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# Plant galls (cecidia) in the neotropical water plant family Podostemaceae induced by larvae of Chironomidae

# (Diptera)

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Novel kinds of plant galls (cecidia) induced in aquatic angiospermous macrophytes by larvae of non-biting midges are reported on for the first time, based on observations involving four species of the neotropical flowering water plant family Podostemaceae. The plants inhabit rocks in waterfalls and cascades and provide unique biocenoses in otherwise inanimate, dangerous environments. Assemblages of invertebrates populating the surfaces of the plants are dominated by larvae of Chironomidae. In addition, several species from the same insect family are found exclusively in leaf cavities surrounded by tissues and structures otherwise unseen on the respective host plants. This study reports on the morphological and histological modifications observed on such galls in Podostemaceae, and discusses some aspects of the plants' interactions with gall inducing chironomids.

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# Introduction

When abnormal growth in a plant is due to the influence of a tissue invading alien organism, it is generally called a gall. Galls induced by larvae of specialized herbivorous insects and mites are termed true galls or cecidia in contrast to those induced by fungi or bacteria (Raman 2007). True galls are evident from special external and tissue structures of the affected plants that are intraspecifically invariable and not found in uninvaded members of the same plant species.

Plant galls (cecidia) caused by plant-animal interactions are fascinating, poorly understood phenomena (Stone & Schönrogge 2003). Although induced by animals, galls are the result of plant tissue growth and, thus, a matter of botany. In spite of this, detailed examinations of their morphology and structure from general botanical perspectives are rare



Fig. 1. Leaf galls in *Marathrum utile*. A. preserved shoots arising from root (R), one with infested, bulged leaf (arrow); B. transversal section of non-infested ensiform leaf blade; C,D. successive transversal sections of leaf showing histological modification of leaf structure: overview of bulging gall with wide cavity (C) surrounded by zone of novel gall tissue, apparent by dense staining (asterisks); note (in D) opening to the outside made by larva; E. transversal section of gall cavity; note gall tissue (centrifugal rows of novel cells without vacuoles and dense cytoplasm) with injured cells at border to cavity (arrow); L=head of larva in horizontal section, mandibles visible at bottom. Scale bars: A = 5 cm,  $B-D = 200 \mu$ m,  $E = 100 \mu$ m.

(Meyer & Maresquelle 1983). Historically, most galls have been described by entomologists, and within botany their study has been a part of phytopathology rather than modified plant structure.

Insect induced galls in aquatic angiosperms are all but unknown. One notable exception is the neotropical Podostemaceae *Mourera fluviatilis* Aublet, which has been found to be inhabited by larvae of *Polypedilum mehinaku* Bidawid & Fittkau, a species of non-biting midges (Diptera, Chironomidae) (Fittkau 1971, Bidawid & Fittkau 1996). A second brief mention of such insects inhabiting galls on another member of Podostemaceae, *Marathrum foeniculaceum* Humb. & Bonpl. (as *M. schiedeanum* (Cham.) Tul.), has come from Costa Rica (Hanson & Gómez-Laurito 2005). Larvae in leaves of *Marathrum utile* Tul. collected from Rio Bogonó, Venezuela, as well as numerous larvae living in the fleshy basal parts of other Podostemaceae (presumably *Mourera fluviatilis* or *Apinagia latifolia* (Tul.) P. Royen), were noted already by Goebel (1893). It is uncertain, however, whether Goebel's report indicates gall formation or mining, the latter being a common phenomenon in aquatic plants (McGaha 1952). These few reports are the only documentations of galls on aquatic angiosperms of which the present authors are aware. Interestingly, they all involved species of Podostemaceae. However, botanical investigations of the structure and histology of those podostemacean galls are unknown.



**Fig. 2.** Leaf galls in *Noveloa coulteriana*. **A.** preserved shoots with numerous infested leaves and chironomid pupae (P) within galls; **B.** stem (St) with five subulate leaves, youngest leaf proximally infested, containing chironomid pupa (P) in distal half of gall and head capsule of cast larval skin (L) in its proximal half; white arrowhead points to one of two openings in the gall wall (photograph by M. Kotrba, ZSM); **C.** longitudinal section of infested leaf, with gall cavity in sagittal section and chironomid larva (*Polypedilum* sp. A) in lateral view (photograph by M. Kotrba, ZSM). Scale bars: A = 15 mm, B = 3 mm, C = 1 mm.

The pantropical Podostemaceae (56 genera, about 300 species) inhabit a curious ecological niche: in waterfalls and cataracts of mainly tropical rivers with seasonal changes in water level, the plants grow attached to rocks by means of a biofilm (Jäger-Zürn & Grubert 2000). They can cover the substrate with dense mats by developing many root-borne shoots in clonal growth, thus can constitute the dominant biotic structure in such habitats. Consequently, Podostemaceae can be important elements of diverse biological communities, providing protection and food for a variety of freshwater animals. For example, invertebrate communities associated with Podostemaceae in Brazil are dominated by insect larvae, especially Chironomidae and Pyralidae (aquatic butterflies) (Léonard & Dessart 1994; Odinetz-Collart et al. 1998, 2001; Tavares et al. 1998).

The fly family Chironomidae is very diverse biologically and ecologically, e.g. as concerns larval habitats and feeding modes. In the majority of species the larvae live aquatically on substrates such as sediments, rocks or submerged parts of plants, and feed more or less opportunistically on detritus, algae or other organic matter. However, various exceptions from these most common patterns occur, including obligate mining in aquatic macrophytes and more or less host-specific parasitism on or in various animal hosts (e.g. Thienemann 1954, Armitage et al. 1995).

Podostemaceae live submerged during the months of high water in the rainy season, often below a water column of several meters. When the water subsides the plants become exposed, flower in a short time and set seed before becoming desiccated on the exposed substratum. After an interval of three to four months during the dry period, at the onset of the rainy season, Podostemaceae grow anew from seedlings. Consequently, animals associating with these plants find predictably changing ecological conditions to which they can adapt their life cycles. Insect density associated with Podostemaceae has been shown to correlate with the monthly precipitation rate, and is lowest at high water levels (Odinetz-Collart et al. 1998: 1084).

Here, we present the first records of insect induced galls in leaves or shoots of four neotropical species of Podostemaceae, subfamily Podostemoideae: *Apinagia riedelii* (Bong.) Tul., *Marathrum utile* Tul., *Noveloa coulteriana* (Tul.) C.T. Philbrick (syn. *Oserya coulteriana* Tul.), and *Podostemum weddellianum* (Tul.) C.T. Philbrick & Novelo. In each case, the observed structures are documented as true galls (cecidia) caused by chironomid larvae. This paper focuses on the modifications to plant host tissue structures of leaves and the shoot axis that are due to the infestations, and on the gall shapes. Detailed descriptions of the previously unknown species and life stages of the chironomid insects involved shall be given elsewhere.

# Material and methods

All plant material was collected at times of low water, i.e. when the Podostemaceae began to flower. Infested plants were noticed for the first time during recent explorations of populations of *Noveloa coulteriana* and *Podostemum weddellianum*; subsequently additional material was found among previously fixed collections by W. Troll (*Apinagia riedelii*) and W. Hagemann (*Marathrum utile*). Collecting localities and corresponding voucher specimens are listed in Table 1. Plants were fixed in FPA (formaldehyde, propionic acid, ethanol) (*Noveloa coulteriana, Apinagia riedelii, Marathrum utile*) or 100 % ethanol (*Podostemum weddellianum*) immediately at the site.

Serial microtome sections (10 µm) of paraplast embedded plant galls from each species (*A. riedelii*: 15 galls; *M. utile*: 4; *N. coulteriana*: 9; *P. weddellianum*: 7 galls) were stained with gentian violet, safranine, methylene blue, and ruthenium red, respectively. Lugol's iodine solution was used for starch identification, and eosin as a protein marker. Macro photographs were made with Leica stereoscopes and the Leica camera application suite; SEM images were made with a Philips XL 30 ESEM TMP scanning electron microscope (critical point dryer: Bal-Tec CPD 030; sputter coater: Bal-Tec SCD 005).

Preparations and identifications of the chironomid gall inhabitants were made by the second author (MS). About 10 galls per plant species were opened and inspected carefully for the presence of insect specimens. Some galls were selected because they appeared to contain associations of several chironomid life stages (see Fig. 2B). Others were opened at random to test whether more than a single chironomid species was present in galls of the particular host plant. Corresponding voucher specimens (museum permanent slide mounts) and additional alcohol preserved material have been deposited at the Zoologische Staatssammlung München (ZSM) in Munich, Germany.

### Results

The plant galls in Podostemaceae described here occur in two types: leaf galls (*M. utile*, *N. coulteriana*, *A. riedelii*) and pith galls (*P. weddellianum*). Within each plant species studied, gall structure was consistent among the samples examined.

# Marathrum utile

*Marathrum utile* possesses compressed, prostrate, distichously foliated shoots. The leaves are up to 30 cm long and ensiform (sword-like, i. e. the blade-like part is the result of longitudinal growth in the midrib area of the leaf). The leaf is subdivided in a proximal, pedicel-like ribbon and a distal wide, parallel-veined, lobate blade.

Infestations occur proximally within the ensiform leaves (Fig. 1A, arrow), near one of the vascular strands, which are positioned one after the other in a row along the leaf's midrib area (Fig. 1B). In external view the infested portion of the leaf appears swollen, much thicker than the non-infested part or a normal leaf blade. A wide, oblong cavity in the center of the bulge (Fig. 1C) is inhabited by the larva (Fig. 1E). The cavity has two openings to the outside (Fig. 1D).

 Table 1. Vouchers of plant material examined (SF=Senckenbergmuseum Frankfurt, Germany; WCSU=Western Connecticut State University, Danbury, CT, USA).

Species	Country	State/ Province	River	Date	Collectors and number	Location of voucher
Apinagia riedelii Tul.	Brazil	Rio de Janeiro	??	??	W. Troll s.n.	SF
Marathrum utile Tul.	Colombia	Boyacá	Junction of Rio Cuisiana and Rio Chichacá, 800-1000 m a.s.l.	14 October 1967	W. Hagemann 18665	SF
Noveloa coulteriana (Tul.) C.T. Philbrick	Mexico	Jalisco	Rio Marabasco, Ayotitlán, 104.303917°W 19.415861°N	25 March 2009	C.T. Philbrick, A. Mora-O. & R. Gyure 6343	WCSU
Podostemum weddellianum (Tul.) C.T. Philbrick & Novelo	Brazil	Rio de Janeiro	Rio São Pedro at Frade near Macaé City, 42°5'13.5" W 22°14'29.7" S	6 July 2011	I. Silva & C. Koschnitzke s.n.	SF

Its walls are histologically distinct (Fig. 1C–D) as a zone of rows of up to 10 strongly stainable cells in centripetal arrangement. Consequently, a 'wreath' of periclinally divided cells envelops the tube-like cavity, slightly overtopping the cavity ends. The expansion of the bulged portion of the leaf mainly results from that special tissue. These cells differ decisively from the typical leaf parenchyma cells by their dense cytoplasm, prominent nuclei and nucleoli, the lack of a vacuole, and by thin cell walls (conditions similar to those described for *Noveloa coulteriana*; see below). The cells adjacent to the cavity are injured (Fig. 1E), i.e. they are without walls towards the cavity, and their protoplasts (when still present) project into the cavity.

#### Noveloa coulteriana

In *Noveloa coulteriana*, numerous leafy shoots arise along the thread-like roots. The subulate leaves are 1–5 cm long and distally forked several times. The ribbon-like proximal part of the leaf is the place where enlargement due to infestation occurs. Above the bulge the leaf retains its normal shape (Fig. 2B). In the flowering period the water levels were decreasing, but the collected shoots with highly infested leaves were still below the water surface (Fig. 2A).

The infested portion of the leaf differs from the usual leaf shape by an oblong, enlarged thickening, which contains a tube-like cavity with the chironomid (Fig. 2C). Insects inside intact galls are often clearly evident from the outside (Fig. 2A,B). The gall cavity has two openings, one at the lower and one at the upper end, visible from the outside of the infested leaf (Fig. 2B).

The gall cavity is about 500 µm wide, and surrounded by special new tissue in the leaf center in the form of a 100-200 µm thick 'wreath' of cells (Fig. 3F). The latter are arranged in rows developed by periclinal mitotic divisions and positioned perpendicular to the border of the elongate cavity. In transverse view, the radially positioned rows are oriented towards the central (vertical) axis of the cavity. The 'wreath' of novel tissue is surrounded by a narrow fringe of smaller cells aligned next to typical leaf parenchyma cells (Fig. 3D). The cells of the new central leaf tissue differ from the usual leaf parenchyma by their strong stainability due to dense cytoplasm content, by large nuclei and distinct nucleoli (Fig. 3G), the lack of vacuoles and thin cell walls (Fig. 3D-F). Such tissue does not occur in the parenchyma cells of gall-free leaves. Common histological staining (eosin) of the induced tissue cells showed high protein content of the dense cytoplasm of the lining cells (Fig. 3C), and an apparent accumulation of starch grains (Lugol's iodine solution) (Fig. 3A, B). Such black stained starch grains are also seen in the guts of larvae found in the galls (Fig. 3B), showing that the insect had fed on the 'wreath' cells, thereby maintaining the cavity (Fig. 3E).

#### Apinagia riedelii

*Apinagia riedelii* has elongate leafy shoots (Jäger-Zürn 2009). The relatively long, ensiform leaves have a broadened, ribbon-shaped proximal part that divides into ribbon-like sections which end in narrow filaments. The proximal parts of the leaves become infested with chironomid larvae.

In contrast to the two preceding species, Apinagia riedelii has erect and elongated, not compressed, rootborne shoots. Each branch and its first leaf (adorsed prophyll) are basally fused to the mother shoot axis. Most infestations were found in the proximal parts, with the gall extending from the axil into the proximal part of the leaf, where the chironomid occupies a wide cavity (Fig. 4A). The latter has two openings to the outside, with the upper one below the top of the cavity. The wide cavity is surrounded by an area of cells that are distinguished from the gall-free leaf parenchyma by their strong stainability (dense cytoplasm) (Fig. 4B,C). They form a 'wreath' of rows of cells arranged centrifugally around the cavity (Fig. 4C). As in the preceding two species, compared to the normal leaf tissue these cells are much enriched with starch grains (staining with Lugol's iodine solution). Cells towards the cavity are injured (Fig. 4F). In contrast to the preceding species, however, Apinagia riedelii develops an additional circular zone of tissue around the gall cavity, between the gall cells and the normal leaf tissue (Fig. 4A,B-E). Many single vascular strands occur here, as shown by their annular tracheary elements (Fig. 4E). The three main vascular strands typical of the leaf of Apinagia riedelii remain distinct.

#### Podostemum weddellianum

In *Podostemum weddellianum*, in contrast to the other three species, midges infest not the leaves but the shoots (Fig. 6), and the shoot galls are of a type different from that seen in leaves of other Podostemaceae.

Gall-free shoots of *P. weddellianum* are compressed and distichously foliated (Jäger-Zürn 2002, as *Crenias weddelliana* (Tul.) CDK Cook & Rutishauser), with about seven delicate lobate leaves (Figs 5A–C, 6A). Infested shoots are elongate, grow upright to about 5 mm length, and look completely different, as the gall occupies nearly the whole shoot and transforms it to a conical shape (Fig. 6B). The distichous phyllotaxy disappears in the galled shoot (Fig. 5D); instead, numerous firm, scaly, leaflet-like appendages develop along the conical gall in an imbricate arrangement (Figs 6B; 7A, C, E). These appendages arise from the gall wall side by side in the form of large bristles or broad spines. They differ from the leaves of uninfested shoots by the lack of a distinct central vascular leaf strand, and by their irregular placement. They are named scales or appendages here, as long as their morphological status is unresolved. They consist of large cells with thick walls in irregular orientation. The abaxial epidermis (pointing to the outside of the gall) is highly papillose (Fig. 7D,E), whereas the adaxial side (towards the gall wall) has smooth cells.

This description suggests that these galls have mature status, which is consistent with the fact that most of the midges found inside had reached the pupal stage (Fig. 6C).

The gall contains a wide chamber, circular in transverse section (Fig. 5D), that is tightly filled by the insect when the latter has pupated. At the base of the gall, the central shoot tissue looks normal, but immediately above this, injured tissue interrupted by cavities connected to the gall chamber appears within the shoot centre (Fig. 5D). Specialized nourishing tissue has not been observed in the basal and central parts in the mature galls under investigation. However, almost all cells lining the cavity, including those in parts of the gall untouched during preparation, were found to be injured, appearing open towards the insect (Fig. 7B). Occasionally, some slightly elongate, intact cells are present.

In mature galls, their wall is firm and thick, consisting of several zones (Fig. 7A). Surrounding the layer of injured cells at the cavity border there is a zone of two to three layers of small, compressed thick-walled collenchyma cells that are prolonged in vertical direction, with a narrow lumen (Fig. 7F). They have an affinity for Ruthenium red stain that indicates pectin content. These cells are generally of

irregularly arranged, intertwined growth (Fig. 7D). Some of them contain a silica crystal, a feature common to the species of *Podostemum*. Cells of the gall wall and the epidermis of the appendages contain silica bodies much more often than the cells of gall-free shoots. The zone of compressed cells is followed by a zone of larger cells that form the scaly appendages (Fig. 7B,D). At the upper end of the gall cavity there are small cells, and the gall is closed and covered with appendages. No openings to the outside were observed on such galls in their closed, insect-inhabited stage. Open galls from which the adult midge has emerged show the appendages near the gall top spread out around a hole about as wide as the gall's lumen (Fig. 8).

# The gall inducing Chironomidae

Table 2 gives an overview of the occurrences of gall forming interactions between Podostemaceae and Chironomidae known at this time.

Within each host species examined here, all chironomid specimens found in the corresponding galls belong to a single species only. While there are six plant host species in five genera, the gall inducers are slightly less diverse, numbering four species in two to three genera. The difference is related to the fact that three of the chironomid species each have been found in only one respective host species (and in one geographic area), whereas *Polypedilum* sp. A is known from galls in three Podostemaceae genera (as well as from west-central Mexico to east-central Brazil).

The three chironomid species other than *Polypedilum mehinaku* are new to science. Genus assignment is still open for two of them, as some taxon limits in the *Endochironomus* complex of genera are unclear. Moreover, especially the immature stages of the species considered here show several morphological characters expressed in more or less reduced form (analogous to adaptations to comparable special habitats known from other chironomid species; e.g. see Gonser & Spies 1997), which renders inference

**Fig. 3.** *Noveloa coulteriana,* transversal sections of parts of galled leaves. **A-C.** histological staining applied to  $\triangleright$  proximal part of infested leaf showing gall cavity with larva (L; in cross section); **A.** leaf cell walls unstained, but starch grains (enriched in gall tissue lining the cavity) stained black with Lugol's iodine solution; **B.** close-up of (A); note starch grains (arrow) within gut of larva (L); **C.** larva (L) and new gall tissue stained with eosin showing protein content; **D-F.** successive transversal sections below and at base of gall cavity: **D.** gall tissue in gall base forming solid aggregation; note irregular growth of leaf cells (arrowhead) and densely stained induced gall tissue side by side with less stained (interpreted as less induced; see text) tissue (arrow); **E.** slightly more distal section showing similar gall tissue (as in D) partly scraped into a hole leading to gall cavity (C); note ungalled leaf parenchyma cells (arrow); **F.** gall cavity (C) lined by densely stained gall tissue arranged in centrifugal rows of cells, with injured cells at cavity border (asterisk); especially note group of less altered and less stained (less induced) cells distinctly demarcated from gall tissue (arrow); **G.** part of gall tissue showing cells with large nuclei and distinct nucleoli. Scale bars: A=200 µm, B=F=100 µm, G=50 µm.





**Fig. 4.** Leaf galls in *Apinagia riedelii*, transversal sections of proximal part of leaf. **A.** part of leaf with gall cavity and larva (L) showing densely stained gall tissue lining the cavity and additional 'wreath' of new, small-celled tissue with vascular elements (arrows) around cavity and gall tissue; **B**-E. close-ups of additional circular vascular elements around cavity (arrow); note (in C) centrifugal rows of cells of gall tissue due to periclinal divisions; **D**. sequences of vascular strands in annular position (arrow); **E**. annular tracheary elements (arrow); **F**. border of gall cavity with injured cells (arrow). Scale bars:  $A, E = 100 \mu m$ ;  $B-C, F = 50 \mu m$ ;  $D = 200 \mu m$ .

on their systematic relations difficult. Consequently, for example, *Endochironomus* complex sp. 1 does not key cleanly to any genus in Spies et al. (2009). Such problems regularly posed by reductions in morphological features could be overcome by draw-

ing molecular data into consideration. However, the vast majority of the very numerous neotropical relatives of the chironomid species in question here (Spies & Reiss 1996) have not been characterized on a molecular level.



**Fig. 5.** *Podostemum weddellianum.* **A.** Line drawing of uninfested, branched leafy shoot with two terminal flowers (f); lobate leaves with one basal stipella each (stl) (from Jäger-Zürn 2002); **B.** transversal section of uninfested, distichously foliated compressed shoot, leaves marked with asterisks; **C.** transversal section of apex (arrow) of uninfested stem; **D.** transversal section of galled shoot at gall base showing cavity (C) and thick-walled cells of firm gall wall. Scale bars: A = 2.5 mm, B-D = 200 µm.

**Table 2.** Podostemaceae species and Chironomidae taxa involved in the formation of true galls, and known geographic distribution of such cases.

Hosts (Podostemaceae)	Gall inducers (Chironomidae)*	Countries (states) with known cases	References
Apinagia riedelii	Polypedilum sp. A	Brazil (Rio de Janeiro)	present study
Marathrum foeniculaceum	Endochironomus complex sp. 1	Costa Rica (San José)	Hanson & Gómez-Laurito (2005)
Marathrum utile	Polypedilum sp. A	Colombia (Boyacá)	present study
Mourera fluviatilis	Polypedilum mehinaku Bidawid & Fittkau, 1996	Brazil (Pará)	Fittkau (1971), Bidawid & Fittkau (1996)
Noveloa coulteriana	Polypedilum sp. A	Mexico (Colima, Jalisco)	present study
Podostemum weddellianum	Endochironomus complex sp. 2	Brazil (Rio de Janeiro)	present study

\* Material of all taxa listed in this column has been examined by the present second author, and is available for study at ZSM. With all galls examined by the second author that still had insect remains in them, the latter evidently belong to a single inhabitant individual. In other words, the gall appears to be caused always by a single midge larva, regardless of the respective gall inducing and host species involved.

Most of the midge specimens taken from previously unopened galls represented relatively late stages of individual development, i.e. the pharate adult prior to emergence, pupa, or final (fourth) instar larva. Larvae in (presumably) third or second instars were encountered rarely, although in several cases galls still contained cast skins left over from one or two such earlier stages along with the corresponding individual that had grown out of them.

# Discussion

The cecidia on aquatic Podostemaceae reported here are an unexpected finding discovered by chance in connection with field work designed for other purposes. Therefore, this paper presents first information and interpretations, whereas a number of questions cannot be addressed yet. For example, we are presently unable to describe the complete life cycle for any of the infesting midge species or the complete sequence of gall development for any of the hosts. The plants were sampled when they could be reached relatively easily, by which season the galls and their inhabitants had developed to relatively advanced stages. Earlier larval and gall developmental stages apparently could be observed only on submerged plants during higher-water seasons.

In spite of these limitations, the following results make interesting new contributions to science. For the first time, true galls are documented in botanical detail from flowering aquatic macrophytes. At least two different types of galls (leaf galls, pith gall) are described, and larvae of Chironomidae (Diptera) are documented as the gall inducing agents in all cases. The numbers of host and infestant taxa involved are increased significantly. The geographic area from which this phenomenon is known is also extended greatly, now ranging from Mexico through northwestern South America (Colombia) to east-central Brazil. However, Podostemaceae remain the only strictly aquatic angiosperms from which such gall formation is known.

In addition, the following further aspects are worth noting.

I. It is evident that the species of Podostemaceae studied here can develop true galls, each caused by a chironomid larva that has settled inside the plant.

Apparently, the actions of the larva stimulate the

plant to produce new types of tissue surrounding the cavity inhabited by the insect. Such tissue does not occur in uninfested plant parts, and only occurs in association with the internal presence of a midge larva. The tissue is therefore identified as new gall tissue, the larva as a galler, and the modified part of the host leaf or stem as a gall (cecidium).

II. The hypothesis that the gall inhabiting larva uses the special tissue for nourishment is supported by several observations. (1) Many walls of cells directly facing the gall cavity show damage consistent with feeding activity by the midge larva. Furthermore, the new tissue lacks an epidermal layer towards the cavity. (2) Other parts of the cavity lining tissue have lower numbers of cells per row and also show that cells next to the cavity were removed. (3) Examinations using common histological stains show high protein and starch content of the new gall tissue. (4) Undigested starch grains (made visible by staining with Lugol's iodine solution) are found in the guts of larvae. There is no indication that the larvae feed on the regular parenchyma of the plant. For example, necrotic cells, a common reaction of plants to damage by herbivores, have not been observed. In this regard, the evidence is inconsistent with attacks by miner insects, which exploit the regular leaf parenchyma of the host plant.

Enlarged nuclei and distinct nucleoli as found in the cells of the special tissue have been found in gall cells elsewhere, described as polytene in structure (Harper et al. 2004), and interpreted as demonstrating that the respective tissue serves as food.

III. It is unknown how the midge larva first invades the plant, how the cavity is established and gall tissue formation is induced. However, the gall tissue at the lower end of the cavity may give some clues. Structural analysis by successive transversal sections of the base of the galled leaf in *Noveloa coulteriana* shows that the new gall tissue at first forms a solid aggregation of cells (Fig. 3D), which afterwards is perforated by the feeding activity of the larva (Fig. 3E–F).

The gall tissue begins in association with the leaf vascular bundle. Such a position has been reported from several other instances of gall formation (Meyer & Maresquelle 1983: 487). It is considered as advantageous to growth into a functional gall for gall midge larvae (Cecidomyiidae) (Morrison & Quiring 2009).

Above the vascular bundle, the novel tissue begins with several gall cells that differ from the other leaf cells by their dense stainability. A few gall cells grow into the solid aggregation of transformed cells in the centre of the infested part, and proceed centrifugally with the formation of rows of daughter cells



**Fig. 6.** Shoot galls in *Podostemum weddellianum*. **A.** two leafy shoots arising from root (R): shoot at left uninfested, with flower (asterisk); shoot at right branched, one branch uninfested, terminating in flower (asterisk), the other modified to a gall (arrow); **B.** outside view of galled shoot with normal leaves below and cone-shaped gall above, the latter irregularly covered with scaly, leaflet-like appendages; **C.** opened gall showing thick gall wall with external scaly appendages, and cavity with chironomid pupa slightly folded out at lower end (photograph by M. Jung-inger). Scale bars: A-C=3.0 mm.

(Fig. 3D). It is the still hypothetical assumption that the stimulating trigger, present in the transformed cells, has proceeded to the daughter cells from one cell to the other and further outward in the tissue (Fig. 3E,F). This assumption may be supported by the observation that groups of transformed cells (visible by their densely stained cytoplasm) are demarcated from other groups of cells that differ from gall cells and might be only slightly induced (Fig. 3F). Distinct borders exist between gall cells and the tissue of less induced or non-induced cells. It is assumed that induced cells have been influenced by the larva while others have not, since distinct portions of gall cells seen intermingled with groups of ungalled or less affected, partially induced tissue and normal tissue cells may reflect the process of gall tissue establishment. It is also possible that only a few plant cells need to be influenced by the larva for gall development to begin. From these few cells, a cascade of periclinally divided descendants (rows of gall cells) could then arise. However, the precise process remains unknown. The number of induced cells increases radially, as mitotic divisions of galled cells show.

The solid aggregation of cells formed at the lower end of the cavity is perforated by force farther distally. The perforation leads to the central gall cavity, showing that the tissue has been destroyed from the centre onward (Fig. 3E). By feeding on the gall tissue, the larva presumably forms the central cavity in which it lives. Later scraping of the established tissue enlarges the cavity, in mature galls reducing the gall cells to a small fringe around the cavity. Similar observations of larvae feeding from the newly formed parenchymatous tissue within a gall chamber are known from dipteran gallers in the family Chloropidae (De Bruyn 1995).

IV. The structures of the leaf galls in *Noveloa coulteriana, Marathrum utile* and *Apinagia riedelii* represent a simple type of galls consisting of a bulged cavity lined by nourishing gall tissue. Leaf modifications with a similar meristematic tissue lining the expanded gall tunnel are described, for example, from *Salix grandiflora* and *Urtica dioica*, where they are caused by the activity of cecidomyiid larvae (Meyer & Maresquelle 1983: 59).

In contrast to *Noveloa coulteriana* and *Marathrum utile*, the gall tissue in leaf galls of *Apinagia riedelii* has a more complex structure, which includes an additional outer area around the cavity with many new vascular strands. Similar zonation has been described, for example, from galls induced by wasps (Hymenoptera: Cynipidae) (Csóka et al. 2005: 596).

The shoot galls in *Podostemum weddellianum* are the most complex of those examined here, and can be referred to as pith galls (closed galls). Although induced by a chironomid as in the other cases, the pith gall in *P. weddellianum* differs considerably from the leaf galls in the other species. Besides the production of novel, evidently nourishing, tissue it also involves alteration of the shoot into a conical shape, with a firm gall wall that is irregularly covered with scaly leaflet-like appendages not expressed anywhere else on these plants. While leaf galls only cause a bulge on the infested leaf, the pith gall represents a complete transformation of the stem of the plant. Thus, the midge larva can be seen as acting like a parasite, suppressing regular development of the flowering shoot and seed production. The dense cover of imbricately arranged scaly appendages on the outside of the gall represents a novel structure. Consequently, there exists a rather complex modification of the original leafy shoot of the host plant by the insect (Raman 2011: 519).

V. The two openings found near opposite ends of the leaf galls in three of the four cases treated here are hypothesized to have been made by the larvae, and to cause a constant flow of fresh water through the gall that keeps the inhabitant well supplied with oxygen. It seems likely that one of the openings marks the spot at which the juvenile larva initially entered the host. The adult females of the chironomid species involved do not have ovipositors or similar structures with which they could deposit eggs inside previously undamaged plants. However, the precise life stage and behaviour during infestation of the respective larvae are unknown. The absence of openings from closed galls of Podostemum weddellianum and the relatively much more solid gall walls raise interesting issues as they would seem to make it more difficult for the gall inhabitant to maintain oxygen supply and to exit the gall upon adult emergence. However, the midges involved in this case (Endochironomus complex sp. 2) do not show special morphological features pertaining to these processes; further details remain to be investigated.

VI. The habitats of the host plants (in swift current for much of the year) may account for why nearly none of the chironomids discussed here have been collected outside of specific samples of Podostemaceae. The single exception are some adults of *Polypedilum mehinaku* that were caught in light traps by Fittkau (1971).

Prior to the present study, chironomid larvae were implicated as causing gall formation in only two cases (see introduction and Table 2). Moreover, these interactions were mentioned only briefly, in works that largely addressed other topics (Fittkau 1971, Bidawid & Fittkau 1996, Hanson & Gómez-Laurito 2005). This may partly explain why the Chironomidae have been overlooked in most discussions of insects inducing true galls in plants (e.g. Raman 2009). Nevertheless, the present findings confirm those earlier records beyond reasonable doubt.

Tavares et al. (1998: 2159) reported larvae of Chironomidae and Pyralidae as living "inside the leaf epidermis" of Podostemaceae from Brazil, and



**Fig. 7.** Shoot galls in *Podostemum weddellianum*, SEM images. **A.** sagittal section of gall showing cavity (insect removed), thick gall wall and external scaly appendages (arrow); **B.** close up of (A) showing injured cells (arrow) lining the cavity, and external appendages protruding from gall wall; **C.** median section of gall (insect removed) showing firm wall with few appendages at gall base, and distal slit in gall wall connected to cavity surrounded by appendages (arrow); **D.** median section of gall with insect (in) in cavity showing firm, compressed gall wall from which external appendages arise irregularly; note papillose cells on abaxial side of appendages; **E.** outside view of gall with irregularly positioned scaly appendages; note papillose cells; **F.** part of opened gall with insect (in) in cavity showing gall wall of compressed, elongate cells. Scale bars: A,C=1 mm; B,E=500 µm; D=500 µm; F=200 µm.

interpreted this as a strategy for protection from enemies. Such shelter indeed is another advantage to gall inhabiting larvae, in addition to the nourishment provided by the plant tissue. However, the authors did not report whether their observations pertained to special cecidia or merely to damage caused by insects feeding on, or mining in, regular plant tissue.



**Fig. 8.** Shoot galls in *Podostemum weddellianum* (photograph by Cristiana Koschnitzke). Top view of plant mat in situ recently exposed by falling water level, showing closed galls and open ones with large cavity holes from which the emerged midge adults have exited.

Although the taxonomy of the insect larvae in the above-mentioned works was rarely resolved at the species level, it is clear that members of the insect fauna associated with Podostemaceae have developed several widely differing strategies for interacting with, and benefiting from, their plant hosts. For example, the leaves of Podostemaceae also serve as attachment places for larvae and pupae of Diptera (Simuliidae), and for sheltering cases of Trichoptera (IJ-Z pers. observ.).

The interaction between *Polypedilum* sp. A and its three known Podostemaceae hosts (*Apinagia riedelii*, *Marathrum utile* and *Noveloa coulteriana*) is unusual, as the vast majority of gallers are specific to a single host (Caneiro et al. 2009). In this context it is notable that the cecidia caused by *P*. sp. A in the different hosts all constitute structurally similar galls, even though the three involved plant genera have no close phylogenetic affinities. Tippery et al. (2011) placed *Apinagia riedelii* in their clade B and *Marathrum utile* in the relatively close clade A of the South American Podostemaceae, but the Central American *Noveloa coulteriana* in its own, distant clade E.

The four chironomid species known to induce galls in Podostemaceae are not seen as very closely related either. Two of them represent separate divisions in the genus *Polypedilum*, the remaining two species belong to members of the *Endochironomus* complex of genera. Both *Polypedilum* and the *Endochironomus* complex also include numerous members with various other larval feeding modes (see, e.g., Thienemann 1954, Armitage et al. 1995, Spies et al. 2009). Consequently, it is apparent that the midges' capacity to induce galls, and the corresponding responses in the Podostemaceae hosts, have evolved several times independently.

When larval mouthparts in the gall inducing chironomid species are compared to those in probable close relatives that do not induce galls, e.g. in members of the *Endochironomus* complex with larvae living as plant miners, the differences observed are gradual rather than substantial. This is neither surprising nor evidence against the phenomena observed and discussed in the present work, as feeding on tissue surfaces can work the same way in a fully enclosing gall as in the tunnel part of a mine.

# **Concluding remarks**

The four species of Podostemaceae studied here differ in geographic distribution. Noveloa coulteriana is restricted to western Mexico (Novelo & Philbrick 1997). Marathrum utile occurs from Honduras to Colombia and Venezuela (Novelo et al. 2009). Apinagia riedelii ranges from south of the Amazon River in Brazil to the Brazilian state of Rio Grande do Sul and adjacent Argentina. Podostemum weddellianum is largely restricted to the Brazilian states of Minas Gerais, Rio de Janeiro, Santa Catarina, Rio Grande do Sul and adjacent regions (Philbrick & Novelo 2004). These distribution areas together represent a considerable portion of the range of Podostemaceae in the neotropics. However, only relatively few of the four species' populations, and only a small share of the taxa in the family and region, could be treated here. Therefore, it is expected that many interesting results associated with these and other such gall phenomena are still awaiting their discovery. One main aim of the present paper is to stimulate more such research.

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