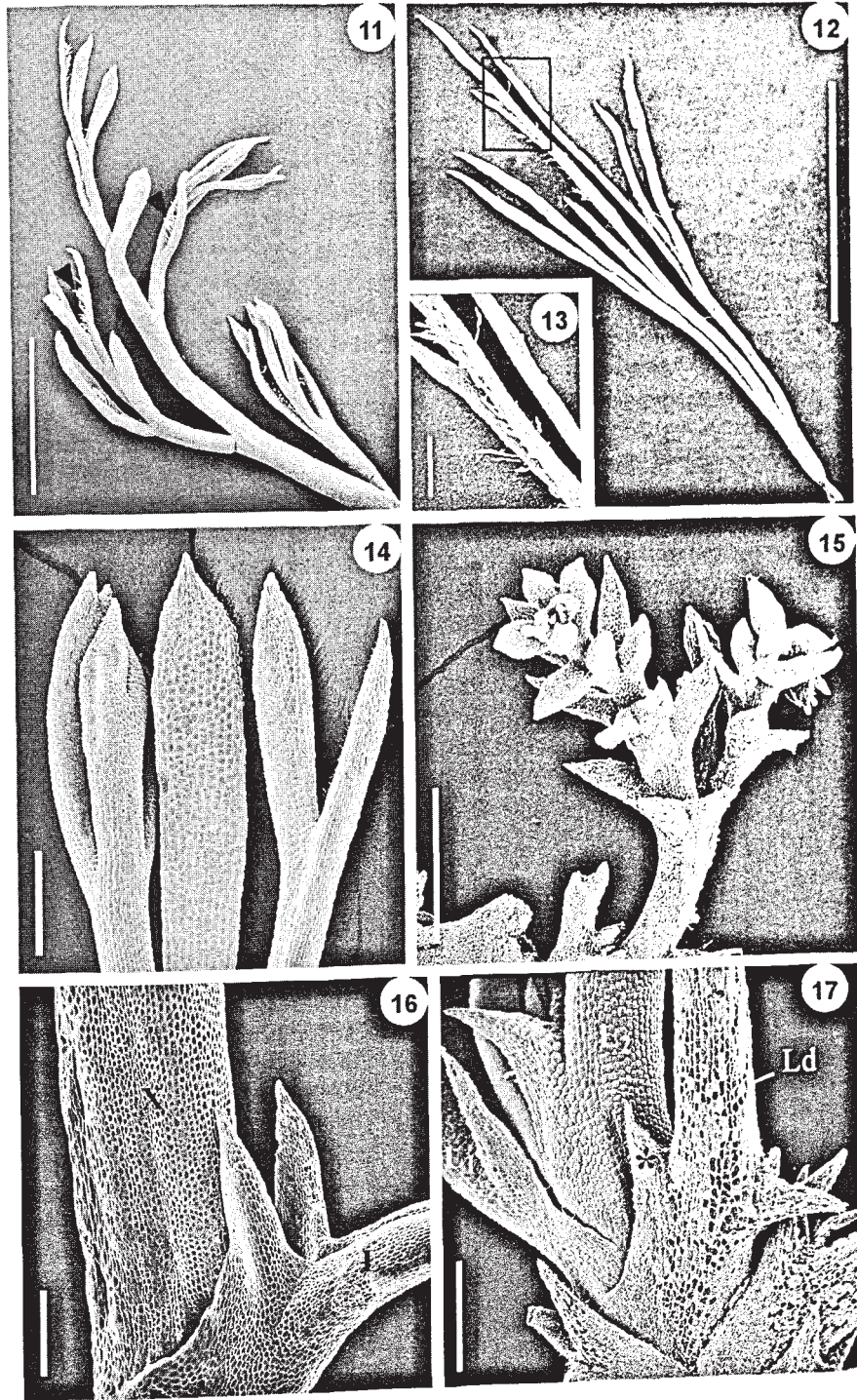
Figs. 7-10. Root-born shoots of *Podostemum distichum*. — 7 (CTP 5006) Overview of root-born shoot, proximal leaves (L3-L4) with short scales in pseudo-whorls (*), distal leaves (L5-L7) with stretched rachides and irregularly arranged subulate scales; bar = 2 mm. 8 (CTP 5008) Root-born fertile shoot showing flower bud in spatella (Fc) emerging from right sheath of a double-sheathed leaf (Ld); proximal shoot portion with remnants of leaf sheaths only (L1-L5), daughter shoot (with leaf sheaths 1'-5') emerging from left sheath of double-sheathed leaf; root (R) provided with finger-like holdfast (H); bar = 1 mm. 9 (CTP 5079) Fertile shoot with two flowers in spatellas (Fc) and two double-sheathed leaves (Ld), bases of other leaves are single-sheathed (L1-L4; L1'-L2'); bar = 1 mm. 10 (CTP 5019) First leaf of young endogenous shoot bud breaking through marginal root cortex, root (R) seen from below; bar = 250 μ m.

8

9

10

w of
istal
ar =
erg-
em-
rom
ar =
ible-
ar =
nar-



Stipules: Two or three (rarely four to six) prominent triangular stipular teeth, attached to a subamplexicaul leaf sheath (Figs. 16, 82). Leaf bases and basal parts of stipules (sometimes even whole stipules) persist after leaves have fallen away (Fig. 23).

Position of flowers: Young flowers are covered by a spathe, which is typically 2–3 times as long as broad (Figs. 8, 9). The presence of flowers is correlated with double-sheathed leaves. One sheath may contain a flower bud, whereas the other sheath contains a new leafy module, which can produce after few single-sheathed leaves another flower together with the next double-sheathed leaf. Thus, a sympodially branched shoot can have more than one flower (Fig. 9).

Flowers: Androecium consisting of two stamens with a common stalk (andropodium). There are three ligulate tepals, two adjacent to the base of the andropodium and the third in the fork between the two filaments (Figs. 18, 20), anthers with two slightly unequal thecae containing pollen in dyads. The ovary consists of two locules which are topped by two entire linear stigma lobes (Fig. 18). The flower has a pedicel which elongates after anthesis until fruit is mature (Fig. 22).

Fruit: The bilocular ovary of *Podostemum distichum* is smooth during anthesis (Fig. 18). Afterwards an anisolobous capsule is formed with two slightly asymmetrical valves which are both sclerified to some degree. The capsule shows eight ribs, i.e. 3 ribs per valve besides the sutural ribs at the border-line between the two valves (Fig. 21). Only the slightly larger valve is persistent on the flower stalk (pedicel), the smaller one is caducous (Fig. 22).

Seeds: The small seeds in the two locules are numerous but not exceeding 40 per capsule (Fig. 22).

2. *Podostemum irgangii* (Figs. 24, 25)

Podostemum irgangii has a maximum stem length of only 2–4 cm, while *P. distichum* is highly variable with respect to shoot length and often much longer than 5 cm. Leaf and stipule characters usually differ from *P. distichum* and allow to recognize *P. irgangii* as distinct species (Philbrick & Novelo 2001, 2004).

Page 13:

Figs. 11–17. Polymorphic leaf blades and stipulate leaf sheaths of *Podostemum distichum*. — 11–12 (11: CTP 5003, 12: CTP 5079) Compound leaflets of nearly mature pinnate leaves, resembling *P. atrichum* Chodat & Vischer, lacking scales along rachis; bar = 1 mm. 13 (CTP 5079) Close-up of ultimate segments with hairs along one sector; bar = 300 µm. 14 (CTP 5005) Ultimate leaflet divisions with subsequent bifurcations and trifurcations; bar = 500 µm. 15 (CTP 5006) Ultimate blade divisions with scales arranged in pseudo-whorls along rachis, resembling *P. distichum* sensu stricto; bar = 500 µm. 16 (CTP 5195) Base of single-sheathed leaf (L) with 2 attached stipular teeth (X = stem); bar = 500 µm. 17 (CTP 5003) Double-sheathed leaf (Ld) in nearly terminal position of shoot tip. Shoot growth continues with single-sheathed leaves (L₁, L₂) in left stipular sheath (asterisk); bar = 500 µm.

ular
asal
llen

h is
re-
ud,
fter
le-
wer

alk
the
(20),
ary
fig.
ure

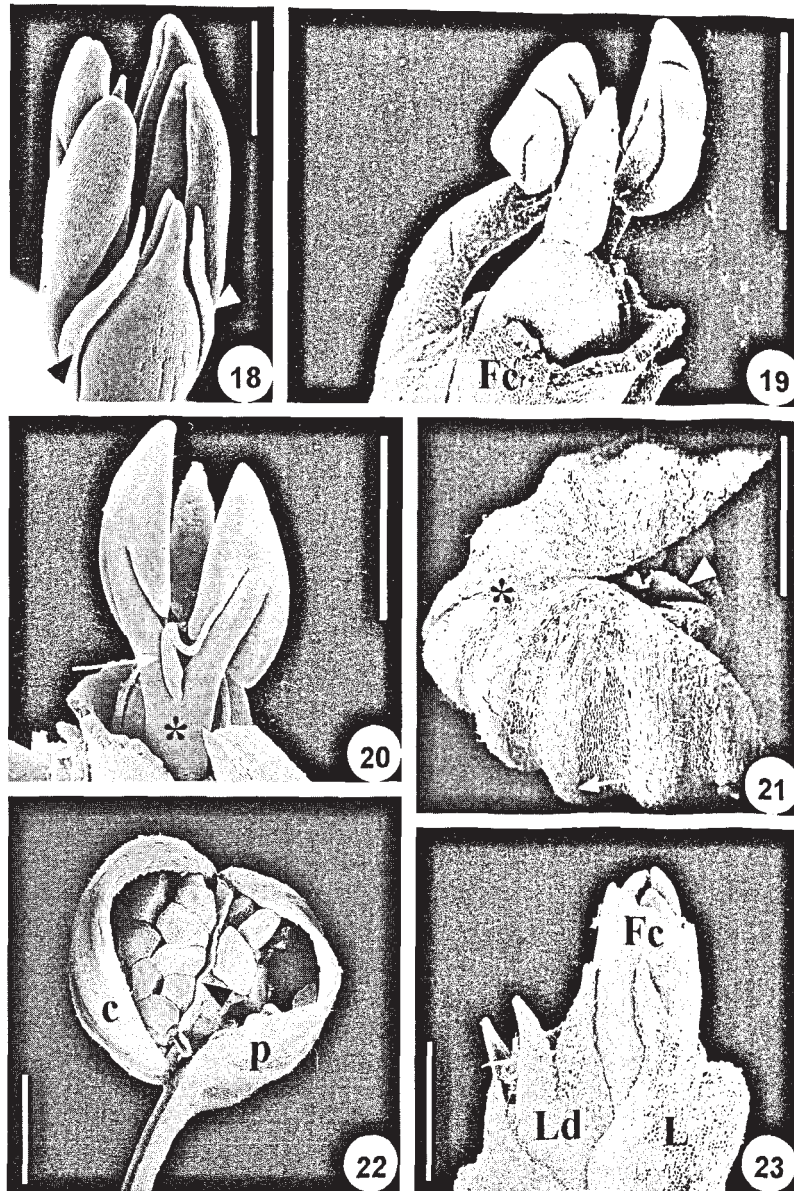
ing
tly
ws
een
ver

40

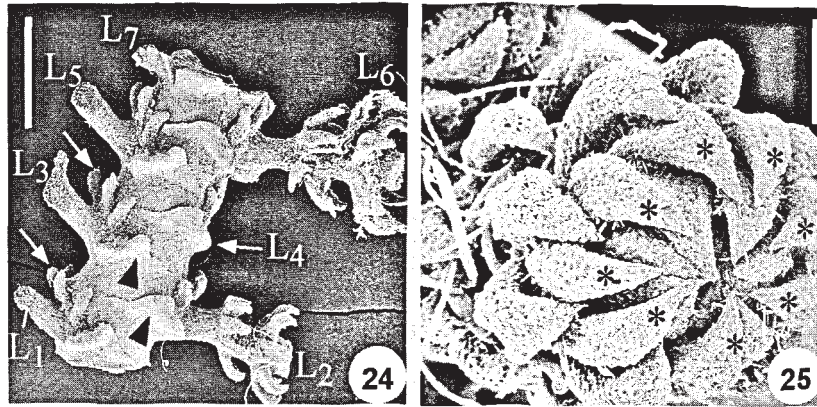
ile
ger
ow

—

m.
ate
m.
m.
ns;
in
IP
=
not
ith



Figs. 18–23. Reproductive structures of *Podostemum distichum*. — 18 (CTP 5020) Flower bud after removal of spathe. Note two thread-like tepals (arrowheads), ovary with two stigmas, and two anthers with slightly unequal thecae; bar = 500 μ m. 19–20 (CTP 5078) Two opposite views of flower bud after removal of upper spathe portion. Arrow points to third tepal in fork where stamens are fused into andropodium (asterisk); bars = 1 mm. 21–22 (CTP 5003) Top view and lateral view of stalked mature capsule, dehiscing on one side only (as artifact of CPD prior to scanning electron microscopy). Capsule with eight ribs, three per valve (arrow) and two sutural ribs, one of the latter indicated by asterisk in Fig. 21. Note septum (arrowhead), one valve is caducous (c), one is persistent (p) on the capsule stalk; bars = 500 μ m. 23 (CTP 5004) Young flower in spathe (Fc, artificially opened at the top) surrounded by single-sheathed leaf (L) and double-sheathed leaf (Ld), both with blade already dropped; bar = 1 mm.



Figs. 24–25. *Podostemum irgangii* (CTP 5441). — 24 Shoot portion showing leaf bases (L1–L7) with two types of stipular segments: lateral lobes (arrowheads) and intrapetiolar teeth (arrows); bar = 1 mm. 25 Tip of young leaf seen from above showing rachis scales arranged in whorls (or nearly so), scales of a single whorl are marked with asterisks; bar = 250 μ m.

Leaves: Unlike *P. distichum* the leaves are never forked into more than two segments (rachides). The distichously arranged leaves usually consist of a single rachis from which several 6- to 11-merous whorls of triangular to subulate scales arise (Fig. 24). The leaves are 3–7 mm long and provided with 5–14 whorls of scales. Young leaf tips show these verticillate scales quite nicely (Figs. 25, 82).

Stipules: Two types of stipules are present. Two ear-like expansions are observable on either side of the leaf insertion area. They can be called detached lateral stipules, whereas the stipules of *P. distichum* are attached to the leaf sheath. Additional 3–9 stipular teeth (i.e. narrow scales) are arranged in a row along the adaxial insertion line of the petiole adjacent to the stem (Fig. 24).

Podostemum ceratophyllum clade

This clade comprises *P. ceratophyllum*, *P. comatum*, and *P. rutifolium*. Within this clade *P. ceratophyllum* is sister to *P. comatum*, *P. rutifolium* subsp. *rutifolium* is sister to *P. rutifolium* subsp. *riccifforme*. The mainly North American species *Podostemum ceratophyllum* is omitted in the following description as its (developmental) morphology has already been described by Warming (1881), Hammond (1936, 1937) and Rutishauser et al. (2003).

According to Philbrick & Novelo (2004) the three closely related mainly S Brazilian species *P. fruticulosum*, *P. ostenianum* and *P. rutifolium* as recognized by Royen (1954) have to be lumped into one species, *P. rutifolium*. In addition, they described *Podostemum riccifforme* as subspecies of *Podostemum rutifolium* based on their morphological similarity. The two subspecies of *Podostemum rutifolium* have a suite of morphological characters (see description below) in

c
n
r
n
1
a
b
r
s
T
g
b
b
s
o
s
o
s
s
b
T
1
r
n
3
a
c
n
l
d
c
r
(j
a

common. They are not clearly distinguishable from each other as smaller specimens of *P. rutifolium* subsp. *rutifolium* closely resemble *P. rutifolium* subsp. *ricciiforme* (Philbrick & Novelo 2004). The two subspecies were kept separate mainly because of their disjunct distribution.

1a. *Podostemum rutifolium* subsp. *rutifolium* (Figs. 26–31)

Roots: Ribbon-like 0.2–3.4 mm wide. Root-born shoots and daughter roots arise endogenously along the root flank whereas holdfasts (finger-like or branched) arise exogenously from the root, but positionally associated with root-born shoots (Figs. 27, 28).

Shoots: The shoots are prostrate to erect, up to 9 cm long, usually much shorter. They arise in opposite or subopposite pairs along the ribbon-like root. The stems are branched or unbranched with internodes short or slightly elongated (Figs. 27, 28).

Leaves: They are up to 2 cm long, simple or few times forked with ribbon-like segments. Similar to *P. comatum*, but unlike *Podostemum distichum* all bifurcations of the blade lie in the same plane (Fig. 26). All leaf blades of a shoot show distichy, they are again flattened in the same plane, somewhat similar to the orientation of the ensiform leaves of *Acorus* and *Iris* (Fig. 28). The ultimate leaf segments which may be coiled to some degree are provided with a patch of hairs on the upper side, i.e. on the surface facing away from the substratum in prostrate shoots (Fig. 28). Leaf tip can be obtuse or acute (Fig. 26).

Stipules: Each leaf base is provided with two lateral stipules as part of a subamplexicaul sheath. The two stipules usually are fused into one bifid boat-shaped structure in front of the leaf to which they belong (Figs. 29–31). Thus, the stipules are similar to those of *P. comatum* (Fig. 82).

1b. *Podostemum rutifolium* subsp. *ricciiforme* (Figs. 32–37)

Roots: Ribbon-like, 0.2–1.0 mm wide, provided with an asymmetrical root-cap (Figs. 32, 33), with daughter roots and root-born shoots arising endogenously (Fig. 34, also Koi et al. 2006, their figs. 18–23).

Shoots: The shoots are prostrate or ascending, up to 2.5 cm long (rarely up to 3.5 cm), branched or unbranched. They arise pair-wise and opposite or rarely alternate along the margin of the ribbon-like root (Fig. 34). Shoot branching is correlated with the occurrence of a double-sheathed leaf at the end of a shoot module (sympodial unit). Each sheath of a double-sheathed leaf gives rise to a leafy daughter module (Figs. 35, 36). Similar shoot branching (combined with double-sheathed leaves) also occurs in *P. rutifolium* subsp. *rutifolium*.

Leaves: Entire to once forked, up to 8 mm long. Blade (or its segments) clearly flattened and broadened towards the tip (Fig. 32). Leaf tips of subsp. *ricciiforme* are always obtuse whereas those of subsp. *rutifolium* are often acute.

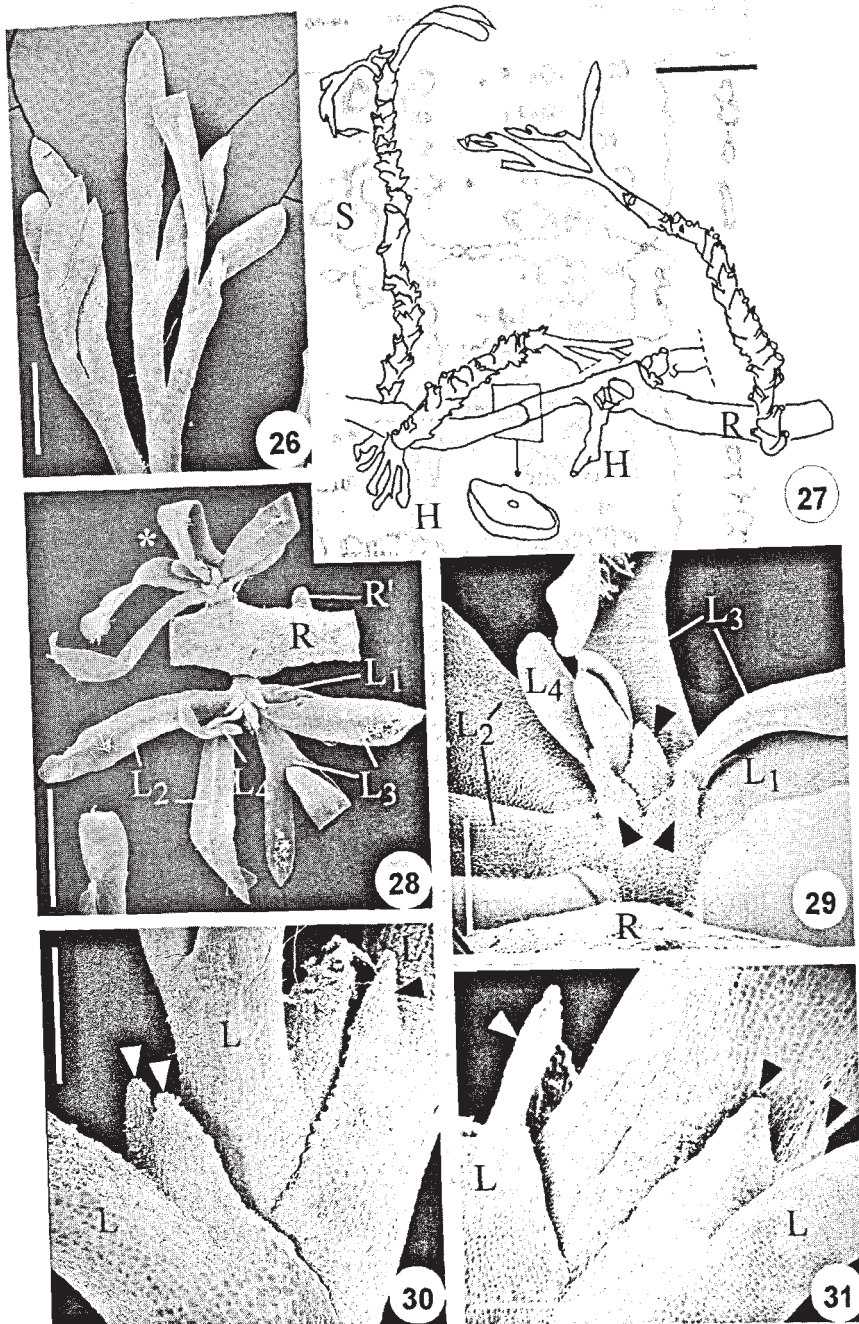
Stipules: Each amplexicaul leaf sheath extends into an entire median (intrapetiolar) stipule which is boat-shaped (Fig. 35), similar to *P. ceratophyllum* and usually not bifid as in *P. rutifolium* subsp. *rutifolium* (Fig. 82).

ases
iolar
cales
ar =

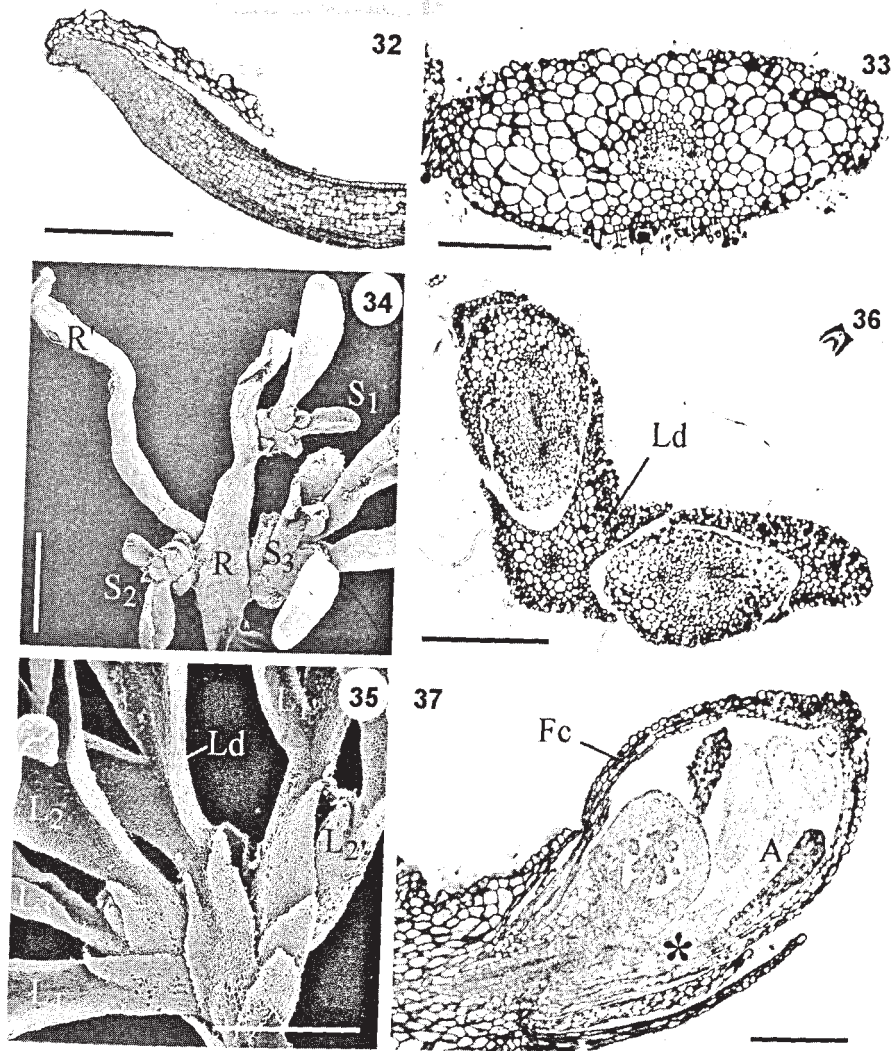
two
ngle
ales
ls of
32).
are
hed
ath.
; the

thin
ium
cies
vel-
81),

inly
zed
ion,
ium
um
) in



Figs. 26–31. *Podostemum rutifolium* subsp. *rutifolium*. — 26 (CTP 5018) Ultimate divisions of repeatedly forked leaf blade; bar = 1 mm. 27 (CTP 5021) Drawing of a plant with creeping root (R), holdfasts (H) and elongated stems (S); bar = 4 mm. 28 (CTP 5008) Ribbon-like root portion (R) with two endogenous shoots, seen from above. Forked leaves of one shoot labeled with L₁–L₄. Note presence of lateral root (R'); bar = 2 mm. 29 (CTP 5008) Root (R)-born shoot with forked leaves L₁–L₄ (same as shoot marked with asterisk in Fig. 28) seen from the upper side; arrowheads point to lateral stipules; bar = 500 μ m. 30–31 (30: CTP 5018, 31: CTP 5025) Close-ups of shoot portions with distichously arranged leaf bases (L). Lateral stipules (arrowheads) are fused in front of leaf blade; bars = 500 μ m.



Figs. 32-37. *Podostemum rutifolium* subsp. *ricciiforme* (Novelo 1426). — 32 Longitudinal section of root tip with asymmetrical cap; bar = 300 μ m. 33 Transversal root section. Note adhesive hairs along lower side; bar = 300 μ m. 34 Ribbon-like root with endogenous shoots (S_1 - S_3) and endogenous lateral root (R'); bar = 1 mm. 35 Branched shoot with double-sheathed leaf (L_d) in terminal position, left daughter shoot with leaves L_1 - L_3 and right daughter shoot with leaves L_1' - L_2' ; bar = 1 mm. 36 Transversal section of double-sheathed leaf (L_d) with daughter shoots in left and right sheath; bar = 500 μ m. 37 Longitudinal section of root-born short-shoot consisting of few bract-like leaves and flower in spathe (F_c). Note dorsiventral floral symmetry with androecium (A) on lower side and bilocular ovary on upper side. Note the presence of a central tepal arising from the andropodium (asterisk); bar = 250 μ m.

divi-
with
Rib-
aves
CTP
risk
 μ m.
ar-
rs =

2. *Podostemum comatum* (Figs. 38–44)

Philbrick & Novelo (2004) combined Royen's (1954) *P. comatum* sensu stricto, *P. dimorphum* and *P. undulatum* into *P. comatum*.

Roots: Similar to *P. rutifolium*, 0.8–2.6 mm wide ribbons.

Shoots: *Podostemum comatum* has the largest shoots of all species of *Podostemum*. They are dimorphous with sterile and fertile shoots having different size. The sterile (vegetative) shoots are pendulous and up to 72 cm long. They are often twisted and may have furrows (Fig. 43). Fertile shoots are erect and much shorter, only up to 1 cm long, sometimes without or only with a few leaves arising from the root. The stems are often slightly flattened in the same plane as the two leaf rows are inserted and usually contain three (or two) parallel vascular stem bundles, each bundle with a (nearly) circular outline (Figs. 42, 43). Elongate shoots which are sterile (i.e. lacking flowers) during the raining season may finally produce a few flowers in their basal zone. This reproductive capacity seems to be due to preformed double-sheathed leaves which (first) give rise to a prominent shoot. Much later (at the end of the rainy season) the flowers appear in the second sheath of the double-sheathed leaves (Figs. 39, 40).

Leaves: Different in size on sterile and fertile shoots. Leaves are up to 5–8 cm long on sterile shoots, repeatedly forked, with ultimate divisions clearly flattened and spatulate, up to 5 mm long (Figs. 38, 41), petioles 5–20 mm long. Leaves on fertile shoots are usually much shorter with a forked blade up to 1 cm or lacking the blade.

Stipules: There is an entire or bifid (rarely trifid) stipular sheath (i.e. intrapetiolar stipule, attached to the leaf base), one per single-sheathed leaf and two of them per double-sheathed leaf (Figs. 39, 40, 42, 82).

***Podostemum muelleri* grade**

Podostemum muelleri and the "Crenias" species studied (*P. ovatum*, *P. scaritiginum*, and *P. weddellianum*) form a grade at the base of the *Podostemum ceratophyllum* clade (Fig. 79). The three species of "Crenias" included in this

Figs. 38–44. *Podostemum comatum*. — 38 (CTP 5045) Ultimate leaf divisions, bar = 2 mm. 39–40 (CTP 5027) Two opposite views of shoot tip, showing double-sheathed leaf with blade dropped (Ld) and flower bud in spatella (Fc). Arrows point to bifid stipular sheath towards the well-developed shoot with massive stem (X); bars = 1 mm. 41 (CTP 5022) Transversal section of two ultimate leaf divisions; bar = 200 μ m. 42 (CTP 5022) Longitudinal stem (X) section with leaf insertion. Note presence of 3 parallel stem vascular bundles, one of them supplying the leaf (L). Arrowhead points to stipular outgrowth; bar = 3 mm. 43 (CTP 5022) Transversal section of stem below leaf node. Note presence of 3 stem bundles. The "L" bundle is the leaf trace (see Fig. 42); the two "X" bundles remain in the stem; bar = 1 mm. 44 (CTP 5022) Close-up of stem vascular bundle, showing phloem element with sieve plates; bar = 10 μ m.

sensu

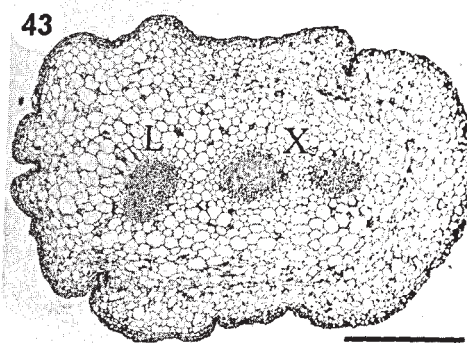
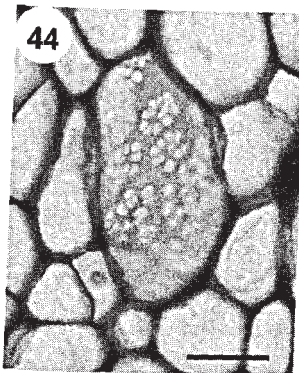
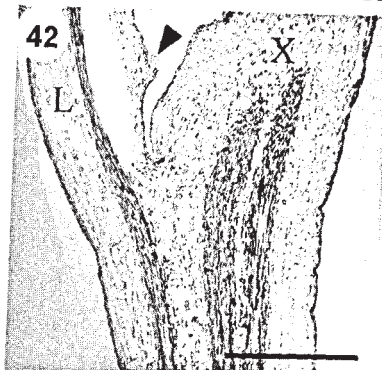
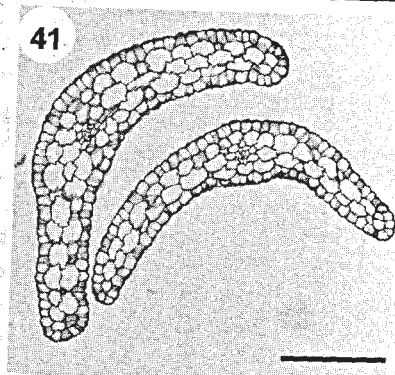
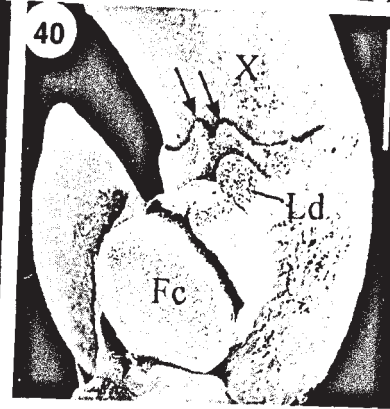
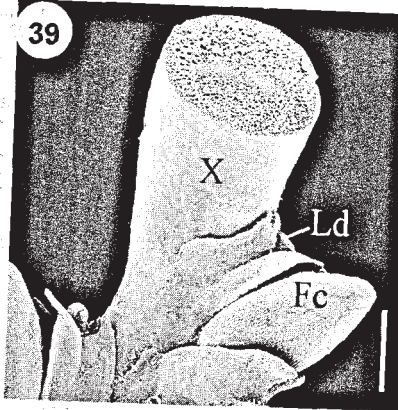
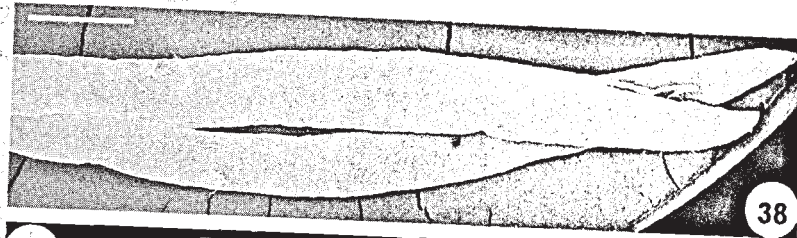
Pod-
ferent
ey are
much
rising
e two
stem
ngate
nally
to be
inent
cond

8 cm
ened
s on
king

(i.e.
and

ca-
um
his

= 2
eaf
lar
[P
2)
u-
h;
of
in
g



study form a clade, which is sister to the *Podostemum ceratophyllum* clade. We describe these species here as a group, even though they form a paraphylum (grade).

The vegetative body of *P. muelleri* is somewhat similar to the the "Crenias" species *P. ovatum*, *P. scaturiginum* and *P. weddellianum*. Both, *Podostemum muelleri* and "Crenias" share two unique synapomorphies: (I) The leaf sheaths are asymmetrical with one conspicuous stipule attached to the sheath half which is situated towards the upper side of the dorsiventral shoot (Fig. 81). (II) There are only inconspicuous capsule ribs in *Podostemum muelleri* while the species in "Crenias" lack capsule ribs completely, as opposed to all other *Podostemum* species which have conspicuous (prominent) capsule ribs. *Podostemum scaturiginum* and *P. weddellianum* have two palmately branched stigmas, whereas *P. muelleri* and *P. ovatum* have entire stigmas similar to all remaining *Podostemum* species.

1. *Podostemum muelleri* (Figs. 45–56)

Podostemum muelleri as proposed by Philbrick & Novelo (2004) comprises *P. dentatum*, *P. galvone*, *P. muelleri* sensu stricto and *P. uruguayense*, as distinguished by Royen (1954).

Roots: The roots are usually narrow ribbons, up to 1 mm wide (rarely broader). The root tip is provided with an asymmetrical cap (Fig. 46). Daughter roots and root-born shoots arise from endogenous buds along the root flanks (Figs. 45, 53). The roots are attached to the rock by adhesive hairs and finger-like holdfasts (Figs. 52, 53).

Shoots: Ascending to erect, up to 7 cm long, frequently branched, branching and flower position correlated with double-sheathed leaves, similar to other *Podostemum* species (Fig. 48).

Leaves: Leaf and stipule characters in *P. muelleri* are clearly different from the *Podostemum* species described so far. Typical for *P. muelleri* are rigid, ovate to elliptical sheaths, which are flattened and lie in the same plane in which the two distichously arranged leaf rows are inserted (Figs. 47, 48, 54, 55). Cross-sections clearly show that the two rows of V-shaped leaf sheaths are inserted opposite each other (Figs. 54, 55). The obliquely inserted leaf bases pass abruptly into a usually entire (rarely forked) linear blade up to 8 cm long (Figs. 48, 49). There may be some hairs along one sector of this linear blade (Fig. 50).

Leaf sheaths and stipules: There is usually only one stipule per leaf attached to the asymmetrical leaf sheath on the upper (front) side of the shoot (Figs. 47, 54). Careful inspection of the leaf sheaths, however, allows to observe an inconspicuous tooth-like stipule on the lower (back) side of the shoot (Fig. 80). Double-sheathed leaves have two opposite sheaths and two prominent stipules facing the upper shoot side (Fig. 55).

Fruits: Capsules are provided with 3 ribs per valve. The capsule ribs, however, are quite inconspicuous. The capsule splits into two unequal valves, one is caducous and one is persistent on the top of the capsule stalk (Fig. 51).

Figs.
with
label
metr
plane
Leaf
Ferti
shoo
pres
leaf
one
tially
(aste

. We
 lum

 ias"
 num
 aths
 hich
 e are
 s in
 spe-
 uri-
 reas
 ste-

 ises
 tin-

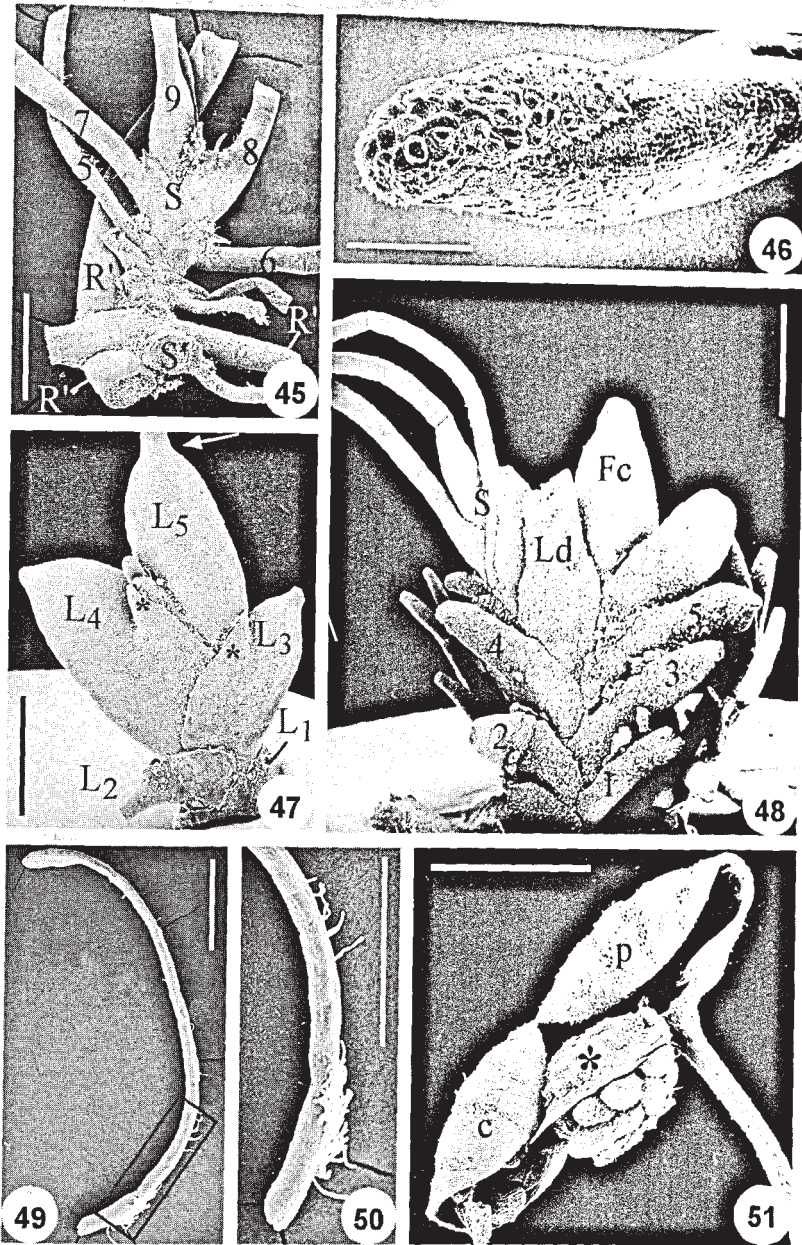
 rely
 nter
 nks
 like

 ing
 her

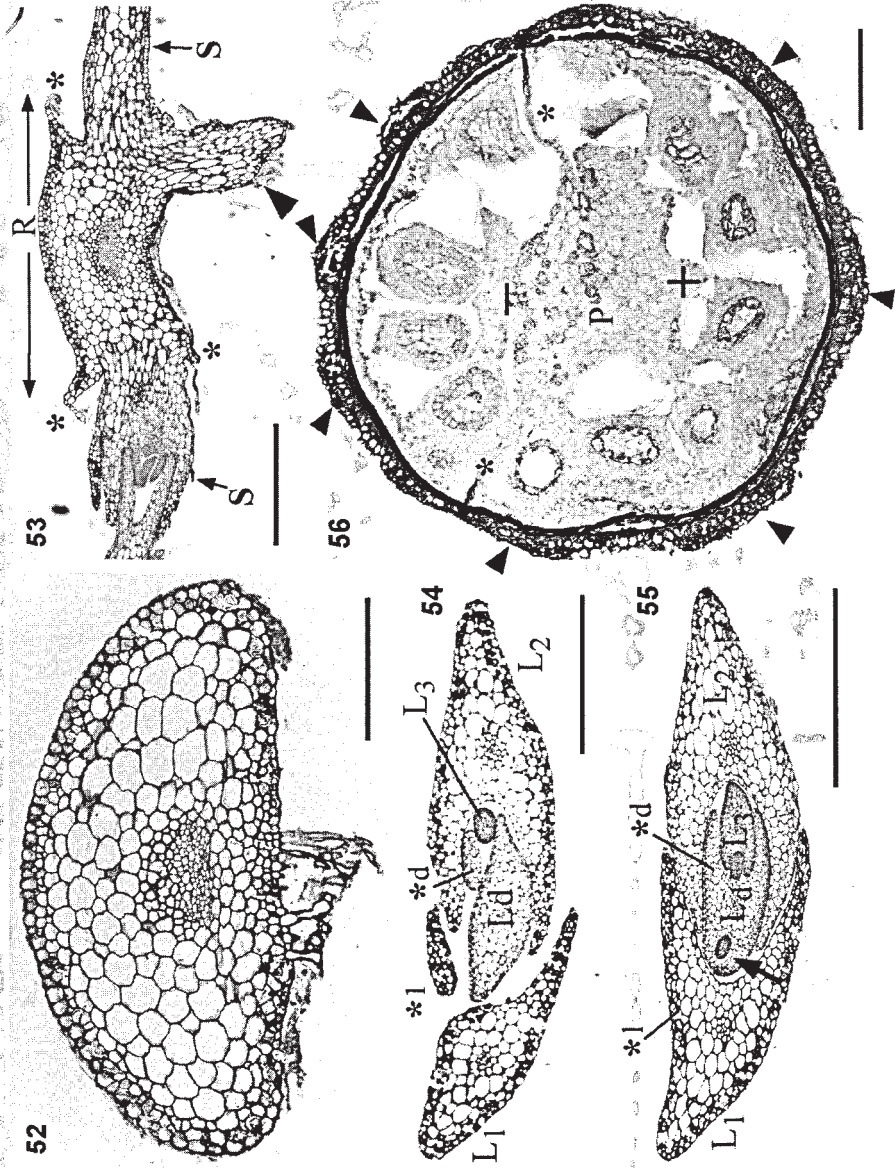
 om
 e to
 wo
 ns
 ach
 lly
 be

 eaf
 not
 an
 O).
 les

 w-
 is



Figs. 45-51. *Podostemum muelleri*. — 45 (CTP 5010) Root (R)-born shoot pair (S/S') with adjacent pair of endogenous daughter root (R'), only upper leaves (5-9) of shoot S labelled, opposite shoot (S') cut away; bar = 1 mm. 46 (CTP 5023) Root tip with asymmetrical root cap; bar = 250 μ m. 47 (CTP 5023) Broad leaf bases (L₁-L₅) flattened in one plane with only one stipular segment (asterisk) per leaf as observable from the front side. Leaf base narrows abruptly into filiform leaf blade (arrow); bar = 1 mm. 48 (CTP 5023) Fertile shoot seen from back (reverse) side, stipules are not observable on this side. First shoot module (with leaves 1-5) ends up into double-sheathed leaf Ld and flower Fc; note presence of branch = second shoot module (S); bar = 1 mm. 49 (CTP 5023) Entire filiform leaf blade; bar = 1 mm. 50 (CTP 5023) Close-up of leaf blade portion showing hairs along one side; bar = 1 mm. 51 (CTP 5023) Capsule with persistent capsule valve (p) and partially dropped caducous valve (c); note the seeds still attached to placenta with septum (asterisk); bar = 500 μ m.



2. Jä
 sti
 w.
 (F
 pl
 pl
 fo
 al
 at
 in
 3.
 w
 li
 fl
 L
 d
 (I
 al
 —
 F
 ra
 b:
 sl
 tu
 b:
 sl
 b:
 st
 d:
 sl
 tu
 b:
 c

2. *Podostemum weddellianum* (syn. *Crenias weddelliana*, Figs. 57–59)

The developmental morphology of this species was recently described by Jäger-Zürn (2002). Our fixed material (CTP 5000) did not contain reproductive structures.

Roots: narrow ribbons usually less than 1 mm wide, root tips provided with asymmetrical caps, adhesive hairs on the lower side of the root ribbon (Figs. 57–59)

Shoots: Ascending to erect, dorsiventral, up to 2 cm long, with distichous phyllotaxis, branched or unbranched.

Leaves: inserted in two rows, the leaf laminas being arranged in the same plane. Leaves are up to 2.5 cm long. The leaf blades are linear, entire or a few times forked. Ultimate divisions are linear to spatulate, occasionally with some hairs along one side.

Leaf sheaths and stipules: The leaf sheaths are overlapping and obliquely attached to the stem. There is only one stipule per leaf, attached to the leaf sheath in a submarginal position (Fig. 80).

3. *Podostemum ovatum* (syn. *Crenias glazioviana*, Figs. 60–64)

Our fixed material (CTP 5001) did not contain reproductive structures. Thus, we studied only vegetative stages.

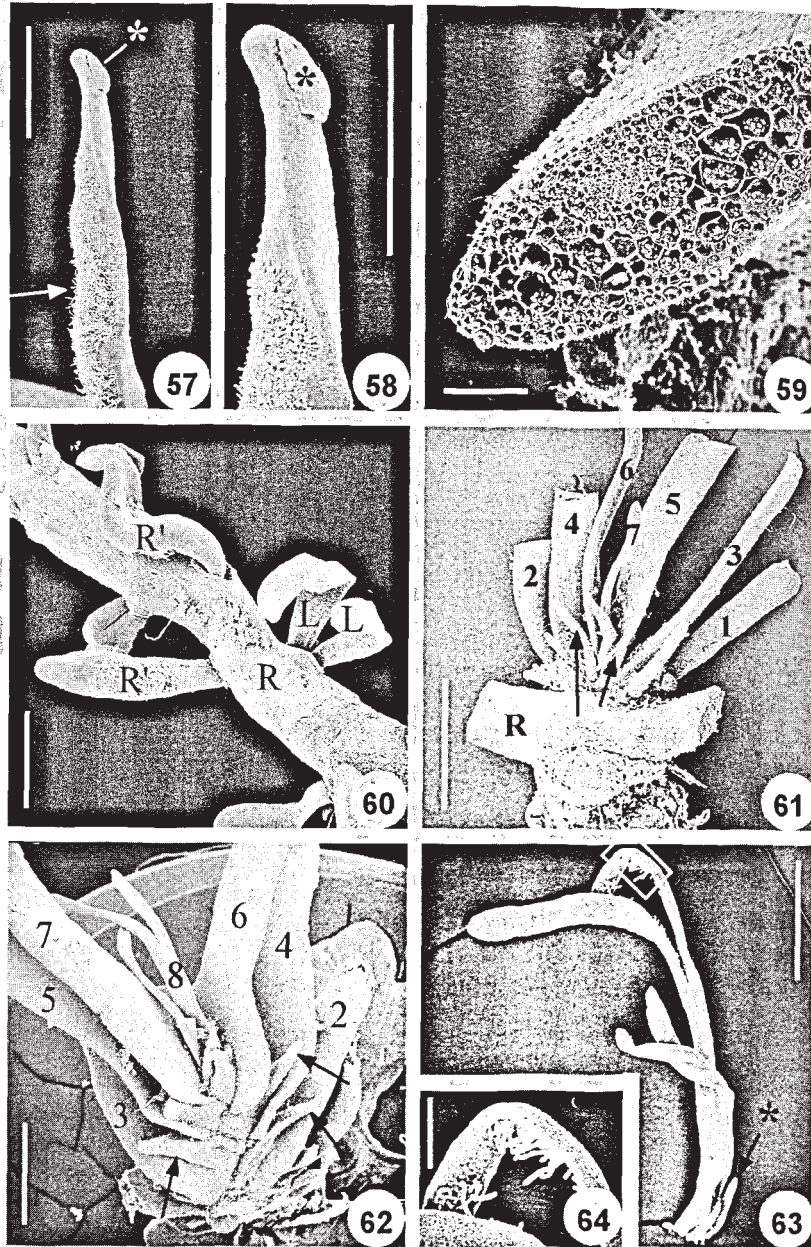
Roots: narrow ribbons up to 0.8 mm wide (Fig. 60), similar to *P. weddellianum*. Lateral roots and shoots arise from endogenous buds along the root flanks (Figs. 60, 61).

Shoots: up to 1.5 cm long (Fig. 62), otherwise similar to *P. weddellianum*.

Leaves: lying more or less in the same plane as the two leaf rows are inserted. Leaves are up to 1.2 cm long, the leaf blades are forked one or two times. Ultimate divisions are linear to spatulate, occasionally with some hairs along one side (Figs. 63, 64).

Sheaths: The leaf sheaths are overlapping and asymmetrically arranged along the shoot like in *P. weddellianum* (Fig. 80). Unlike *P. weddellianum* the leaf

Figs. 52–56. *Podostemum muelleri* (CTP 5023). — 52 Transversal section of dorsiventrally flattened root with central vascular bundle. Note adhesive hairs along lower surface; bar = 200 μ m. 53 Transversal section of root (R) with two endogenously formed lateral shoots (S). Note leaves and their sheaths on left side. Asterisks indicate portions of ruptured root cortex. Arrow-head points to finger-like holdfast with bunch of adhesive hairs; bar = 500 μ m. 54, 55 Two consecutive transversal sections of dorsiventrally flattened shoot tip, with two single-sheathed leaves (L_1 , L_2) in distichous arrangement and a double-sheathed leaf (L_d) in between. Top = front side, bottom = back side of shoot. A single stipular lobe (*1) belongs to leaf L_1 , another stipule (*d) belongs to the right sheath of the double-sheathed leaf L_d . A new leaf (L_3) is formed in this sheath. Arrow points to the left sheath of L_d containing a young leaf; bar = 500 μ m. 56 Transversal section of nearly mature capsule, with unequal size (\pm) of the two locules and 8 inconspicuous ribs (indicated by arrowheads); two of the ribs next to the sutures with septum (asterisk); central placenta (P) is surrounded by young seeds; bar = 200 μ m.



Figs. 57–59. *Podostemum weddellianum* (syn. *Crenias weddelliana*, *Mniopsis weddelliana*, CTP 5000). — 57–58 Root tip with asymmetric cap (asterisk) and adhesive hairs on ventral side (arrow) where the root was attached to the substratum, bar = 500 μ m. 59 Ribbon-like root, cells filled with starch grains; bar = 100 μ m.

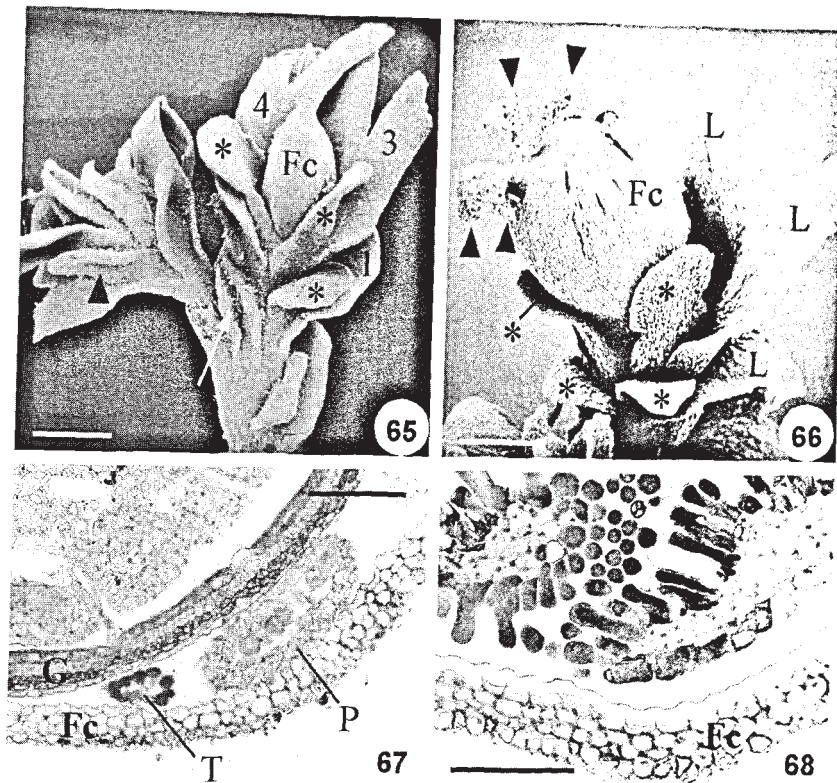
Figs. 60–64. *Podostemum ovatum* (syn. *Crenias glaziioviana*, *Mniopsis glaziioviana*, CTP 5000). — 60 Root portion (R) with endogenous shoot consisting of two leaves (L) and endogenous daughter roots (R'); bar = 1 mm. 61–62 Two root (R)-born shoots seen from front side (away from substratum), showing distichous phyllotaxis, leaves (1–8) with broad leaf bases, entire or forked blades, arrows point to subulate stipules (one per leaf); bars = 1 mm. 63 Nearly mature leaf with blade twice forked, note presence of subulate stipule (asterisk) attached to the leaf base; bar = 1 mm. 64 Close-up of leaf segment, showing hairs along one sector; bar = 250 μ m.

sheaths are widened and spoon-shaped ("ovate") and narrowed towards the blade (Fig. 32).

Stipules: The submarginal insertion of a single stipule asymmetrically on one side of the leaf sheath is similar to *P. weddellianum* (Figs. 61, 62, 80).

4. *Podostemum scaturiginum* (syn. *Crenias scaturigina*, Figs. 65–77)

Roots: narrow ribbons, usually less than 1 mm wide. They are provided with adhesive hairs along the lower surface which is slightly concave in transversal sections (Fig. 75).



Figs. 65–68. *Podostemum scaturiginum* (syn. *Crenias scaturigina*, *Mniopsis scaturigina*, CTP 5602). — 65 Fertile shoot with terminal flower in spathe (Fc) and branch (arrowhead); white arrow points to position of double-sheathed leaf. There are four single-sheathed leaves (1–4) below the flower, each one with a single stipular lobe (asterisks); bar = 1 mm. 66 Flower in anthesis, arrowheads point to branched stigma lobes emerging from ruptured spathe (Fc), anthers not visible. Note presence of leaves (L) with stipular lobes (asterisks); bar = 500 μ m. 67 Transversal section of ovary wall (G = gynoeceium) and spathe (Fc = flower cover). Note presence of tepal (T) and pollen sac (P); bar = 40 μ m. 68 Transversal section of branched stigma lobes inside spathe (Fc). Stigma surface is covered with elongate (hair-like) papillae; bar = 40 μ m.

adel-
hairs
 μ m.

CTP
len-
rom
with
caf);
ilate
ow-

Shoots: up to 1.7 (rarely 3) cm long, ascending from root flank. They arise from endogenous buds in root cortex, similar to other *Podostemum* species. Mature vigorous stem bases, however, lack any evidence of endogenous origin from the root (Fig. 76). They get their vascular supply (including some tracheids with spiral thickenings) from the root bundle (Fig. 77).

Leaves: sessile and arranged along two rows (i.e. distichous phyllotaxis). The leaves are up to 3 (rarely more) mm long, usually entire or once forked (Figs. 65, 66). The rounded blade lobes are flattened and about twice as long as wide, occasionally with some hairs along one side.

Sheaths: Similar to other members of the *Podostemum muelleri* grade, i.e. *P. muelleri* and the "Crenias" species described above (*P. ovatum*, *P. weddellianum*), the leaf sheaths are overlapping and obliquely attached to the stem (Figs. 80, 81). The oblique leaf insertion seems to be more pronounced in this species than in the other members of the *P. muelleri* grade (see Discussion below).

Stipules: Similar to other members of the *Podostemum muelleri* grade, each leaf has a prominent stipule pointing towards the front side of the shoot (Fig. 81). In *P. scaturiginum* the stipule is a rounded lobe, resembling the proper leaf blade but only half as long (Figs. 65, 66, 80). Unlike other members of the *P. muelleri* grade the stipule in *P. scaturiginum* is nearly free (detached) from its associated leaf. There is only a very short tissue zone common to both the stipule and its associated leaf sheath, as shown by a series of transversal sections (Figs. 69–74, 80).

Flower position along shoots: Shoot branching as well the occurrence of flowers is correlated with double-sheathed leaves, as typical for most Podostemoideae. One sheath may contain a flower bud, whereas the other sheath

Figs. 69–77. *Podostemum scaturiginum* (syn. *Crenias scaturigina*, *Mniopsis scaturigina*, CTP 5602). — 69–71 Three consecutive transversal sections above, at and below insertion area of leaf sheath and its single stipular lobe (asterisk) which is winged (keeled). The stipular lobe is already attached to the leaf base when it starts to fuse with the stem (X); bar = 300 μ m. 72, 73 Two consecutive transversal sections clearly and slightly above insertion area of leaf sheath and its single stipular lobe (asterisk) which is flat. The stipular lobe is attached to the leaf base slightly above leaf insertion at stem (X); bar = 300 μ m. 74 Transversal stem section (below insertion of terminal flower) with basal parts of two dorsiventrally organized leaves: L₁ at insertion level with stipular lobe (asterisk 1) attached to sheath in submarginal position, L₂ clearly above insertion level. Arrow with dashed line shows where the still free stipular lobe (asterisk 2) will attach to sheath of L₂ next to lower stem node; bar = 200 μ m. 75 Transversal root section. Note adhesive hairs along lower surface, and a weak central vascular bundle; bar = 200 μ m. 76 Transversal section of root and longitudinal section of strong root-born stem arising from right flank. There is (nearly) no sign left indicating its endogenous origin from root. Note adhesive hairs, weak root bundle (asterisk) from which the stem bundle is deriving (arrow-head); bar = 200 μ m. 77 Close-up of bundle in root-stem transition area. Note presence of tracheids with spiral thickenings; bar = 120 μ m.

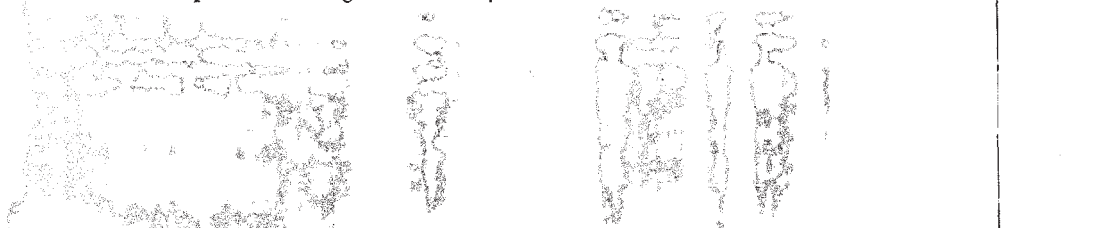
69

70

71

72

73



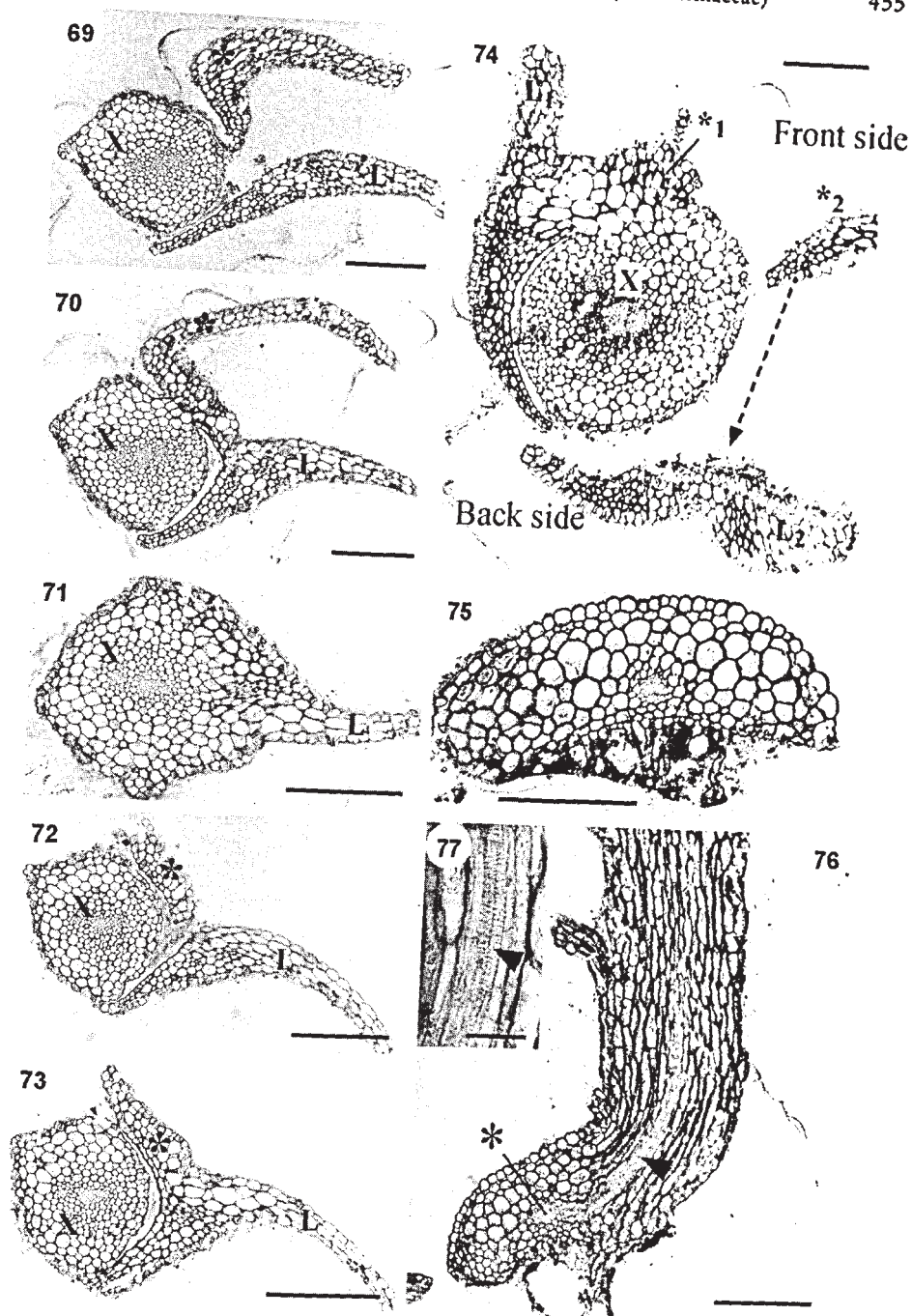
ey arise
species.
s origin
acheids

otaxis).
forked
ong as

de, i.e.
eddel-
stem
n this
low).
, each
3, 81).
blade
elleri
iated
id its
-74,

ence
nost
eath

ina,
ion
The
X);
er-
be
m.
wo
at-
ith
L2
rs
c-
k.
ve
);
f



contains a new leafy module (Fig. 65). The latter can produce another flower (associated with the next double-sheathed leaf) after few single-sheathed leaves. Rarely we observed short root-born shoots with a single terminal flower in *P. scaturiginum* and no branching.

Flowers and capsules: Flower buds are first covered by a spathella which irregularly ruptures from the top, allowing the palmately lobed stigmas to be exposed to the air (Fig. 66). The spathella is 3 or 4 cell layers thick and devoid of any vascular and fibre strands (Figs. 67, 68), similar as in most podostemoid members. The stigma lobes are covered with prominent elongate papillae (Fig. 68), similar to all other "Crenias" members, whereas *P. muelleri* and other *Podostemum* species have short stigmatic papillae only. Similar to all "Crenias" members *P. scaturiginum* lacks any ribs in the capsule walls. Even the ovary wall of flowers prior to anthesis do not show any sign of fibre strands. The thin ovary wall only consists of 4–5 cell layers throughout (Fig. 67, compare Fig. 56: *P. muelleri*).

Cladistic analysis

Data set

The length of the aligned sequences was 757 bp for ITS and 1441 bp for the *trnK* intron. ITS-1 had an aligned length of 254 bp, ITS-2 had an aligned length of 227 bp (minimum) in our data matrix. The ITS data provided 199 parsimony-informative characters, the *trnK* intron provided 72 parsimony-informative characters, and the morphological data provided 30 parsimony-informative characters. Due to the high sequence divergence the outgroup sequences were represented by "?"-characters in two short region in ITS-1 (approx. 120 bp) and ITS-2 (approx. 70 bp).

Morphological data

Cladistic analysis of species of *Podostemum* (including "Crenias") based on morphological characters yielded 262 most parsimonious trees (length = 67, Consistency index [CI] = 0.8507, Retention index [RI] = 0.9000). The strict consensus tree (Fig. 78) confirms a close relationship of *P. distichum* and *P. irgangii*. These species form a clade which is characterized by the tendencies to increase the number of stipular lobes (teeth) per leaf and to produce three-dimensional compound leaves with whorls or pseudo-whorls of scales along the rachides. *Podostemum muelleri* and the three "Crenias" species (*P. ovatum*, *P. scaturiginum*, and *P. weddellianum*) form a clade with dorsiventral stems, asymmetrical leaf sheaths, and single stipules restricted to the front side of the sheath as synapomorphies (Figs. 61, 80). A third monophyletic group is formed by *P. ceratophyllum*, *P. comatum* and *P. rutifolium* which is characterized by similar stipule and leaf shapes (Fig. 82).

Fig
ical
sim
abc
cie:

wil
col
(Fi
tric
eq
Th

flower
leaves.
wer in

which
s to be
void of
mem-
ig. 68),
other
"enias"
y wall
ovary
ig. 56:

or the
gth of
nony-
native
char-
repre-
ITS-2

ed on
Consensus
These
num-
ound
mum
, and
aths,
phies
llum,
d leaf

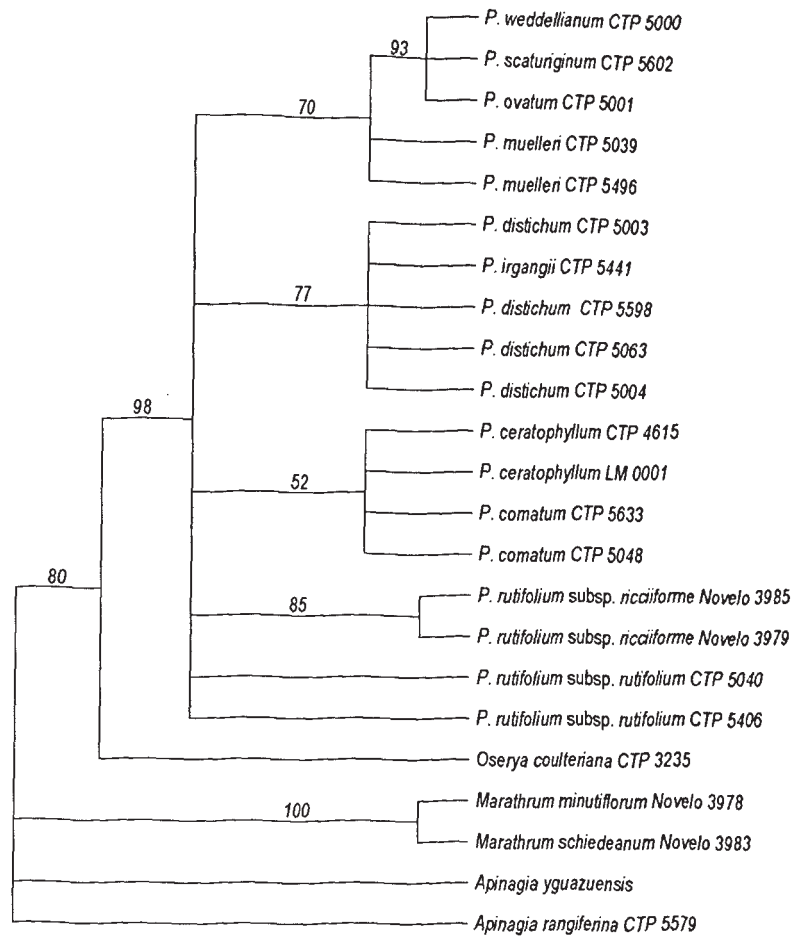


Fig. 78. Phylogeny of the genus *Podostemum* (including "Crenias") based on morphological data (see Appendices 1 and 2). Strict consensus tree calculated from the 262 most parsimonious trees yielded from the morphological data. The bootstrap support is indicated above branches (when higher than 50 %). Terminals are labelled with the respective species names alongside with their collection number.

Molecular data

The separate analysis of the ITS data yielded 4 equally parsimonious trees with a length of 423 steps (CI = 0.7069; RI = 0.8297). The topology of the strict consensus tree (data not shown) was almost identical to the combined tree (Fig. 79), only the node grouping *P. ceratophyllum* and *P. comatum* was not retrieved (unresolved). The separate analysis of the *trnK* intron data yielded 2675 equally parsimonious trees with a length of 99 steps (CI = 0.9091; RI = 0.9379). The strict consensus was not resolved.

Combined analysis

The morphological tree comprised two incongruences with respect to the molecular tree: (I) "Crenias" and *P. muelleri* form a clade (support 70 %), and (II) *P. ceratophyllum* and *P. comatum* are sister to all other species including "Crenias", as opposed to *P. distichum* and *P. irgangii* in the molecular tree. However, the backbone of the morphological tree is only poorly supported for the ingroup with three nodes receiving 29 %, 11 % and 30 % bootstrap support. Consequently, the combination of the morphological and the molecular data sets is not precluded, even though the 70 % bootstrap support in the morphological data for "Crenias" and *P. muelleri* falls just on the border of what was defined here *a priori* as the cut-off criterion for "hard" incongruency.

The analysis of the molecular (ITS, *trnK* intron) in conjunction with the morphological data yielded 3 MPTs (length = 595; CI = 0.7513; RI = 0.8491). The differences between the three MPTs were restricted to the *P. distichum* clade, with the remaining topology staying constant in all MPTs. The examination of the data revealed that the molecular data provide only one phylogenetical informative character. Thus, the differences are due to a lack of characters that could support a stable topology within the *P. distichum* clade. One of these three trees is shown in Fig. 79.

Two major clades were resolved in the strict consensus tree. The first clade comprises *P. distichum* and *P. irgangii* (100 % bootstrap support). The second clade comprises *P. ceratophyllum*, *P. comatum*, *P. muelleri*, *P. ovatum*, *P. rutifolium*, *P. scaturiginum*, and *P. weddellianum* (62 % bootstrap support). *Podostemum ceratophyllum* is sister to *P. comatum* (82 % bootstrap). *Podostemum rutifolium* subsp. *rutifolium* is sister to *P. rutifolium* subsp. *riccifforme* (83 % bootstrap). The clade comprising *P. ceratophyllum*, *P. comatum*, and *P. rutifolium* (the *P. ceratophyllum* clade) receives 99 % bootstrap support. The node placing *Podostemum muelleri* as sister to this clade receives 73 % bootstrap support. The three species of "Crenias" included (*P. ovatum*, *P. scaturiginum*, and *P. weddellianum*) form a clade (100 % bootstrap), which is sister to all species of *Podostemum* except *P. distichum* and *P. irgangii* (62 % bootstrap), thus rendering *Podostemum* paraphyletic. In addition, *P. distichum* is paraphyletic with *P. irgangii* nested within the four included accessions. The ITS sequence of one accession of *P. distichum* (CTP 5598) from Goias is strikingly different from the remaining accessions (including *P. irgangii*) with 29 autapomorphies. Unfortunately we could not study the morphology of the CTP 5598 collection due to a lack of fixed material.

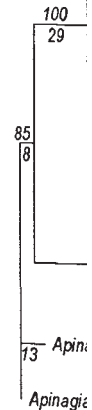


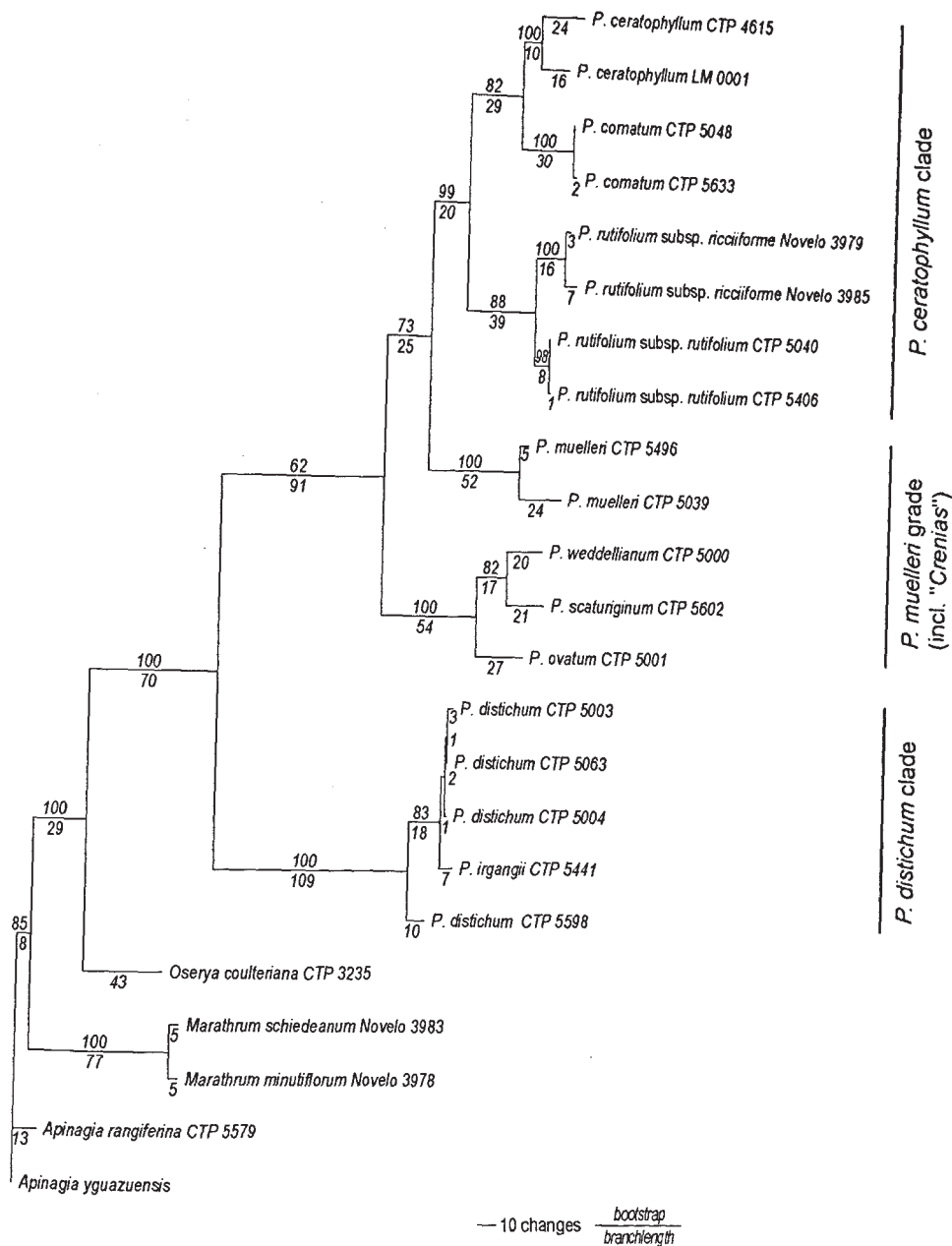
Fig. 79. Phylogeny of the genus *Podostemum* (including "Crenias") based on combined molecular and morphological data. One of the three most parsimonious trees (MPTs) yielded from the combined molecular (ITS, *trnK* intron) and morphological data. The

bo
len
na
est

to the
nd (II)
uding
How-
or the
port.
ta sets
ogical
here

mor-
e dif-
with
data
ative
ort a
vn in

clade
cond
tifo-
oste-
num
3 %
ium
cing
The
del-
do-
ring
with
one
the
tu-
to a



bootstrap support is indicated above branches (when higher than 50 %), and branch-length is indicated below branches. Terminals are labelled with the respective species names alongside with their collection number. The branch length is proportional to the estimated number of changes.

ned
Ts)
the

4. Discussion

Morphological characters

Roots, exogenous holdfasts and endogenous shoots

The creeping roots of *Podostemum* (including "Crenias") are thread-like to ribbon-like, i.e., slightly to strongly flattened ribbons. It seems that all *Podostemum* spp. (including "Crenias") have root tips provided with an asymmetric cap. Daughter roots in *Podostemum* (repeating the growth behavior of the main root) usually arise endogenously. In several other Podostemaceae (e.g. the Asian genera *Hydrobryum* and *Zeylanidium*) daughter roots are usually initiated exogenously along the main root, similar to what is shown (as exception) for *P. distichum* in Fig. 6 (see also Ota et al. 2001, Hiyama et al. 2002, Rutishauser & Moline 2005, Koi et al. 2006). In addition to adhesive hairs the *Podostemum* roots often show exogenous, finger-like outgrowths which serve as multicellular holdfasts, when the root itself cannot reach the rocky substrate. Hammond (1937, p. 21) compared the holdfasts to root tendrils that are "very sensitive to both gravity and contact, and they soon become firmly attached to the substratum by an adhesive secretion." Holdfasts (also called 'haptera') are positionally associated with shoot buds which are endogenously formed along the root (Rutishauser et al. 2003). The root-born shoots arise in an often opposite or subopposite pattern along the root margin.

Phyllotaxis and leaf arrangement of vegetative shoots

As typical for most podostemoids the leaves are arranged along two rows (orthostichies) in vegetative shoots. The two rows are usually arranged opposite each other, i.e. in one plane, with divergence angles of c. 180° between two consecutively initiated leaves. This type of phyllotaxis, called 180° leaf arrangement by Philbrick & Novelo (2004), is found in some but not all *Podostemum* species. Jäger-Zürn (2002) gave emphasis on the fact that in *Podostemum weddellianum* the two leaf orthostichies of the front side are only 130° apart from each other, equalling an angle of 230° when the dorsiventral shoots are viewed from the back (or reverse) side. According to Philbrick & Novelo (2004, p. 8) most members of the *P. muelleri* group (especially *P. muelleri*, *P. ovatum*, *P. scaturiginum* and *P. weddellianum*) have dorsiventral stems with alternate leaves arising at an angle less than 150°. All other *Podostemum* spp. (including *P. saldanhanum* as member of "Crenias") have 180° leaf arrangement (Philbrick & Novelo 2004). Looking at shoot cross-sections of *P. muelleri* (Figs. 54, 55), however, we observe angles of 180° rather than "less than 150°", as mentioned by Philbrick & Novelo (2004): It seems that also the leaves of *P. scaturiginum* (Fig. 74) are inserted opposite each other (i.e. approaching an angle of 180°) rather than showing dorsiventral distichy with an angle of only 130° towards the front side (i.e. the stipule side). Developmental studies will show which divergence angles really occur within the

sh
of
me
me
Ru
Ha
ok
ne
As
lac
lea

xy
fir
wa
Pe
ph
in
in
ca
(F
m
th
ro
C
("
la
ve

er
fo
Ti
A
ha
(F
w
(s
ca
m

shoot tip and to which degree any type of dorsiventral distichy is only the result of secondary shifting of the leaves after their inception. Then we may also find more evidence for the hypothesis that there is no permanent shoot apical meristem in *Podostemum* spp. and other podostemoids (as discussed by Rutishauser et al. 2003). While describing *P. ceratophyllum* shoot tips, already Hammond (1937, p. 27) wrote: "The leaves arise each from the base of the second older leaf, i.e. from the next older leaf in its rank." This observation coincides with new findings by Imaichi et al. (2005) and Koi et al. (2005) who have shown that in Asian podostemoids (e.g. *Cladopus*, *Zeylanidium* spp.) a shoot apical meristem is lacking. New leaves are formed endogenously on the adaxial side of a pre-existing leaf.

Mechanical and vascular tissue in stems

The vascular tissue in most Podostemaceae lacks a clear differentiation into xylem and phloem (Schnell 1967, 1998). Typical phloem elements are difficult to find (Romano & Dwyer 1971, Jäger-Zürn 2000). The presence of transversal cell walls resembling sieve-plates, however, indicates the existence of phloem in *Podostemum* stem bundles (Fig. 44, Ancibor 1990). The existence of proper phloem in Podostemaceae, however, needs to be proven by callose-specific staining (R. Aloni, personal comm.). More easily observable are weak xylem elements in Podostemaceae. Vessels seem to be lacking, only tracheids with annular or helical wall thickenings are known from, e.g., *Apinagia*, *Mourera* and *Podostemum* (Fig. 77; Rutishauser & Grubert 1994, 2000). — The flexible stems of *Podostemum comatum* (with lengths up to 72 cm) are slightly flattened and provided with three (rarely two) vascular bundles in one plane (Figs. 42, 43). Each bundle is surrounded by mechanical tissue (mainly collenchyma), as already described by Chodat & Vischer (1917) who compared these floating stems with flexible cables ("valeur mécanique d'un câble flexible"). Comparative studies will show if similar stem bundle arrangements are also found in other *Podostemum* species with very long (> 30 cm) and flexible shoots such as *P. distichum*.

Leaf sheaths and stipules

Shape and arrangement of leaf sheaths, stipules and blades show many differences in *Podostemum* and have been found to be a reliable source of information for the taxonomical treatment of the genus (Warming 1881, 1888, Royen 1954, Tur 1997, Philbrick & Novelo 2004). In many Podostemoideae (e.g. species of *Apinagia*, *Ledermanniella*, *Marathrum*) the leaf sheaths are stipular because they have one or two lobes or teeth that extend beyond the leaf insertion area (Rutishauser & Grubert 2000, Ameka et al. 2003). However, interpreting exactly what a stipule is in Podostemaceae as compared to other eudicots can be difficult (see paragraphs below). More general question about the morphological significance of stipules in various eudicots, including contrasting (or complementary) morphological interpretations were already discussed by Rutishauser (1999).

Symmetrical leaf sheaths and stipular arrangements in *Podostemum ceratophyllum* clade and *P. distichum* clade

The leaves of these two clades are arranged in a distichous order, with each leaf inserted transversely (i.e. perpendicular to the stem), as usual in most flowering plants (Troll 1941). The stipules in these *Podostemum* spp. are entire or lobed extensions of broadened, boat-shaped leaf sheaths (Rutishauser et al. 2003, Philbrick & Novelo 2004). The apical sheath extensions are entire in *P. ceratophyllum* and (occasionally) also in *P. rutifolium* subsp. *riccifforme* whereas they are divided into two apical teeth (i.e. two fused lateral stipules) in *P. rutifolium* subsp. *rutifolium*, less so in subsp. *riccifforme*. The boat-shaped stipular sheaths are entire or — more often — provided with two (or three) teeth in *P. comatum*, also belonging to the *P. ceratophyllum* clade (Fig. 82).

Philbrick & Novelo (2001, 2004) accepted *Podostemum irgangii* as a species clearly distinguishable from the somewhat polymorphic species *P. distichum*. Our combined phylogenetic analysis shows that both species are closely related (Fig. 79). Both species have symmetrical leaf sheaths and, thus, are comparable with the stipular arrangements in the *P. ceratophyllum* clade (Fig. 82). *Podostemum distichum* usually has stipules attached to a leaf sheath. There may be two stipular teeth (i.e. two apical extensions of the leaf sheath) per leaf (Fig. 16). Unlike other *Podostemum* spp. both *P. distichum* and *P. irgangii* can increase the number of stipular teeth. In *P. distichum* there are 2–6 triangular teeth per leaf, with the two lateral stipules usually longer than those in between (i.e. the intrapetiolar ones). *Podostemum irgangii* behaves similarly, but the leaf sheath is lacking (Fig. 82). Thus, the stipular appendages in *P. irgangii* are not attached to a leaf sheath, they arise along the insertion line of the leaf base. The two lateral auriculate stipules are clearly detached from the leaf base. There are additional 3–4 (rarely up to 9) intrapetiolar stipular teeth (i.e. narrow scales) which are arranged in a row along the adaxial insertion line of the petiole adjacent to the stem (Figs. 24, 82). According to Philbrick & Novelo (2004) *Podostemum irgangii* shares with some but not all *P. distichum* populations the presence of additional scales (subulate teeth) higher up along the blade axes (i.e. rachides). These scales can be arranged in whorls along the rachis, typically so in *P. irgangii* (Fig. 25), less so in certain *P. distichum* populations (Figs. 7, 15). Further studies need to be done in order to find a possible correlation between the presence of rachis scales and additional (intrapetiolar) stipular scales in *P. distichum*. We need to know if only the *P. distichum* populations having rachis scales are also provided with supernumerary stipular teeth (i.e. with up to 9 stipules per leaf), whereas the *P. distichum* populations without such rachis scales have leaf sheaths with 2 (–4) stipular teeth only. Such a correlation of scale presence in *P. distichum* would provide an argument in favour of the view that the supernumerary stipular teeth in *P. distichum* and *P. irgangii* are equivalent to rachis scales, i.e. homologous to "stipellae" sensu J ger-Z rn (2002, see next paragraph).

of
P.
as
all
(J 
bl
lo
(h
th
at
(F
st
H
(r
a
m
st
(F
(i
ta
D
st
st
P.

w
"s
cc
P.
(i
T
k
si

gi
(c
d
o
o
(c

mum

Shoot dorsiventrality with asymmetrical leaf sheaths and stipule loss on the back side in the *Podostemum muelleri* grade

h leaf
ering
lobed
2003,
rato-
they
lium
eaths
atum,

pecies
hum.
lated
rable
loste-
two
. Un-
the
leaf,
. the
ath is
d to a
ateral
ional
re ar-
stem
angii
ional
cales
, less
done
s and
only
rnu-
hum
teeth
rgu-
hum
ensu

As visualised by schematic drawings (Figs. 80, 81) each shoot in the members of the *Podostemum muelleri* group (including *P. muelleri*, *P. saldanhanum*, *P. scaturiginum* and *P. weddellianum*) is strongly dorsiventral and provided with asymmetrical and obliquely inserted leaf sheaths. This shoot dorsiventrality allows to distinguish a front (upper) side and a back (lower, reverse) side (Jäger-Zürn 2002, Philbrick & Novelo 2004). The oblique leaf insertion resembles the succubous leaf insertion in foliose liverworts (Jungermanniales). When looking at the front (upper) side the proximal margin of each leaf (L₂) covers (hides) the distal margin of the next older leaf (L₁) in the same row (Fig. 81). In the *P. muelleri* group the asymmetrical leaf sheaths are associated with a clearly attached or nearly detached stipule per leaf on the front side of the shoot only (Fig. 80; see also Warming 1881, 1888, Philbrick & Novelo 2004). A second stipule per leaf sheath is usually lacking in members of the *P. muelleri* group. However, we rarely observed such additional rudimentary stipules on the back (reverse) side of *P. muelleri* shoots. Thus, there are leaf sheaths in *P. muelleri* with a conspicuous attached stipule on the front side of the shoot (often in a submarginal position!) whereas the same sheath carries an inconspicuous marginal stipule only or (more often) lacks any sign of stipule on the back side of the shoot (Fig. 80). Thus, the dorsiventral stipular arrangement in the *P. muelleri* group (including "Crenias") can be derived from symmetrical leaf sheaths with two attached lateral stipules as found, e.g., in *P. comatum* and *P. rutifolium* (Fig. 82). — Double-sheathed leaves in the *P. muelleri* group usually have two prominent stipular lobes on the front side of the shoot. Two additional but inconspicuous stipules were occasionally found on the back side of double-sheathed leaves in *P. muelleri*, but not in "Crenias" members (Rutishauser, unpublished results).

Jäger-Zürn (2002) described leaf and stipule arrangement in *Podostemum weddellianum* (syn. *Crenias weddelliana*). She interpreted the stipule as a "stipella", i.e. as the lowermost leaflet of the blade. Her interpretation did not consider the morphological significance of the stipular appendages of other *Podostemum* spp., also not those of other members of the *P. muelleri* group (including "Crenias") which appear as putative relatives of *P. weddellianum*. Thus, we do not know if Jäger-Zürn intended to interpret all stipular appendages known in the whole genus *Podostemum* sensu Philbrick & Novelo (2004) as possibly derivable from leaflets or not.

Royen (1954) and Royen & Reitz (1971) described for example *P. scaturiginum* (syn. *Crenias scaturigina*, Fig. 80), as having stems with leaves in four rows (orthostichies). It seems that Royen (1954, p. 225) misinterpreted the nearly detached stipules as two additional rows of leaves. He wrote: "Leaves in 4 orthostichies, the largest leaves in the outer orthostichies, and the two other orthostichies with smaller leaves." The stipules of *P. scaturiginum* are nearly free (detached) from its associated leaf. Moreover, the stipules of this species are ovate

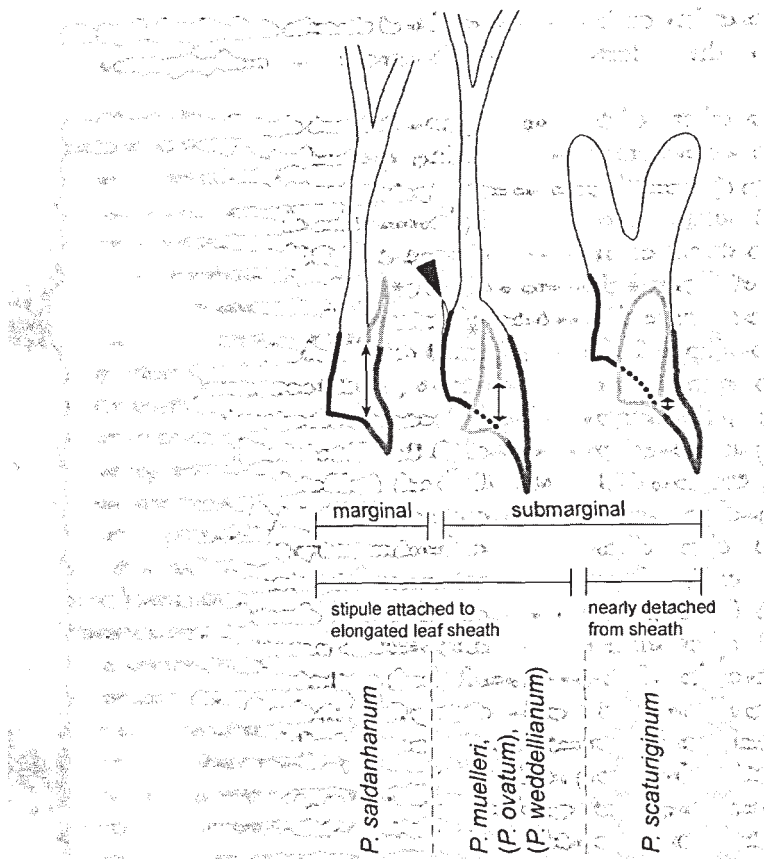


Fig. 80. Asymmetrical leaf sheaths and oblique leaf insertion along the stem in *Podostemum muelleri* group (including "Crenias"). The three schematic drawings correspond to patterns observable in the various species of the *Podostemum muelleri* group: Oblique leaf insertion is indicated by back side sheath lobe (bold black line) inserted more distally than its front side lobe (dark grey line). There is usually only one stipule (asterisk = bright grey line) per leaf sheath, restricted to the front side of the dorsiventral shoot. A second (rudimentary) stipule on the back side of the leaf sheath (arrowhead) is occasionally found in *P. muelleri*. The front stipule can be marginal as part of an elongated leaf sheath (left drawing, double-arrow indicates length of common sheath). Or, the front stipule is inserted in a submarginal position, i.e. on the sheath surface adjacent to the front margin. The submarginal front stipule is clearly attached to the leaf sheath (middle drawing) or nearly detached from it (right drawing). The length of the common sheaths are indicated by double-arrows. *Podostemum scaturiginum* has short (often bilobed) blades (right drawing) and prominent stipules, whereas all other species of the *P. muelleri* group have leaf blades that are much longer than their basal sheaths and stipules. The leaf sheaths of *P. ovatum* and *P. weddellianum* are intermediate between *P. muelleri* (with which they have the clearly attached submarginal inserted stipules in common) and *P. scaturiginum* (with which they share the nearly detached stipules). Note that the sheaths shown in this figure belong to single-sheathed leaves. Double-sheathed (ditheous) leaves are more complicate (Fig. 55).

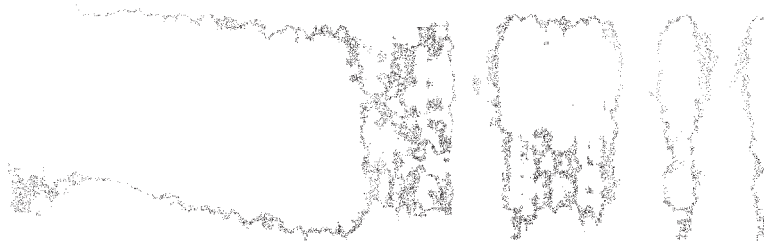


Fig. 81 *muelleri*: one row points its back obliqu (Junge row cc shadin sheath stipule sheath *P. sald. rather*

Fig. 8 leaf i Podo 81). I necti as pa are ir hype pron to th to th

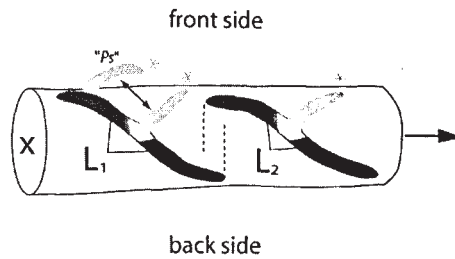


Fig. 81. Oblique insertion of asymmetrical leaves along a stem portion in *Podostemum muelleri* group (including "Crenias"). This scheme shows the insertion of two leaves of one row. The stem (X) is drawn as horizontal cylinder growing from left to right (arrow points towards shoot tip) with its front side presenting the stipules (asterisks) above and its back side (usually lacking stipules) below. Apart from the presence of stipules this oblique leaf insertion resembles the succubous leaf insertion in foliose liverworts (Jungermanniales). When seen from the front side the proximal margin of a leaf (L_2) in a row covers (hides) the distal margin of the next older leaf in the same row. Grey and black shadings of the leaf insertion zones are identical to those used in Fig. 80: front lobe of leaf sheath in dark grey, its back lobe in black, stipule insertion in light grey. Note that the stipule can be inserted next to the stem (i.e. leaf node area) or higher up as part of the leaf sheath itself (depending on the species, see Fig. 80 and text). Double-arrow: Except for *P. saldanhanum* ("Ps") each front side stipule is attached to its leaf sheath in a submarginal rather than marginal position.

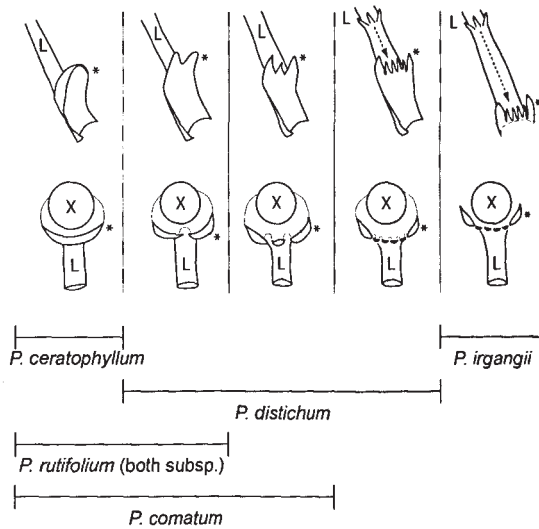


Fig. 82. Stipule types in *Podostemum* spp. with symmetrical leaf sheaths and transversal leaf insertion (*P. ceratophyllum* clade and *P. distichum* clade). Here included are all *Podostemum* species, except for the members of the *P. muelleri* group (compare Figs. 80, 81). Each stipule type is drawn from the side (upper row of drawings), showing its connection to the leaf sheath. The lower row of drawings illustrates the various stipule types as part of a transversal-section through the leaf node (stem). Free stipular lobes or teeth are indicated by asterisks, L= base of leaf blade, X= stem. The dashed arrows illustrate the hypothetical derivation of the supernumerary "stipular teeth" between the two more prominent lateral stipules (asterisks) by a dislocation (descendence) of rachis scales down to the leaf base. In polymorphic species such as *P. comatum* and *P. distichum* there are up to three stipule types observable. For more informations see text.

in
corre-
group:
serted
tipule
entral
ad) is
gated
front
front
draw-
hs are
blades
group
e leaf
(with
) and
it the
athed

lobes resembling the leaf blade which is only twice as long as the stipule. In the other members of the *P. muelleri* group (incl. "*Crenias*") the leaves are much longer and the stipules inconspicuous teeth (Fig. 80). Thus, *Podostemum scaturiginum* resembles to some degree dorsiventral shoots of *Selaginella* spp. with their obvious anisophylly.

Non-axillary shoot branching associated with double-sheathed leaves

In *Podostemum* and other Podostemoideae the root-born shoots first produce leaves with one sheath each in a distichous order. These single-sheathed leaves were called 'monothecous' by Warming (1881, 1899). They are equivalent to the leaves usually found in flowering plants. Many members of the subfamily Podostemoideae, however, are able to switch to another leaf type, having two sheaths opposite each other or nearly so (see, e.g. Figs. 17, 35, 36). These leaves which were called double-sheathed or 'ditheous' by Warming (1881, 1899) are a unique feature and evolutionary novelty of the Podostemaceae, not known from any other group of flowering plants (Jäger-Zürn 1994, 2002, Rutishauser 1997). Chodat & Vischer (1917) presented arguments in favour of the view that the double-sheathed leaves in *Podostemum* result from the congenital fusion of two single-sheathed leaves, somewhat comparable to Siamese twins who are fused along their back side. Double-sheathed leaves often occur in a (seemingly) terminal position leading to bifurcation (Y-shaped branching) of the shoot axis when both sheaths are occupied by daughter shoots (Fig. 35; Rutishauser et al. 2003). It is a peculiarity of many Podostemoideae that the leaf sheaths are arranged in the same plane as the leaf blade spreads its segments. The 'outer' sheath of a double-sheathed leaf is often smaller than the 'inner' one (Troll 1941, Jäger-Zürn 2002).

Due to this special branching mode most podostemoid taxa have lost axillary branching that is typical for most seed plants, showing a lateral branch (shoot bud) being subtended by a more proximal leaf along the same mother shoot axis. There are only few podostemoids with such an axillary branching pattern, e.g. *Diamantina* and *Saxicolella* (Ameka et al. 2002, Rutishauser et al. 2005). Warming (1881), Troll (1941) and Jäger-Zürn (1994, 2002) as well as Engler (1928) proposed different interpretations in order to derive the non-axillary shoot branching (associated with the double-sheathed leaves) of Podostemaceae-Podostemoideae from the typical axillary branching. These views will not be repeated here. They are summarised and discussed in Rutishauser et al. (2003).

Flower position along shoots

Shoots in *Podostemum* (including "*Crenias*") produce several distichously arranged leaves with a single sheath each. Only few *Podostemum* shoots end up with a terminal flower at the tip, similar to what is usually observable in many podostemoid genera from Asia (Rutishauser 1997). Vegetative shoots in *Podostemum* usually turn into reproductive ones by adding a double-sheathed leaf at their growing tip. One sheath of the double-sheathed leaf gives rise to a

fl
fe
th
le
(F

"
th
st
tr
rr
rr
b
W
o
(i
u

P.
ci
o
d
e
F
st
&
b
"
P
1
c
F

s
b
t:
ii
a

flower. The other sheath gives rise to a daughter shoot module which consists of few to several single-sheathed (monothealous) leaves in distichous order prior to the formation of — again — a seemingly terminal double-sheathed leaf and at least one flower. This pattern can be repeated resulting in higher-order modules (Rutishauser et al. 2003).

Flowers

Flower morphology is quite invariable within *Podostemum* (including "Crenias") providing little useful characters for species delimitation, except for the stigma shape (Royen 1954, Philbrick & Novelo 2004). The perianth in most species of *Podostemum* (including "Crenias") consists of three nearly linear or triangular tepals. Two of them flank the basally fused filaments of the two stamens on either side. Unlike many other Podostemoideae with an andropodium, most species of *Podostemum* (including "Crenias") have an additional third tepal born at the top of the andropodium in the fork between the two filaments (Fig. 20; Warming 1888, Engler 1928, Royen 1954, Cook 1996). The third tepal in the fork of the andropodium is sometimes missing in members of the *P. muelleri* group (including *P. muelleri*, *P. scaturiginum* and *P. weddellianum*), also in certain populations of *P. distichum* (see "*P. glaziovianus*" in Royen 1954).

Species of *Podostemum* have typically a bilobed stigma (Fig. 18), except for *P. saldanhanum*, *P. scaturiginum* and *P. weddellianum* (i.e. most "Crenias" species) which are provided with an up to six-lobed stigma (Fig. 66). The occurrence of palmately branched or cristate stigmas does not seem to be a good character to distinguish genera. Also in other podostemoid genera there are species with two entire stigma lobes and species with palmately branched or cristate stigma lobes. For example, the South Asian genus *Zeylanidium* contains species with bilobed stigmas and species with an additional subdivisions of each stigma lobe (Mathew & Sateesh 1997).

Stigma surface seems to be more reliable for separating the "Crenias" members from all species of *Podostemum* sensu Royen (1954). The stigma lobes of all "Crenias" species are covered with prominent elongate papillae (Fig. 68), whereas *P. muelleri* and other species have short stigmatic papillae only (Warming 1881, 1888). This feature is not mentioned by Philbrick & Novelo (2004) although it is carefully drawn in their figures of *P. ovatum*, *P. saldanhanum*, *P. scaturiginum* and *P. weddellianum*.

Capsules

Capsule morphology was traditionally used to classify the subfamily Podostemoideae. Presence and number of capsule ribs (due to sclerified longitudinal bundles) were and are important taxonomic characters, especially for the delimitation of genera (see Engler 1928, Royen 1954, Cook 1996, Cook & Rutishauser in press). The ribs usually become more conspicuous once the capsule is mature and the epidermis and outer parenchymatous cortex have been lost. In earlier

days species with smooth capsules and species with clearly ribbed capsules were not accepted as part of the same genus in Podostemaceae. Using a wider genus concept, there are now some podostemoid genera such as *Cladopus*, *Podostemum* and *Zeylanidium* which all comprise species with smooth besides species with ribbed capsules (Cook & Rutishauser 2001, Rutishauser & Pfeifer 2002, Philbrick & Novelo 2004).

Capsules of members of the *Podostemum ceratophyllum* clade and the *P. distichum* clade have eight prominent ribs, at least when the capsule is mature and the parenchymatous cell layers of the capsule wall between the fibrous strands have dropped off (Fig. 21). The *Podostemum* species belonging to "Crenias", such as *P. scaturiginum* lack any ribs in the capsule walls (Fig. 67). Already the ovary wall of flowers prior to anthesis does not show any sign of fibre strands. The thin ovary wall consists of 4–5 cell layers throughout. A somewhat intermediate condition is found in *P. muelleri* capsules (Figs. 51, 56), having eight inconspicuous ribs, i.e. three per valve plus the rib-like sutures.

Phylogenetic analysis

Our results from the combined data (Fig. 79) support the infrageneric relationship of *Podostemum* (including "Crenias") as proposed by Philbrick & Novelo (2004). The support for the topology resulting from the combined data is generally good, and the topology is congruent with the pattern seen in the morphological variation. The combination of the molecular and the morphological data increases the bootstrap support for the topology. Even the support for the node grouping "Crenias", the *P. ceratophyllum* clade, and *P. muelleri* in a clade is higher (62 % bootstrap) compared to the support from only the molecular data (53 % bootstrap). Thus, the morphological data seems to support the same general topology as the molecular data although the morphological data favours a grouping (70 % bootstrap) of *P. muelleri* with "Crenias" (see also Philbrick & Novelo 2004).

Infrageneric relationship of *Podostemum* (including "Crenias")

One of the notable results is the inclusion of *Crenias* in *Podostemum*, a finding that Philbrick & Novelo (2004) already demonstrated in a cladistic analysis of morphological data. The two genera have been recognized as closely related being similar in their vegetative and floral morphology. They have been traditionally distinguished based on two reproductive characters: "Crenias" has smooth capsules and (except for *Podostemum ovatum*, syn. *Crenias glazioviana*) two palmately branched stigmas, whereas *Podostemum* in the narrow sense of Royen (1954) has ribbed capsules and two linear stigmas. Another interesting result is the disjunct distribution of the sister-species *P. ceratophyllum* (Dominican

F
A
F
g
s
d
t
a
P

it
t
c
f
s
s
F
t
s
a
v
v
c
F
F
(
(
a
f
t
s
c
c
t
F

c
i
t
r
s
a
l
l

Republic to NE Canada) and *P. comatum* (southeastern Paraguay, northeastern Argentina, western Uruguay and southern Brazil), and the two subspecies of *Podostemum rutifolium*: Subspecies *rutifolium* is known from southeastern Paraguay, northeastern Argentina, western Uruguay and southern Brazil, whereas subsp. *riccifforme* is known from Mexico, Belize, Costa Rica and Colombia. These disjunct distribution patterns might be due to long-distance dispersal by migratory birds. The myxospermy typical for all Podostemaceae could provide a suitable mechanism for long-distance dispersal (Grubert 1970, 1976, Philbrick & Novelo 1997).

We follow Philbrick & Novelo (2004) in their view that the structural plasticity within the group is best described by recognising *P. irgangii* as distinct species but closely related to *P. distichum*. Nevertheless, a review of the morphological characters of the recently described new species *P. irgangii* shows that the leaf form is overlapping with *P. distichum*, with which it grows sometimes on the same rock (Philbrick & Novelo 2004). An important difference between the two species is the possession of axillary supernumerary stipules of *P. irgangii*, whereas *P. distichum* sensu Philbrick & Novelo (2004) has a variable number of stipular teeth attached to the elongated leaf (Fig. 82). The taxonomic value of the 3-dimensional structure of the leaf pinnae (including scales) in both species is of questionable quality as the highly variable leaf morphology of *P. distichum* encompasses very similar forms. *Podostemum irgangii* always has verticillate rachis scales, whereas they may lack in *P. distichum* or being subverticillate or irregular. Moreover, the teeth in the leaf axil of *P. irgangii* can be interpreted as being equivalent to proximal scales along the leaf rachis, thus only the two detached lateral stipules of *P. irgangii* may be the "true" stipules, whereas the supernumerary axillary teeth (Fig. 82) are descended rachis scales, equalling "stipellae" sensu Jäger-Zürn (2002). The molecular analysis revealed that *P. irgangii* is nested within several accessions of *P. distichum*. Thus, the inclusion of *P. irgangii* as a condensed dwarf form in *P. distichum* resulting in a monophyletic clade is appealing. In the combined tree one accessions of *P. distichum* (CTP 5598) is sister to all other accessions of *P. distichum* and *P. irgangii* sitting on a comparable long branch. This distinct accession is from a different Brazilian State (State of Goias) than the rest of the accessions of *P. distichum* (State of Rio Grande do Sul; see Table 1). Unfortunately, no fixed material was available of this accession for comparative morphological studies.

According to Philbrick & Novelo (2004) *Podostemum* subsp. *riccifforme* is a dwarf form of subsp. *rutifolium*. They kept it as subspecies because it is geographically distinct, and the leaf tips of subsp. *riccifforme* are always obtuse whereas those of subsp. *rutifolium* are often acute. Moreover, the stipules of subsp. *riccifforme* are entire, hood-like or with 2 flattened triangular teeth, whereas the stipules in subsp. *rutifolium* have always 2 flattened triangular teeth. The stipules are also important for distinguishing *P. rutifolium* subsp. *rutifolium* from *P. comatum* when flowers are absent. Larger leaves of *P. comatum* have the stipular teeth attached on leaf sheaths 2–3 mm long, whereas stipular teeth of

P. rutifolium subsp. *rutifolium* usually are attached on shorter leaf sheaths. But when only small leaves (<3 cm) and no flowers are present the two taxa are difficult to separate (Philbrick & Novelo 2004).

Podostemum flagelliforme could not be included in this study, due to a lack of material. This is unfortunate as this peculiar species might be of great importance as it has been formerly placed in the monotypic genus *Devillea*, and is the only species of *Podostemum* that has pollen monads instead of dyads. *Podostemum* (including *Devillea*) as proposed by Philbrick & Novelo (2004) would be the only New World genus of Podostemoideae with both monads and dyads (depending on the species). Otherwise, all podostemoid genera (except the large African genus *Ledermanniella*) have uniformly either monads or dyads (Cook & Rutishauser, in press).

Conclusion

The morphology of *Podostemum* (including “*Crenias*”) was compared with the results obtained from the phylogenetic analysis of molecular data, and the best current estimate of the phylogeny based on combined morphological and molecular data was presented. The comparative morphological data and the phylogenetic data proved to provide a complementary source that helped to elucidate the infrageneric relationship and morphological variation within *Podostemum*. Our results suggest that the former genus *Crenias* is nested in *Podostemum*, and that Philbrick & Novelo (2004) rightfully sank it into *Podostemum*. That *P. muelleri* and the “*Crenias*” species are not forming a monophylum according to the molecular data is somewhat unexpected given their shared suite of distinct morphological characters. Also, it is interesting that in two instances two closely related taxa — *P. ceratophyllum* and *P. comatum*, the two subspecies of *P. rutifolium*, respectively — have very disjunct distributions. Moreover, these two taxa-pairs are sister clades to each other. We can only speculate that they might share characters which could have facilitated long-distance dispersal. Nevertheless, the question of species delimitation remains not completely answered in this — now slightly less — enigmatic plants. Nothing is known about the population structure and gene flow in and possibly between species, but these data are essential to assess the species boundaries. This is illustrated by the case of *P. distichum* from Southern Brazil and adjacent regions. Without any data on gene flow we do not know if the summation of a considerable amount of sequence divergence since colonization (including long-distance dispersal) took place among different populations of *P. distichum* (e.g. accession CTP 5598 from Goias, Brazil) although their morphologies seem to be quite similar, or if one population of the widespread *P. distichum* turned on a divergence trajectory leading to a new species, i.e. *P. irgangii* in Parana and Santa Catarina (Brazil). Thus, the study of gene flow and the genetic variation within and among populations of the *P. distichum* clade (including *P. irgangii*) is badly needed.

Biol
J. G
Scar
and
Scie
Swi:

Am

Anc

Chc

Coc

—

Coc

Cus

Do:

Eld

Eng

Fel:

Gru

—

Gu

Ha:

—

Hiy

Acknowledgments

The technical assistance (scanning electron microscopy) of U. Jauch (Institute of Plant Biology, University of Zurich) is gratefully acknowledged. We thank M. Moody, T. Mione, J. Gabel (University of Connecticut, Storrs, USA), and C. Soros (University of Toronto, Scarborough, Canada) for their valuable help. Financial support was provided by the G. and A. Claraz Foundation (Zurich, Switzerland). This work was supported by National Science Foundation grant number DEB 9629767 to C. T. Philbrick and D. H. Les, and by Swiss National Science Foundation grant No. 3100AO.105974 to R. Rutishauser.

References

- Ameka, G.K., Clerk, G.C., Pfeifer, E. & Rutishauser, R. 2003: Developmental morphology of *Ledermanniella bowlingii* (Podostemaceae) from Ghana. — *Pl. Syst. Evol.* 237: 165–183.
- Ancibor, E. 1990: Anatomía de las especies Argentinas de *Podostemum* Michaux Podostemaceae: — *Parodiana* 6: 31–47.
- Chodat, R. & Vischer, W. 1917: La végétation du Paraguay. VI. Podostémacées. — *Bull. Soc. Bot. Genève, Sér. 2*, 9: 165–196.
- Cook, C.D.K. 1996: Aquatic plant book. 2nd revised ed. — The Hague: SPB Academic Publ.
- 1999: The number and kinds of embryo-bearing plants which have become aquatic: a survey. — *Perspect. Pl. Ecol. Evol. Syst.* 2: 79–102.
- Cook, C.D.K. & Rutishauser, R. (in press): Podostemaceae. — In: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants*. — Berlin: Springer.
- Cusset, C. 1992: Contribution à l'étude des Podostemaceae: 12. Les genres asiatiques. — *Bull. Mus. Hist. Nat. (Paris Sect. B, Adansonia)* 14: 13–54.
- Doyle, J.J. & Doyle, J.L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue — *Phytochem. Bull.* 19: 11–15.
- Eldenäs, P.K. & Linder, H.P. 2000: Congruence and complementarity of morphological and *trnL-trnF* sequence data and the phylogeny of the African Restionaceae. — *Syst. Bot.* 25: 692–707.
- Engler, A. 1928: Reihe Podostemales. — In: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, 2nd ed., 18a: 1–68, 483–484. [published as volume in 1930] — Leipzig: Verlag W. Engelmann.
- Felsenstein, J. 1985: Confidence-limits on phylogenies — an approach using the bootstrap. — *Evolution* 39: 783–791.
- Grubert, M. 1970: Untersuchungen über die Verankerung der Samen von Podostemonaceen. — *Internat. Rev. Hydrob.* 55: 83–114.
- 1976: Podostemaceen-Studien. Teil 2. Untersuchungen über die Keimung. — *Bot. Jahrb. Syst.* 95: 455–477.
- Gustafsson, M.H.G., Bittrich, V. & Stevens, P.F. 2002: Phylogeny of Clusiaceae based on *rbcL* sequences. — *Int. J. Pl. 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.
- Hiyama, Y., Tsukamoto, I., Imaichi, R. & Kato, M. 2002: Developmental anatomy and branching of roots of four *Zeylanidium* species (Podostemaceae), with implications for evolution of foliose roots. — *Ann. Bot.* 90: 735–744.

- Igersheim, A. & Cichocki, O. 1996: A simple method for microtome sectioning of prehistoric charcoal specimens embedded in 2-hydroxyethyl methacrylate (HEMA). — *Rev. Palaeobot. Palynol.* 92: 389–393.
- Imaichi, R., Hiyama, Y. & Kato, M. 2005: Leaf development in absence of a shoot apical meristem in *Zeylanidium subulatum* (Podostemaceae): evolutionary implications. — *Ann. Bot.* 96: 51–58.
- Jäger-Zürn, I. 1994: Morphologie der Podostemaceae. IV. Zur Kenntnis der dithekischen Blätter bei *Podostemum subulatum* Gard. (Podostemoideae). — *Beitr. Biol. Pflanzen* 68: 391–419.
- 2000: Developmental morphology of *Podostemum munnarensense* as compared to related taxa. — *Bot. Jahrb. Syst.* 122: 341–355.
- 2002: Comparative studies in the morphology of *Crenias weddelliana* and *Maferrina indica* with reference to *Sphaerotherylax abyssinica* (Podostemaceae). — *Bot. J. Linn. Soc.* 137: 63–84.
- Jäger-Zürn, I. & Grubert, M. 2000: Podostemaceae depend on sticky biofilms with respect to attachment to rocks in waterfalls. — *Int. J. Pl. Sci.* 161: 599–607.
- Johnson, L.A. & Soltis, D.E. 1994: *matK* DNA-sequences and phylogenetic reconstruction in *Saxifragaceae* s. str. — *Syst. Bot.* 19: 143–156.
- 1998: Assessing congruence: empirical examples from molecular data. — In: Soltis, D.E., Soltis, P.S. and Doyle, J.J. (eds.), *Molecular systematics of plants II: DNA sequencing*, p. 297–348. — New York: Kluwer Academic Publishers.
- Kita, Y. & Kato, M. 2001: Intrafamilial phylogeny of the aquatic angiosperm Podostemaceae inferred from the nucleotide sequence of the *matK* gene. — *Pl. Biol.* 3: 156–163.
- 2004: Molecular phylogeny of *Cladopus* and *Hydrobryum* (Podostemaceae, Podostemoideae) with implications for their biogeography in east Asia. — *Syst. Bot.* 29: 921–932.
- 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 Podostemaceae and the evolution of root dorsiventrality. — *Amer. J. Bot.* 93: 682–692.
- Koi, S., Imaichi, R. & Kato, M. 2005: Endogenous leaf initiation in the apical-meristemless shoot of *Cladopus queenslandicus* (Podostemaceae) and implications for evolution of shoot morphology. — *Int. J. Pl. Sci.* 166: 199–206.
- Les, D.H., Philbrick, C.T. & Novelo R., A. 1997: The phylogenetic position of river-weeds (Podostemaceae): Insights from *rbcL* sequence data. — *Aquatic Bot.* 57: 5–27.
- Mason-Gamer, R.J. & Kellogg, E.A. 1996: Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). — *Syst. Biol.* 45: 524–545.
- Mathew, C.J. & Satheesh, V.K. 1997: Taxonomy and distribution of the Podostemaceae in Kerala, India. — *Aquatic Bot.* 57: 243–274.
- Novelo R., A. & Philbrick, C.T. 1997: Taxonomy of Mexican Podostemaceae. — *Aquatic Bot.* 57: 275–303.
- Okada, H. & Kato, M. 2002: Pollination systems inferred from pollen-ovule ratio of some species of Podostemaceae. — *Acta Phytotax. Geobot.* 53: 51–61.
- Ota, M., Imaichi, R. & Kato, M. 2001: Developmental morphology of the thalloid *Hydrobryum japonicum* (Podostemaceae). — *Amer. J. Bot.* 88: 382–390.
- Philbrick, C.T. 1981: Some notes regarding pollination in a New Hampshire population of *Podostemum ceratophyllum* Michx. Podostemaceae. — *Rhodora* 83: 319–321.
- Philbrick, C.T. & Crow, G.E. 1983: Distribution of *Podostemum ceratophyllum* Michx. Podostemaceae. — *Rhodora* 85: 325–341.
- Philbrick, C.T. & Novelo R., A. 1995: New World Podostemaceae: ecological and evolutionary enigmas. — *Brittonia* 47: 210–222.
- 1997: Ovule number, seed number and seed size in Mexican and North American species of Podostemaceae. — *Aquatic Bot.* 57: 183–200.

- his- — — 2001: A new species of *Podostemum* (Podostemaceae) from the States of Parana and Santa Catarina, Brazil. — *Novon* 11: 92–96.
- Rev. — — 2004: Monograph of the genus *Podostemum* Podostemaceae: — *Syst. Bot. Monogr.* 70: 1–106.
- sical Romano, G.R. & Dwyer, J.D. 1971: A demonstration of phloem in the Podostemaceae. — *Bull. Torrey Bot. Club* 98: 46–53.
- s. — Royen, P. van 1954: The Podostemaceae of the New World. Part III. — *Acta Bot. Neerl.* 3(2): 215–263.
- hen Royen, P. van & Reitz, P.R. 1971: Podostemaceae. *Mniopsis, Podostemum*. — In: Reitz, P.R. (ed.), *Flora Illustrada Catarinense*, p. 16–33. Itajai, Santa Catarina: Herbario Barbosa Rodrigues.
- izen Rutishauser, R. 1995: Developmental patterns of leaves in Podostemaceae compared with more typical flowering plants: saltational evolution and fuzzy morphology. — *Canad. J. Bot.* 73: 1305–1317.
- ated — 1997: Structural and developmental diversity in Podostemaceae river-weeds. — *Aquatic Bot.* 57: 29–70.
- rria — 1999: Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness of organ identity. — *Int. J. Pl. Sci.* 160: S81–S103.
- inn. Rutishauser, R. & Grubert, M. 1994: The architecture of *Mourera fluviatilis* (Podostemaceae). — *Bot. Helv.* 104: 179–194.
- pect — — 2000: Developmental morphology of *Apinagia multibranchiata* (Podostemaceae) from the Venezuelan Guyanas. — *Bot. J. Linn. Soc.* 132: 299–323.
- tion Rutishauser, R. & Moline, P. 2005: Evo-devo and the search for homology ("sameness") in biological systems. Special issue on "Evolutionary Developmental Biology: New Challenges to the Homology Concept?" (Richter, S., Olsson, L., Eds.). — *Theory Biosci.* 124: 213–241.
- ltis, Rutishauser, R., Pfeifer, E., Moline, P. & Philbrick, C.T. 2003: Developmental morphology of roots and shoots of *Podostemum ceratophyllum* (Podostemaceae) — *Podostemoideae*. — *Rhodora* 105: 337–353.
- se- Savolainen, V., Fay, M.F., Albach, D.C., Backlund, A., van der Bank, M., Cameron, K.M., Johnson, S.A., Lledó, M.D., Pintaud, J.C., Powell, M., Sheahan, M.C., Soltis, D.E., Soltis, P.S., Weston, P., Whitten, W.M., Wurdack, K.J. & Chase, M.W. 2000: Phylogeny of the eudicots: a nearly complete familial analysis based on *rbcl* gene sequences. — *Kew Bull.* 55: 257–309.
- do- Schnell, R. 1967: Etudes sur l'anatomie et la morphologie des Podostémacées. — *Candollea* 22: 157–225.
- biol. — 1998: Anatomie des Podostémacées. — In: Carlquist, S., Cutler, D. F., Fink, S., Ozenda, P., Roth, I. & Ziegler, H. (eds.), *Encyclopedia of plant anatomy: extreme adaptations in angiospermous hydrophytes*, p. 197–283. — Berlin: Borntraeger.
- ae, Soltis, D.E., Mort, M.E., Soltis, P.S., Hibsich-Jetter, C., Zimmer, E.A. & Morgan, D. 1999: Phylogenetic relationships of the enigmatic angiosperm family Podostemaceae inferred from 18S rDNA and *rbcl* sequence data. — *Mol. Phylogen. Evol.* 11: 261–272.
- 3ot. Swofford, D.L. 2000: PAUP*. Phylogenetic Analysis Using Parsimony *and Other Methods, ver. 4b10. — Sunderland (Massachusetts): Sinauer Associates.
- 006: Troll, W. 1941: Vergleichende Morphologie der höheren Pflanzen. — Vol.1/3. Berlin: Borntraeger.
- ste- Tur, N.M. 1997: Taxonomy of Podostemaceae in Argentina. — *Aquatic Bot.* 57: 213–241.
- less — 1999: Podostemaceae (9). — In: Spichiger, R. & Ramella, L. (eds.), *Flora del Paraguay*, p. 5–35. — Geneva: Cons. J. Bot.
- 1 of Ueda, K., Hanyuda, T., Nakano, A., Shiuchi, T., Seo, A., Okubo, H. & Hotta, M. 1997: Molecular phylogenetic position of Podostemaceae, a marvelous aquatic flowering plant family. — *J. Pl. Res.* 110: 87–92.
- eds lec- e in atic me oid ion hx. lu- can

- Warming, E. 1881: Familien Podostemaceae I. — Kongel. Danske Vidensk. Selsk., Naturvidensk Math. Afh. 2: 1–34 (plates I–VI). 14.
- 1882: Familien Podostemaceae II. — Kongel. Danske Vidensk. Selsk., Naturvidensk Math. Afh. 2: 77–130 (plates VII–XIV). 15.
- 1888: Familien Podostemaceae. III. — Kongel. Danske Vidensk. Selsk., Naturvidensk Math. Afh. 4: 443–514. 16.
- 1891: Familien Podostemaceae. IV. — Kongel. Danske Vidensk. Selsk., Naturvidensk Math. Afh. 7: 133–179. 17.
- 1899: Familien Podostemaceae. V. — Kongel. Danske Vidensk. Selsk., Naturvidensk Math. Afh. 9: 105–154. 18.
- Weber, M. & Igersheim, A. 1994: 'Pollen buds' in *Ophiorrhiza* (Rubiaceae) and their role in Pollenkitt release. — Bot. Acta 107: 257–262. 19.
- White, T.J., Bruns, T. & Taylor, J. 1990: Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. — In: Innis, M.A., Gelfand, D.H. & Snindky, J.J. (eds.), PCR Protocols: A Guide to Methods and Applications, p. 315–322. — San Diego: Academic Press. 20.
- Wiens, J.J. 1998: Combining data sets with different phylogenetic histories. — Syst. Biol. 47: 568–581. 21.

Accepted for publication February 16, 2006 22.

Addresses of the authors: 23.

Philip M. Moline, Evelin Pfeifer, and Rolf Rutishauser*, Institut für Systematische Botanik, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland. 24.

Don Les, Department of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Road, Unit 3043, Storrs, CT 06269–3043, U.S.A. 25.

C. Thomas Philbrick, Department of Biological & Environmental Sciences, Western Connecticut State University, Danbury, Connecticut 06810, U.S.A. 26.

Alejandro Novelo R., Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, México D.F. 04510, Mexico. 27.

* author for correspondence, e-mail: rutishau@sybot.unizh.ch 28.

Appendix 1 29.

The 30 morphological characters used in the present study. 30.

1. showiness of flower: 0 = white to pink; 1 = greenish.
2. shape of tepals: 0 = linear, often elongate; 1 = triangular, often short.
3. tepal number: 0 = 6–12 as complete whorl; 1 = 2 or 3 on one side.
4. perianth symmetry: 0 = radial, whorl around ovary; 1 = confined to one side.
5. stamen whorl(s): 0 = incomplete or lacking; 1 = at least one complete whorl.
6. max. stamen number: 0 = 5–12; 1 = 3–4; 2 = two.
7. andropodium with 3rd tepal: 0 = absent; 1 = present.
8. pollen union: 0 = monads; 1 = dyads.
9. shape of stigma lobes: 0 = each lobe entire, linear; 1 = lobes branched (multilobed).
10. papillae on stigma lobes: 0 = papillae short (stigma surface smooth); 1 = papillae elongated (stigma surface hairy).
11. pedicel after anthesis: 0 = present, > 15 mm; 1 = present, 1–15 mm.
12. ribs of mature capsule: 0 = absent; 1 = present.
13. valve symmetry: 0 = 2 equal valves (isolobous); 1 = 2 unequal valves, 1 persist..

- lsk.,
14. maximal seed no. per capsule (according to Philbrick & Novelo 2004): 0 = 2 seeds; 1 = 100 seeds; 2 = 200 seeds.
- :nsk
15. holdfast formation: 0 = endogenous (same bud as shoot); 1 = exogenous, associated with shoots (if present at all).
- :nsk
16. elongate stem: 0 = not obvious (leaves in rosette); 1 = obvious (stem usually present).
- :nsk
17. stem form (cross-section): 0 = cylindrical or nearly so; 1 = flattened, dorsiventral, disk-like.
- :nsk
18. vegetative stem length: 0 = <10 cm; 1 = >10 cm (occasionally up to 40 cm).
- :nsk
19. flower position: 0 = flowers embedded in (often) flattened stem base; 1 = flowers not embedded in stem pocket, usually along elongated stem.
- le in
20. maximum leaf length: 0 = 1.3 cm; 1 = 2–5 cm; 2 = >6 cm.
- ibo-
21. blade division: 0 = pinnate; 1 = forked twice or more; 2 = simple or forked only once.
- ds.),
22. blade division: 0 = in one plane (never with rachis scales); 1 = in 3 dimensions (usually with rachis scales).
- \ca-
23. scales on leaf rachides: 0 = no additional scales present; 1 = irregular to pseudo-whorls; 2 = clearly whorls.
- 3iol.
24. blade vernation (young leaves): 0 = tips of leaf divisions straight; 1 = tips coiled (circinate).
25. stipule type: 0 = intrapetiolar sheathing stipule; 1 = 2 lateral + 0–2 intrapetiolar teeth; 2 = 2 lateral + >2 intrapetiolar teeth; 3 = prominent tooth on front side only (see Figs. 80–82).
- sche
26. sheath asymmetry: 0 = transversal leaf insertion; 1 = oblique (succubous) leaf insertion (see Fig. 81).
- :cti-
27. form of ultimate leaf divisions (cross-section): 0 = circular or elliptical without groove; 1 = elliptical with hairy groove; 2 = 3–4 times wider than thick.
- tern
28. type of distichy: 0 = not dorsiventral (two leaf rows forming an angle of ca. 180°); 1 = dorsiventral (two leaf rows forming an angle of 130–150°).
- dad
29. stipule (on back side): 0 = present; 1 = absent.
30. stipule insertion (relative to leaf base): 0 = marginal; 1 = submarginal to almost detached from sheath (see Figs. 80–82).

Appendix 2

The 30 morphological characters from habit, leaves, flowers, and fruits used in the present study for phylogenetic analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Podostemum ceratophyllum</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	1	0	1	1	2	1	0	0	0	0	0	18&2	0	0	0
<i>Podostemum comatum</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	1	0	1	1	2	1	0	0	0	0&1	0	18&2	0	0	0
<i>Podostemum distichum</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	18&2	0	2	0	1	0	0	0
<i>Podostemum irgangu</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	1	0	0	1	1	2	1	2	0	2	0	1	0	0	0
<i>Podostemum muelleri</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	1	0	0	1	1	2	0	0	0	3	1	2	0&1	0&1	0&1
<i>Podostemum ovatum</i>	1	0	1	1	0	2	1	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	3	1	2	1	1	1
<i>Podostemum rufifolium</i>	1	0	1	1	0	2	1	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	3	1	2	1	1	1
subsp. <i>riciforme</i>	1	0	1	1	0	2	1	1	0	1	1	1	1	1	1	0	0	1	0	0	2	0	0	0	0&1	0	2	0	0	0
<i>Podostemum rufifolium</i>	1	0	1	1	0	2	1	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	2	0	0	0
subsp. <i>rufifolium</i>	1	0	1	1	0	2	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	2	0	0	0
<i>Podostemum scaturiginum</i>	1	0	1	1	0	2	1	1	1	1	1	1	1	1	1	1	0	0	1	0	2	0	0	0	3	1	2	1	1	1
<i>Podostemum weddellianum</i>	1	0	1	1	0	2	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	3	1	2	1	1	1
<i>Apinagia cf. rangiferina</i>	1	0	1	1	0	1	0	0	0	1	1	1	0	2	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
<i>Apinagia yugaziensis</i>	1	0	1	1	0	2	0	0	0	0	0	1	0&1	2	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
<i>Marathrum minusiflorum</i>	0	1	0	0	1	0	0	0	0	0	0	1	0	2	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0
<i>Marathrum schiedeanum</i>	0	1	0	0	1	0	0	0	0	0	0	1	0	2	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0
<i>Oxera coulteriana</i>	1	0	1	1	0	2	1	1	0	0	1	1	1	1	1	0	0	1	0	0	2	1	0	0	1	0	0	0	0	0

Klacke
126: 4

The fo
(Genti
morph
have tu
clade c

Keywo

Co
phyllo
from
first cl
by Er
Miqu
transf
next t
and K
tion o
specie
follow
Ba
by Kl
lanthe
lanthe

DOI: