2007

Structural Attributes of the Hypogeous Holoparasite Hydnora Triceps Drège & Meyer (Hydnoraceae)

Kushan U. Tennakoon
Old Dominion University

Jay F. Bolin
Old Dominion University

Lytton J. Musselman
Old Dominion University, lmusselm@odu.edu

Erika Maas

Original Publication Citation

Tennakoon, K. U., Bolin, J. F., Musselman, L. J., & Maass, E. (2007). Structural attributes of the hypogeous holoparasite *Hydnora triceps* Drège & Meyer (Hydnoraceae). *American Journal of Botany, 94*(9), 1439-1449. doi:10.3732/ajb.94.9.1439

American Journal of Botany 94(9): 1439–1449. 2007.

STRUCTURAL ATTRIBUTES OF THE HYPOGEOUS HOLOPARASITE *HYDNORA TRICEPS* DRÈGE & MEYER (HYDNORACEAE)[1]

KUSHAN U. TENNAKOON,[2,3] JAY F. BOLIN,[3] LYTTON J. MUSSELMAN,[3,5] AND ERIKA MAASS[4]

[2]Department of Botany, University of Peradeniya, Peradeniya 20400, Sri Lanka; [3]Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529 USA; and [4]Department of Biology, University of Namibia, Windhoek, Namibia

The morphology of the hypogeous root holoparasite *Hydnora triceps* is highly reduced, and as with many holoparasites, the vegetative body is difficult to interpret. The vegetative body of *H. triceps* has been historically considered a "pilot root" studded with lateral appendages known as "haustorial roots." We found the vegetative body of *H. triceps* to consist of a rhizome with a thickened root-cap-like structure that covered a vegetative shoot apical meristem. From the apical meristem, procambial strands originated and developed into endarch collateral vascular bundles arranged radially around a pith without an interfascicular cambium. Xylem vessels had scalariform pitting and simple perforation plates. A continuous periderm without root hairs was observed. Increase in girth was attributed to cork and fascicular cambia. "Haustorial roots" or bumps on the surface of the vegetative body were exogenous, contained meristems and were the origins of vegetative branching, budding, and haustoria. The haustoria of *H. triceps* were cylindrical and penetrated the host root stele. Phloem and xylem elements were observed within the endophyte, and direct xylem to host-xylem contacts were observed. The arrangement of vascular tissues and xylem anatomy of *H. triceps* are likely plesiomorphic features in light of Hydnoraceae's placement in the Piperales.

Key words: anatomy; haustoria; holoparasite; homeosis; *Hydnora*; Hydnoraceae; parasitic plants.

The Hydnoraceae is a root holoparasitic family and includes some of the most intriguing plants known. Without a direct requirement for sunlight, root holoparasites frequently have unusual adaptations and morphologies, quite distinct from autotrophic angiosperms (i.e., Kuijt, 1966, 1969; Gedalovich-Shedletzky and Kuijt, 1990; Mauseth et al., 1992; Hsaio et al., 1993, 1994). The multiple origins of parasitism in the tree of life provide an example of both convergent evolution and morphological novelty in the diverse vegetative morphologies of root holoparasites.

The taxonomic position of the Hydnoraceae has been uncertain because of its morphological reduction. The Hydnoraceae are the only angiosperms with no leaves or scales of any sort. Despite a paucity of characters, Cronquist (1981) placed the Hydnoraceae within the Rafflesiales, and more recently Takhtajan (1997) considered the Hydnorales and Rafflesiales related to the Aristolochiaceae. Alternatively, Cocucci and Cocucci (1996) proposed a relationship to the Annonaceae and Mitrastemonaceae based on floral characters, embryology, and geographic distributions. With molecular data, Nickerent et al. (2002) placed the Hydnoraceae in the Piperales with the Aristolochiaceae.

Hydnora spp. are found in the semiarid regions of Africa and in the southern Arabian peninsula (Musselman and Visser, 1989). More than 12 species have been described (Vaccaneo, 1934; Harms, 1935). Based on an extensive review of world herbarium specimens, Musselman (1991) recognized *H. africana* Thunb., *H esculenta* Jum. & H. Perrier, *H. johannis* Becc. (= *H. abyssinica* A. Braun = *H. solmsiana* Dinter), and *H. triceps* Drège & Meyer as distinct species. Subsequently *H. sinandevu* Beentje & Q. Luke was described from Tanzania (Beentje and Luke, 2001), bringing the total number of currently recognized *Hydnora* species to five.

The most remarkable of this extraordinary group of plants is *H. triceps*, one of the few dicotyledonous plants with its entire life belowground. *Hydnora triceps* was first described in 1833 by Drège in the northwestern Cape region of South Africa. It was thought extinct until it was rediscovered by Johann Visser in 1988, with additional discoveries in southern Namibia (Maass and Musselman, 2004). This species exclusively parasitizes the shrub *Euphorbia dregeana* Meyer (Visser, 1989; Maass and Musselman, 2004). Other *Hydnora* species parasitize a range of *Euphorbia* and *Acacia* species, and the flowers of these species emerge from the soil making them easier to detect than *H. triceps*.

Because of their cryptic nature and seasonal appearance, *Hydnora* species are rarely encountered and collected. Herbarium material preserves poorly, and this has contributed to taxonomic confusion. Except for the information available on the natural history of *Hydnora* (Harms, 1935; Musselman and Visser, 1987, 1989; Visser, 1989; Musselman, 1991; Maass and Musselman, 2004), information on the growth and morphogenesis of this unique group of parasitic plants is limited (but see Schimper, 1880; Leemann, 1933; Cocucci and Cocucci, 1996). Because of its subterranean flowering, nearly all aspects of *H. triceps* biology remain to be explored. Moreover, diamond mining and other human activities threaten its existence.

The vegetative body of *Hydnora* is belowground. On the ridges of the angular or terete body, haustoria and flower buds develop. The extreme reduction of the vegetative body of *Hydnora* and the absence of clearly distinguishable stem, root, or leaf parts limit the number of useful taxonomic characters. In a study focused on the sister genus *Prosopanche* De Bary, Schimper (1880) considered the vegetative body rhizome-like because of its root and shoot characters. Coccuci and Coccuci (1996) termed the main vegetative body of *Prosopanche* a rhizome, based on the stem-like arrangement of vascular

[1] Manuscript received 28 September 2006; revision accepted 26 June 2007.

The project was supported by the Mary Payne Hogan Endowment at Old Dominion University (ODU), USA. This work would not have been possible without the facilities of the Department of Biology, University of Namibia. The authors acknowledge the assistance of electron microscopist K. Al-Arid (ODU). K.U.T. conducted part of this investigation under the auspices of a Fulbright Hays Senior Fellowship.

[5] Author for correspondence (e-mail: lmusselm@odu.edu), fax: + 757 683 5283

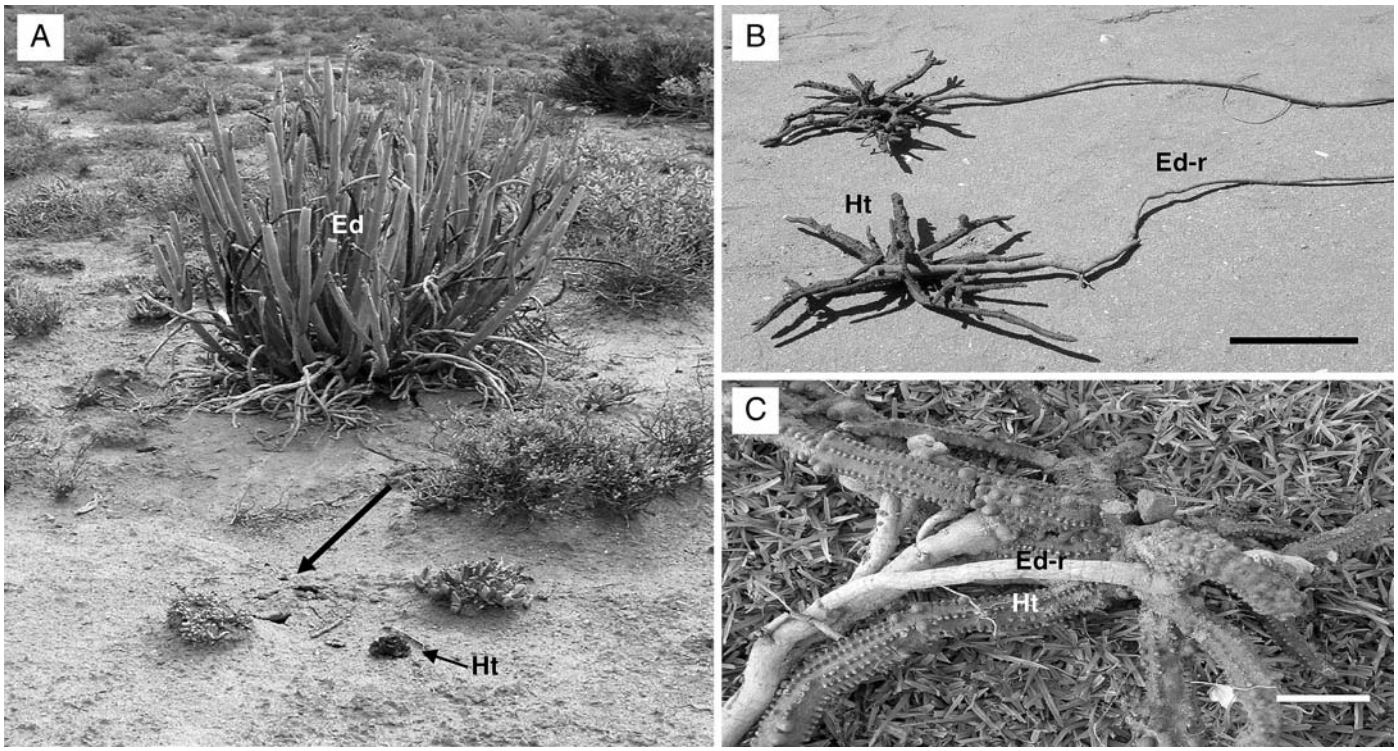

Fig. 1. General features of the host plant *Euphorbia dregeana* and *Hydnora triceps* in sandy Richtersveld coastal plain. **(A)** Approximately 1.5 m tall host plant *E. dregeana* [**Ed**]. In foreground, dried perianth part of *H. triceps* [**Ht**] excavated by unknown animals and bulge of earth indicating a hypogeous flower (see large arrow). **(B)** Intertangled mass of the subterranean vegetative body of *H. triceps* [**Ht**] parasitizing the roots of *E. dregeana* [**Ed-r**], note articulation. **(C)** The mostly pentagonal vegetative body of *H. triceps* ornamented with "bumps," background is grass lawn. Scale bars: Fig. 1B = approximately 0.5 m; Fig. 1C = 4 cm.

tissues. In Kuijt's (1969, p. 114) influential review of parasitic plants, he used the terms "pilot roots" for the main vegetative body and "haustorial roots" for the tuberous outgrowths (or bumps). His description of the vegetative body was based on gross morphological observations that the vegetative body is "not articulated, and appear[s] to have a caplike tissue protecting the apical meristem." The term "haustorial root" was applied based on its ability to form intimate connections with the host roots. In 1988, when Johann Visser rediscovered *H. triceps* (after being uncollected for almost 80 yr), he regarded the anatomical nature of the vegetative body as obscure (Visser, 1989) and adopted the terminology used by Kuijt (1969).

Classification of many holoparasitic plant organs challenges taxonomists and anatomists because of their great morphological reduction. The morphological convergence and novelty observed among disparate groups of holoparasitic plants is, in our view, among the clearest examples of plant evolution and adaptation. Our goal is to describe the vegetative and haustorial anatomy of this highly reduced group of plants in context of its placement in the Piperales and sister to the Aristoloceaceae; we use *H. triceps* as the example.

MATERIALS AND METHODS

Study region and field observations—Plants were collected from the Richtersveld region of South Africa in February 2004 and January, September, and December 2005. The study area was the farm Gemsbokvlei, located approximately 20 km east of Port Nolloth, Northern Cape Province (29°18.301′ S, 17°01.098′ E), where the coastal dunes meet the first line of mountains at Oograbiesberg. The Richtersveld in general and Oograbiesberg in particular are recognized as centers of endemism (Williamson, 2000). The remarkable flora of this area is dominated by a diverse assemblage of leaf succulent Aizoaceae and shrubby *Euphorbia* species. The high rate of local endemism is a function of topography and climatic extremes. Precipitation occurs almost entirely during the winter and varies from less than 50 mm annually at the coast to 300 mm near Steinkopf, approximately 90 km inland (Williamson, 1996). The study site averages less than 80 mm of precipitation per year (N. Kotze, farmer, personal communication, 2005) but receives a near daily misting of cool Atlantic fog. At the study site dense fog moves nightly from the coast until it meets the west slope of Oograbiesberg, then quickly dissipates after sunrise. Temperatures at the study site fluctuate dramatically. During field excursions in September 2005, shaded air temperatures recorded by a HOBO Datalogger (Onset Computer Corporation, Bourne, Massachusetts, USA) ranged from 3.1 to 41.9°C over 24 h.

To evaluate host specificity and to estimate the rate of parasitism, we established six 10×100 m transects in a random stratified pattern running east–west from a north–south baseline. Because of its hypogeous nature, *H. triceps* was difficult to detect. Nevertheless, *Euphorbia* spp. were generally well spaced making positive identification of the host possible after excavation of the main haustorial cluster. When flowering, plants can be located by following the fetid odor originating from their osmophores. Alternatively, plants were located by searching for cracks or small mounds of soil near the base of *E. dregeana* shrubs (Fig. 1A). Occasionally, *H. triceps* was located by digging under dried flowers or fruits scattered on the soil surface, presumably excavated by jackals or other mammals. Most plants were observed on the sandy coastal plains, but some *H. triceps* were observed on the stony slopes and ridgelines of the Oograbiesberg.

Anatomy—*Light microscopy*—We randomly collected material from different parts of the vegetative body and the haustoria of *H. triceps* plants

still attached to *E. dregeana*. Plant parts were thoroughly washed to remove soil and debris. To ensure a representative study, we fixed and sectioned 30 segments of the vegetative body from approximately 15 individuals (mean = 8.8; range 4–14 mm) and 40 haustoria (mean = 6.9; range 4–15 mm).

In the field, plant material was fixed and stored in a solution of 40% formaldehyde, glacial acetic acid, 95% ethanol, in distilled water (FAA) prepared at a ratio of 2 : 1 : 10 : 7 (see Berlyn and Miksche, 1976). The fixed plant material was dehydrated in an ethanol–xylene series (Johansen, 1940; Berlyn and Miksche, 1976) and embedded in Paraplast Plus Tissue Embedding Medium (Fisher Scientific Co. L.L.C., Houston TX) tissue embedding medium at 58°C before sectioning. Serial sections were cut at 8–12 μm on a Spencer A 820 Microtome (American Optical Corp., Buffalo NY), affixed to microscope slides with Haupt's adhesive (Johansen, 1940), and stained. Because of the large quantities of tannin in *H. triceps* and latex in *E. dregeana* tissues, of the several staining procedures initially tried, 1% aqueous safranin-0. 1% fast green (in 95% alcohol) (Johansen, 1940) and 0.05% aq. Toludine blue O in citrate-phosphate buffer (pH 5.5) (Berlyn and Miksche, 1976) gave best results. Representative sections were photographed using a Biophot photomicroscope (Nikon Inc., Garden City NY) and Camedia C 4000 digital camera (Olympus Corp., Melville NY).

Scanning electron microscopy—For scanning electron microscopy, small pieces of the vegetative body and haustorial tissues were fixed in 3% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M Na cacodylate buffer at pH 7.4. The materials were brought into the laboratory in the fixative and left for approximately 1 mo before being further processed. Then the materials were postfixed in cacodylate-buffered 1% OsO_4 for 2 h, washed again for 2 h, and dehydrated with a stepwise series of increasing ethanol–acetone concentrations.

Specimens for scanning electron microscopy were prepared in a critical point dryer CPD 7501 (Polaron Instrument, Newhaven, UK), affixed to a specimen holder, and coated with gold. Some specimens were observed with SEM using a modified version of the wax-embedded slide preparations described earlier. Thin wax-embedded sections (8–12 μm) were affixed to cover glasses. Then paraplast was cleared from the sections with a double xylene wash. Then samples were air-dried and coated with gold. The specimens were observed with a Leo 435VP scanning electron microscope (Carl Zeiss SMT AG Oberkochen, Germany) operating at 15 kV.

RESULTS

Field observations—Field excavations of *H. triceps* revealed that the vegetative body was arranged radially around a central haustorial mass composed of several large haustoria and a tangle of "pilot roots," hereafter referred to as the vegetative body (Fig. 1B, C). The maximum horizontal length of the plants ranged from 0.8 to 1.6 m ($N = 5$). The usually pentamerous or hexamerous vegetative bodies (diameter ranged from 9–30 mm) were invariably thickest in the center of the plant, seemingly the site of the seed germination and attachment. The vegetative body traversed the soil in apparently random directions (Fig. 1B) but maintained an orientation mainly parallel to the soil surface.

Excavated plants bore numerous dry flowers from past seasons and some remnants of fruit as first described by Maass and Musselman (2004). Flowers of the current season and buds were concentrated 15–35 cm from the apical meristem and were not observed on mature portions of the vegetative body. Frequently, distal portions of the parasitized host root beyond the haustoria were dead (Fig. 1B). Despite numerous excavations, no seedlings or small nonflowering *H. triceps* plants were located.

In the sandy flats, *E. dregeana* was the dominant shrub, but other *Euphorbia* spp. co-occurred in declining importance: *E. ephedroides* E. Meyer ex Boiss., *E. mauritanica* L., *E. chersina* N.E. Br., and *E. tuberculata* Jacq. Flowering *H. triceps* was observed on 22% of *E. dregeana* shrubs (182 total host plants scored). Of course, this was likely an underestimate of parasitism because of the plant's exclusively hypogeal habit, only flowering parasites were scored and parasitism was never observed on small hosts (<10 stems per host plant). No other *Euphorbia* spp. were observed as hosts of *H. triceps* at this study site (seven collecting trips) or at the first Namibian *H. triceps* location near Rosh Pinnah (five collecting trips).

Vegetative body morphology and anatomy—The body of *Hydnora* consisted of both vegetative and reproductive structures. With the exception of dermal and conducting tissue, the vegetative body was composed of tannin-containing parenchyma cells. A root-cap-like structure was apparent in the primary growth tip of *H. triceps* (Fig. 2A). The outer portion of this structure appeared to be continuous with the periderm, and the inner portion was derived from the cauline apex of the meristematic region of cells in the growth tip (Fig. 2A–C). The apical meristematic region was characterized by densely stained nuclei, thin cell walls, and several mitotic figures (Fig. 2B). Procambial strands originated from the meristematic region (Fig. 2C) and developed into a cylinder of discrete vascular bundles (Figs. 2F and 3A) separated by ground tissue, with typical stem organization (Fig. 3A). However, unlike stem organization in a typical dicotyledonous stem, the ground tissue that separated the procambial strands of *Hydnora* did not give rise to an interfascicular vascular cambium that joined the separate procambial strands. The number of primary vascular bundles corresponded to the number of sides of the angular vegetative body, mostly pentagonal or hexagonal. The vascular bundles alternated with the ridges of lateral appendages or bumps (Fig. 2F). In the transverse view of the vegetative body, traces of vascular tissue to the bumps were arrayed in a star pattern alternating with the primary vascular bundles (Fig. 2D, F). The cells of the central region of the vegetative body were closely examined for remnant xylem cells to confirm the presence of a true pith and to rule out the possibility of dilated metaxylem. No xylem was found in the pith.

A key morphological feature of *Hydnora* was an external protective layer of suberized cells of the cork/phellem (Figs. 2A, 3E, G). This exogenous layer was continuous over the vegetative body including the apical meristem (Fig. 2A). The suberized cork layer of periderm was approximately 4–6 cells thick throughout the vegetative body except at the growing tip, where it was two to three times as thick (Fig. 2A). The growing tip of freshly excavated *Hydnora* was the only visibly moist portion of the vegetative body; this suggested that the growing tip is lubricated as it penetrates the soil. Cork/phellem was formed to the outside by a cork cambium that presumably originated beneath the epidermal layer (Fig. 3E). The secondary cortex or the cells of the phelloderm produced to the interior of the phellogen were living at maturity, lacked suberin lamellae, and resembled cortical parenchyma cells filled with tannin (Fig. 3E).

The increase in girth (secondary growth) of the *Hydnora* vegetative body just below the growth tip resulted from the activity of two lateral meristems, the vascular cambium and the cork cambium. The fascicular cambium added cells to the secondary xylem and secondary phloem of the collateral vascular bundles (Fig. 3B, C). The xylem consisted of vessels and lignified fibers. A few interspersed fibers were observed within the phloem. The protoxylem tips of the xylem tissue were always directed toward the pith (Fig. 3A, B). Equal amounts of xylem and phloem cells were observed. Further-

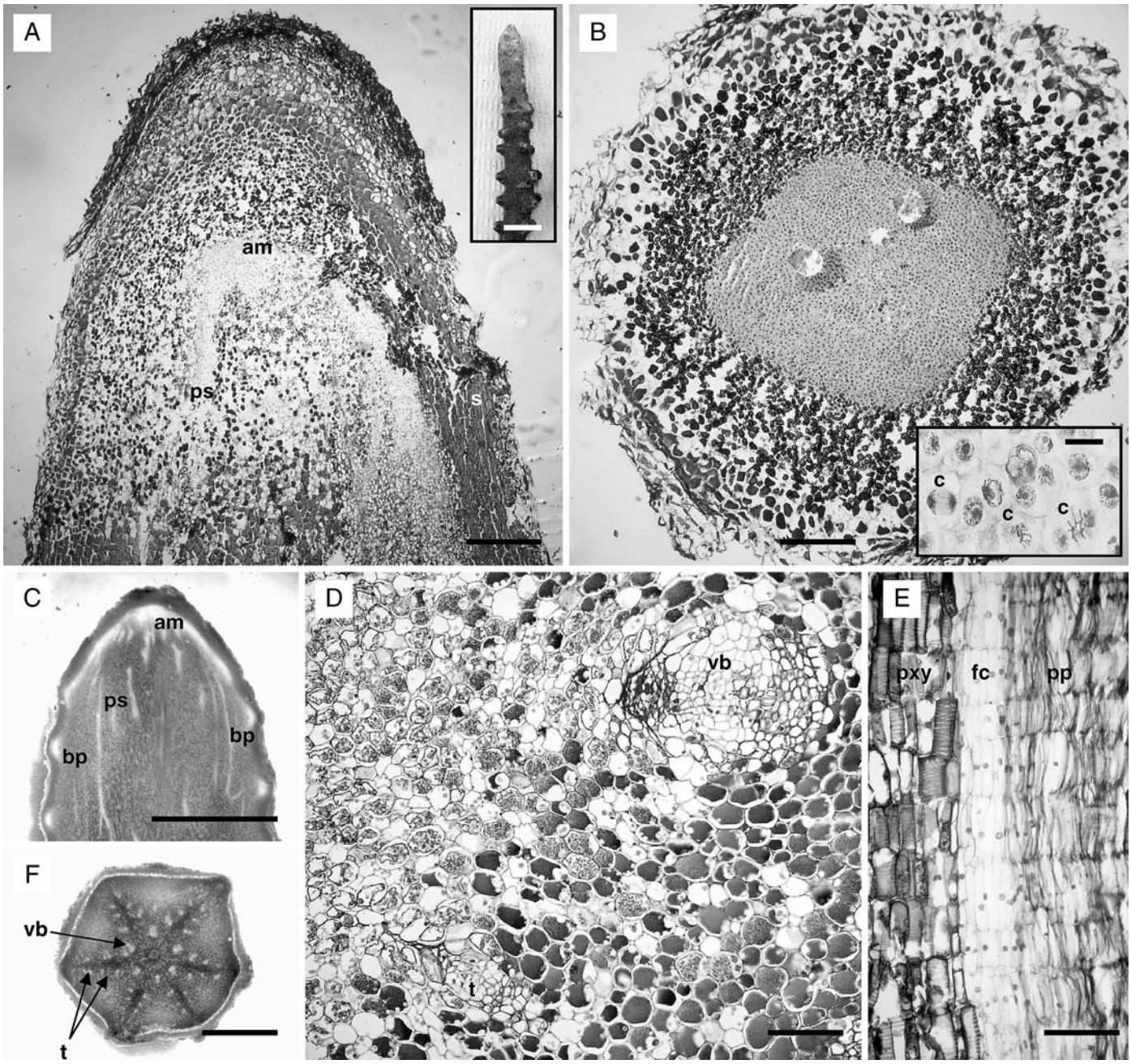

Fig. 2. Light micrographs and hand sections of the growth tip and vegetative body of *Hydnora triceps*. **(A)** Longitudinal section of the *H. triceps* primary growth tip showing distinct cell types: thick suberized layer of the periderm [**s**]. This exogenous suberized layer is continuous in all parts of the vegetative body; apical meristem [**am**]; procambial strands [**ps**] emerge from the meristematic region. Inset shows gross morphology of growth tip. **(B)** Transverse section of *H. triceps* growth tip showing the central meristematic region. The magnified inset shows meristematic cells with their nuclei in various stages of mitosis [**c**]. **(C)** Longitudinal hand section of *H. triceps* primary growth tip showing the apical meristem [**am**], procambial strands [**ps**], and the exogenous origins of the bumps. Note the continuity of the meristematic tissue (light in contrast with the parenchyma) between the apical meristem [**am**] and the primordia [**bp**] of developing bumps, or lateral appendages. **(D)** Transverse section approximately 3 cm below the apical region of *H. triceps* growth tip, showing larger vascular bundle [**vb**] relative to smaller vascular trace [**t**] leading to a "bump" or lateral appendage. Pith is oriented in the lower right corner. **(E)** A longitudinal section of the young vascular strand [primary xylem, **pxy**; fascicular cambium, **fc**; primary phloem, **pp**]. **(F)** Hand transverse section of a hexagonal vegetative body approximately 3 cm below the apical region of *H. triceps* growth tip, note six vascular bundles [**vb**] in collateral arrangement around a central pith alternating with the ridges of bumps. Traces of vascular tissue [**t**] arrayed in star-shaped pattern leading to bump meristems. Scale bars: Fig. 2A = 2.5 mm; Fig. 2A inset = 1 cm; Fig. 2B = 1.25 mm; Fig. 2B inset = 30 μm; Fig. 2C = 5 mm; Fig. 2D = 250 μm; Fig. 2E = 200 μm; Fig. 2F = 4 mm.

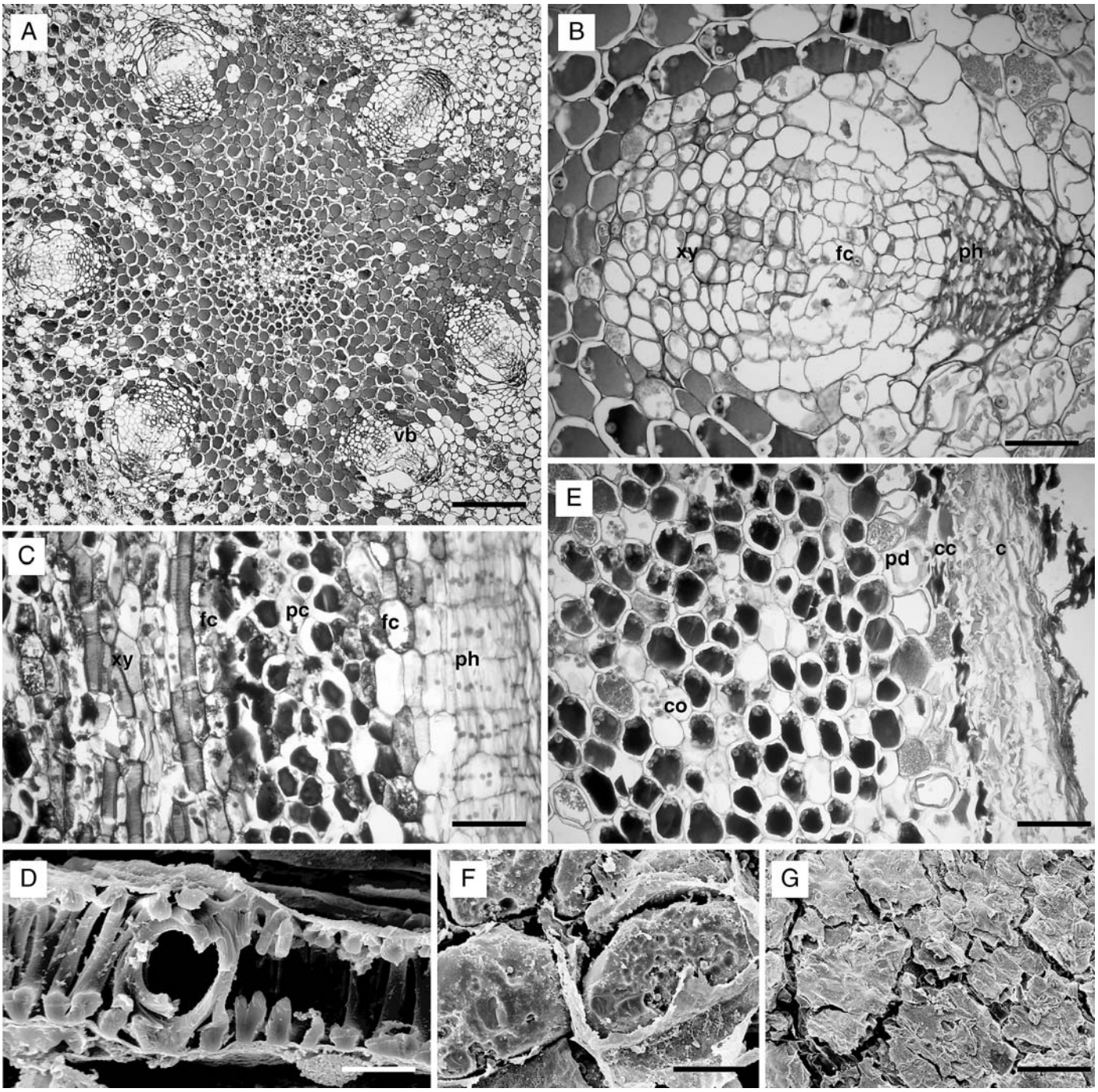

Fig. 3. Light micrographs of the *Hydnora triceps* vegetative body, vascular bundle, and periderm. SEM images of xylem elements, tannin-filled parenchyma, and suberized cork layer. **(A)** A transverse section of mature *Hydnora* vegetative body (taken about 4 cm from the growth tip) showing peripherally arranged vascular bundles [**vb**] depicting typical "stem" characters. Note the eustelic, endarch, and collateral vascular bundles in a cylinder of discrete strands separated by ground tissue. Interfascicular vascular cambium is absent between the vascular bundles. **(B)** Detailed anatomy of a mature vascular bundle [**xy**, xylem; **fc**, fascicular cambium; **ph**, phloem]. **(C)** Detailed anatomy of a longitudinal section of a mature vascular strand showing xylem vessels [**xy**] separated from the phloem [**ph**] by the divided fascicular cambia [**fc**] and parenchyma cells [**pc**]. **(D)** SEM image of xylem elements, note scalariform pit membranes and simple perforation plate **(E)**. Transverse section of the peripheral area of the mature *H. triceps* vegetative body showing the activity of cork cambium [**c**, cork layer; **cc**, cork cambium; **pd**, phelloderm; **co**, cortex]. **(F)** SEM image of the *H. triceps* cortex showing tannin-filled parenchyma cells. **(G)** SEM image of the outermost suberized cork layer in periphery of the main body of *H. triceps*. Note the scaly surface texture. Scale bars: Fig. 3A = 400 μm; Fig. 3A inset = 0.5 cm; Fig. 3B = 70 μm; Fig. 3C = 100 μm; Fig. 3D = 20 μm; Fig. 3E = 110 μm; Fig. 3F = 30 μm; Fig. 3G = 500 μm.

more, because of the absence of an interfascicular vascular cambium, secondary growth of xylem and phloem did not result in the formation of a cylinder of secondary vascular tissues in the main vegetative body. Xylem elements were approximately 90–110 μm long and displayed scalariform pitting (Fig. 3C, D). Perforation plates were simple and 10–12 μm in diameter (Fig. 3D). Endodermis and pericycle layers were not observed. Moreover, in contrast to conventional roots, root hairs or similar protruding structures were not present in any part of the body.

Cortex and pith of *Hydnora* were a mass of tannin-filled parenchyma cells traversed by a ramifying group of collateral vascular bundles. All cells adjacent to the vascular bundles were ordinary tannin-filled parenchyma cells (Fig. 3B, F). Mechanical tissues such as collenchyma, sclereids, fibers, or any other obviously specialized cell types, were absent in the pith and cortex. In the mature vegetative body (>2 cm diameter), parenchymatous cells were observed between distinct xylem only and phloem only bundles (Fig. 3C). The apparently divided fascicular cambium or perhaps thickening meristems among the parenchyma may be responsible for the bifurcation of the vascular bundles and some increase in girth of the mature body.

On the ridges of the mostly pentamerous to hexamerous vegetative body, specialized lateral appendages known as bumps were located (Figs. 2C and 4A). The meristematic tissue of the bump primordia was continuous with the main apical meristem of the vegetative body (Fig. 2C). Each bump contained meristematic cells. The primordia of active bumps either becomes corky and dormant or differentiates into haustoria, lateral branches, or flower buds. Occasionally as the bumps expanded, some of the overlying periderm was stretched and ruptured. After contact with a host root, some of the young bumps formed haustoria (Fig. 4B–D). However, we observed that the vast majority of bumps remained dormant and did not branch, produce haustoria, or flower buds, and were occasionally fused among themselves. The vascular tissues into haustoria and flower buds were bifurcated from the central strands of the procambial tissue (data not shown). Articulation of the vegetative body appeared to be the direct result of bumps developing and expanding, particularly after the dieback of an apical growth tip. SEM investigations confirmed that trichomes, stomata, and lenticels were absent on the *Hydnora* vegetative body (Fig. 3G).

Structure of haustoria—The haustoria of *H. triceps* were generally cylindrical (Fig. 4B). Field excavations showed that a single host root can support multiple *Hydnora* haustoria, and autoparasitism was frequently observed. The cortical folds of haustoria produced a collar comprised of parenchyma cells filled with tannin (Fig. 4C, D).

The body of the *H. triceps* haustorium can be divided into two main regions, an outer cortex (mostly with tannin cells scattered throughout) and a central axial region containing the vascular tissues that penetrated the host root (Fig. 4D). The outermost layer of the haustorium was composed of a well-developed cork layer. The endophyte and the associated cortical fold partly encircled the host root. The haustorium penetrated the host root by periclinal divisions within the converging central mass of tissue. The penetrating endophyte of the haustorium contained three areas: (1) elongate digitate parenchyma cells at the periphery of the advancing endophyte, (2) meristematic cells adjacent to conducting tissue, and (3) the central region filled with tannin (Fig. 4D, E).

Serial transverse sections of the host–parasite interface (Fig. 5A, B) revealed direct connections between parasite xylem and host xylem and between parasite contact parenchyma and host xylem. We did not observe a phloem tissue graft between the two partners. A marked preponderance of elongated, thin-walled parasite contact parenchyma cells were visible in the endophyte tissue that adjoined the host xylem vessels (Fig. 5A). Furthermore, terminating files of *Hydnora* tracheary elements that formed host xylem continuities were observed (Fig. 5B). With SEM, we could not confirm the presence of penetrating oscula, open and often trunk-like haustorial intrusions, into the host xylem tissues (Dörr, 1997).

DISCUSSION

We conducted the first complete field excavations of individual *H. triceps* plants ($N = 5$) that we are aware of. Despite the proximity of *H. triceps* to five *Euphorbia* spp. in the sandy flats of the study area, only *E. dregeana* was observed as a host of *H. triceps*. Extreme host specificity as in *H. triceps* is relatively unusual among parasitic plants and would seem to be a good strategy only for extinction. Notably, *Hydnora africana,* a plant with a broader host range (but excluding *E. dregeana*), was observed parasitizing a few isolated *E. mauritanica* plants within a larger "patch" of *E. dregeana*, an apparent case of niche partitioning.

In many aspects *H. triceps* is a unique holoparasite, its vegetative body difficult to homologize to typical plant organs. At first sight, the vegetative body of *H. triceps* suggests a ramified "pilot root" because of its subterranean habit, presence of a root-cap-like structure at the growth tip, and the absence of external morphological characters such as leaves, stomata, or scales. However, the rest of the vegetative body does not possess other specific root-like structures: there were no root hairs, no endodermis or casparian strip, and no root protostele. The arrangement of primary tissues proximal to the growth tip does not correspond to a typical root structure in any respect; that is, the tissues lack alternate bundles of phloem and xylem vascular strands. The vascular bundles of *H. triceps* vegetative body are collateral (in a single vascular bundle, the phloem is on the same radius as the xylem and positioned external to it) and arranged cylindrically in a stele with a conspicuous pith, thus having all the hallmarks of a typical dicotyledonous stem (Esau, 1953). The main vascular bundles alternated with the ridges of the angular (usually five- or six-sided) vegetative body. Our ongoing investigations have revealed that the related species, *H. africana,* has similar anatomical features in the mature vegetative body. Still, the unusual anatomical features of *Hydnora* make it difficult to interpret the homology of the entire vegetative body.

As suggested by Baird and Riopel (1986a), Sattler (1988), Mauseth et al. (1992), and Hsiao et al. (1994), it may be useful to avoid thinking in terms of separate organ types such as "roots" and "stems" when dealing with highly modified structures like the *Hydnora* vegetative body. Sattler (1988) and Lehmann and Sattler (1992) point out that homeosis (transference of features) in plants generates novelty through the rearrangement of existing parts that can result in "hybrid" structures. The *Hydnora* vegetative body, however, appears to be essentially a rhizome with some root-cap-like features. The

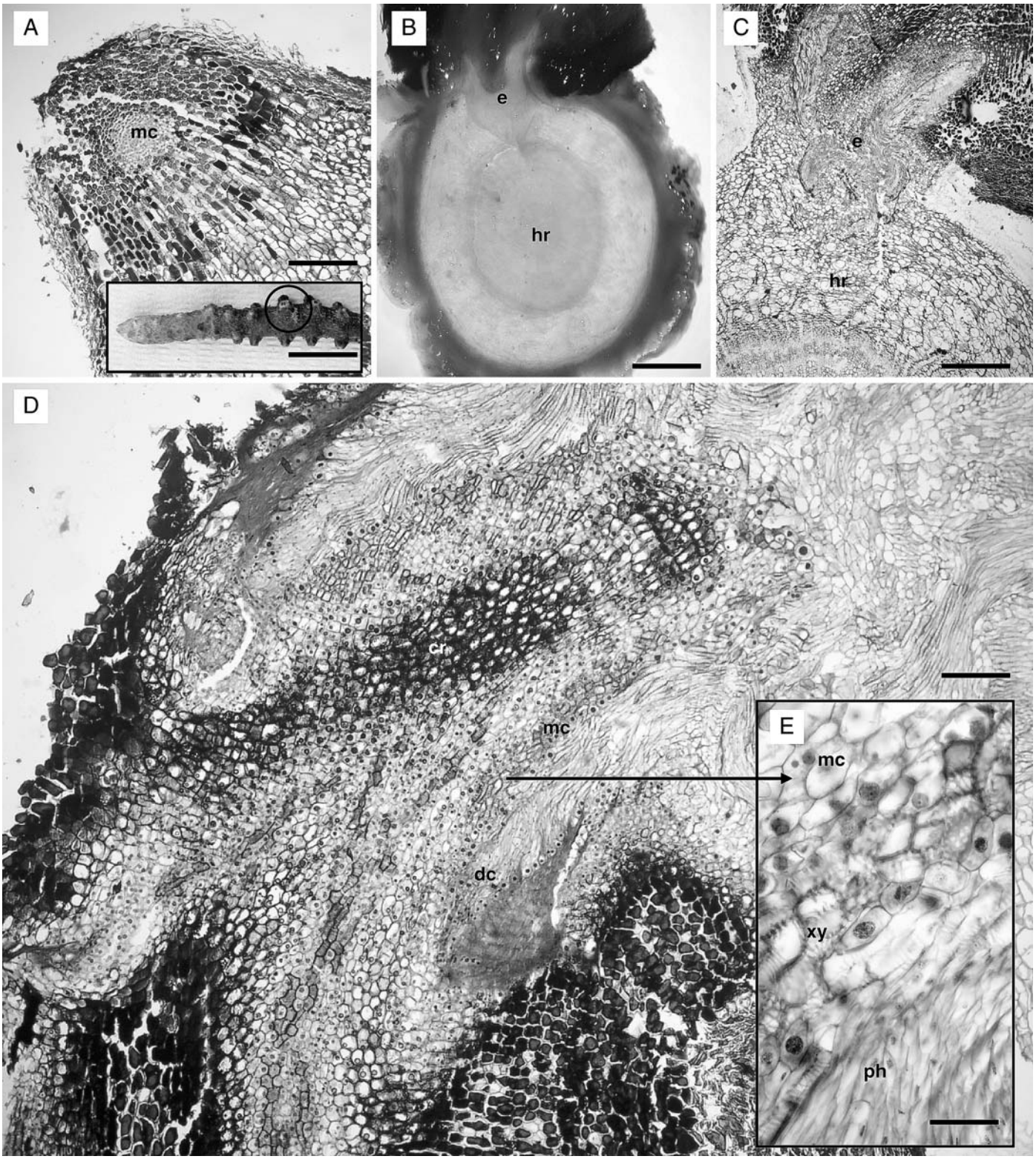

Fig. 4. Light micrographs of *Hydnora triceps* lateral appendages or "bumps" and the endophyte **(A)** An active primodium in a "bump" on the surface of the *H. triceps* vegetative body. Central meristematic cells [**mc**] that can differentiate into either haustoria, flowers, or branches. Note how the overlying cork layer is stretched and ruptured when the dividing cells of the bumps expand outwards. **(B)** Upon contact with a host root [**hr**], the active bumps can develop into an endophyte [**e**]. **(C)** Transverse section through the center of an endophyte [**e**] penetrating a root of *E. dregeana* [**hr**]. **(D & E)** Detailed anatomy of the initial point of contact of *H. triceps* haustorium. Note the three cell types present in the endophyte (1) elongate digitate parenchyma cells [**dc**] at the periphery of the advancing endophyte, (2) meristematic layer of cells adjacent to xylem [**xh**] and phloem elements [**ph**], (3) the central region of the endophyte filled with tannin [**cr**]. Scale bars: Fig. 4A = 0.5 mm; Fig. 4A inset = 0.5 cm; Fig. 4B = 2.5 mm; Fig. 4C = 1 mm; Fig. 4D = 400 μm; Fig. 4E = 60 μm.

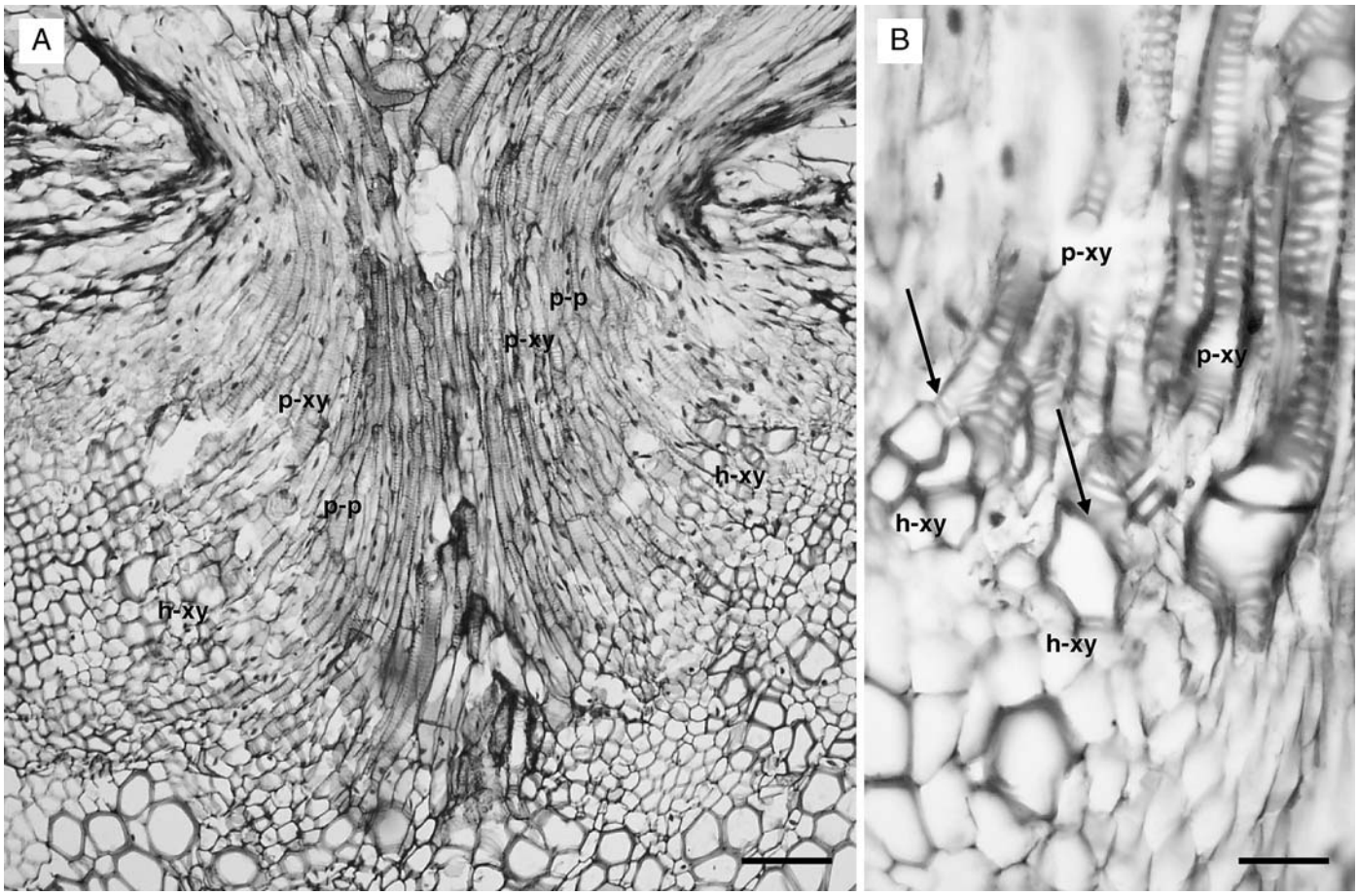

Fig. 5. Light micrographs of the haustorial interface. **(A)** Anatomy of the haustorial interface between *H. triceps* (top center) and *E. dregeana* (bottom) root showing parasite xylem [**p–xy**] and parasite elongate parenchyma [**p–p**] penetrating the host vascular stele and associated host xylem tissues [**h–xy**]. **(B)** An image of a parasite xylem [**p–xy**]–host xylem [**h–xy**] connection, arrows indicate interface. In both micrographs note the different orientation and perpendicular graft of the host and parasite vessels. Scale bars: Fig. 5A = 100 μm; Fig. 5B = 40 μm.

root-cap-like structure may be a product of homeosis or perhaps more simply a continuation of the periderm, with extra thickening contributed by the cauline apex of the meristematic region.

However, the possibility remains of independent initiation of a true root tip just after the seedling establishes on a host root or as a radicle. In some root parasites, a tubercle (a poorly differentiated mass of cells) is formed after initial seedling attachment to the host root (see Baskin and Baskin, 1998). The first development of independent shoot and root structures from the tubercle have been reported for some species of Orobanchaceae and Balanophoraceae (Rangaswamy, 1967; Arekal and Shivamurthi, 1976; Baird and Riopel, 1986b). Nothing is known about the seed germination of *Hydnora* (Kuijt, 1969). Thus, observations of germination and seedling development are needed to completely understand *Hydnora* morphogenesis.

The primary tissues immediately underlying the region of cell division (away from the growing tip), but not sharply delimited from it, constitute the region of elongation. The cells of this area compose a fairly well-defined protoderm, ground meristem, and the procambial strands. This leads to the well-organized eustele-like ring of vascular bundles, similar to a dicotyledonous stem. Thus, *Hydnora* grows in length only near the growing tips and only a very limited portion of the vegetative body is constantly pushed through the soil. During field visits, we observed moisture (possibly mucigel) in the same region, apparently for lubrication through soil. This contributes to the characterization of the apical protective layer as a root-cap-like structure.

The meristematic region of the growing tip of the vegetative body of *H. triceps* was clearly demarcated by cells with densely stained nuclei, thin cell walls, and several mitotic figures. The apical meristematic region was continuous with the bumps (or lateral appendages) just under the dermal layers in the early stages of development (Fig. 2C), demonstrating the bump's exogenous origin. Endogenous bump origin is further excluded by the absence of a pericycle and the lack of evidence for bump emergence from a central stele or inner cortex. Procambial vascular strands that arise from the apical meristematic region directly differentiate into collateral vascular bundles.

The region of elongation is followed by the region of maturation. There are two types of growth in this region, the increase in the diameter of the vegetative body and the differentiation/ramification of "bumps." The increase in

diameter is attributable to the meristematic activity of the fascicular cambium and the cork cambium and perhaps divisions of the matrix parenchyma of the pith and cortex as reported for species in the family Balanophoraceae (Hsiao et al., 1993, 1994). Because of the absence of an interfascicular vascular cambium, the cork cambium undoubtedly plays a greater role for the girth increment of *Hydnora*.

There are still some unsolved problems with the concept of bumps differentiating into haustoria, flowers, or bifurcations of the rhizome. We do not know the precise conditions that dictate the development of either buds or haustoria. We can discount the term "haustorial root" for the lateral appendages or bumps. The bumps are of exogenous origin and are derived from the apical meristem (Fig. 2C), similar to the development of primordia from a vegetative shoot apical meristem. An unusual modification of *H. triceps* is the continuous periderm overlaying the developing bumps and the vegetative shoot apical meristem.

The thick continuous dermal arrangement of the main vegetative body protects *Hydnora* from desiccation in dry desert environments. However, the specific scaly surface texture of the cork cell layer in the periphery of the main body of *Hydnora* must be permeable to gases (similar to transcuticular movement) for respiration. Transdermal water loss was extremely low for buried lengths of the vegetative body (0.19 mg $H_2O \cdot cm^{-2} \cdot h^{-1}$; J. F. Bolin, unpublished data).

Schimper (1880) investigated the vegetative anatomy of *Prosopanche americana* (R. Br.) Baill. (*P. americana* = *P. burmeisteri*) and to a lesser extent the anatomy of *H. abyssinica* and *H. africana*. He considered the vegetative body rhizome-like. Our observations of vascular arrangement were generally congruent with Schimper's observations for *P. americana* and *H. africana,* except we found no evidence to support his observations of numerous mucilage ducts. Moreover, Schimper and other authors considered the vegetative body unarticulated, possibly the result of only working with limited preserved and herbarium material. His figures of *H. abyssinica* depict a terete vegetative body with a scattered vascular bundle arrangement, which is interesting in light of similar vascular arrangements in some Piperaceae (Metcalfe and Chalk, 1954).

Cocucci and Cocucci (1996) interpreted the vegetative body of *Prosopanche* as a rhizome with vascular bundles separated in a "star-like contour." They observed extensive mucilage and tannin ducts within the pith. They also considered *Prosopanche* an annual plant because it lacks secondary structures and thickening meristems. We can discount this notion at least for *Hydnora* spp., which are certainly perennial plants based on our observations of marked plants (between January and December 2005) and the ubiquitous presence of dried fruits and perianth parts from previous seasons associated with mature plants. Cocucci and Cocucci (1996) suggested that occasional branching is due to divisions of the apical meristem. For *H. africana* and *H. triceps*, we observed apical tip dieback followed by development of lateral bumps into branches of the rhizome.

In contrast to the *H. triceps*, which has a rhizome with a root-cap-like structure, other root holoparasites have strongly dissimilar vegetative morphologies. The vegetative structure of the root holoparasite *Pholisma depressum* is clearly a root, based on the presence of a triarch vascular organization, lateral roots, and a thin root cap (Kuijt, 1966, 1967). The unusual main vegetative bodies of *Helosis cayennensis, Langsdorffia hypogaea,* and *Ombrophytum subterraneum* are irregular spherical structures (considered tubers with few typical shoot or root characters) lacking apical meristems, roots, or leaves (Mauseth et al., 1992; Hsaio et al., 1993, 1994).

The structure and function of the penetrating *Hydnora* endophyte was similar to those of some root parasitic Olacaceae, Loranthaceae, and Santalaceae (Kuijt, 1977; Weber, 1980; Beyer et al., 1989; Fineran, 1991) in that it penetrates the host tissues and is vascularized. The intrusive haustorial interface of *H. triceps* contrasts with that of many root holoparasites in the Balanophoraceae. For *Balanophora* spp. (Gedalovich-Shedletzky and Kuijt, 1990), *Helosis cayennensis* (Hsaio et al., 1993), *Langsdorfia hypogaea* (Hsaio et al., 1994), and *Ombrophytum subterraneum* (Mauseth et al., 1992), the host root tissue grows into the parasite tuber and the parasite forms a complex of absorptive strands. The endophyte of the root holoparasite *Pholisma depressum* is similar to the *Hydnora* endophyte in that it penetrates the host root and establishes xylem–xylem continuities (Kuijt, 1966), although no phloem was observed in the *Pholisma* endophyte. The *Hydnora* endophyte could be distinguished from the surrounding host root cortical cells by the presence of tannins in some of its parenchymatous cells and the contrasting perpendicular arrangement of host and parasite vascular tissues. Penetration may be due to a combination of mechanical and enzymatic activities as proposed for many unrelated groups of parasitic plants (Press et al., 1999). The cells that originate from the meristematic zone of the growing endophyte are characterized by dense cytoplasm and clear nuclei. The xylem and phloem of the endophyte were clearly distinguishable. Lumen-to-lumen tracheary/vessel continuities between host and parasite were observed. However, we could not conclusively demonstrate the phloem tissue graft at the host–parasite interface with light microscopy and SEM. Thus we are unable to explain the precise pathway of host phloem-derived solutes into *Hydnora*. A transmission electron microscope study may reveal a phloem element graft.

Our findings contrast with work of Leemann (1933) on the haustoria of *H. abyssinica*. He found no evidence of a vascularized endophyte. However, he noted what he termed "giant cells" in the endophyte, which he interpreted as "active intermediates" for transport. This may be analogous to what we regard as parenchymatous contact cells or elongate digitate cells in the haustoria. The largely parenchymatous surface (contact cells/transfer cells) of the host–parasite interface can be viewed as being principally involved in selective uptake and processing of host-derived solutes as suggested for many shoot parasites, such as *Cuscuta reflexa* (Haupt et al., 2001) and *Amyema* spp. (Pate et al., 1991), and root parasites, such as *Striga hermonthica* (Pageau et al., 2003), *Rhinanthus minor* (Seel and Jeschke, 1999), *Olax phyllanthi* (Tennakoon and Pate, 1997), *Santalum acuminatum* (Tennakoon et al., 1997), and *Orobanche cernua* (Hibberd et al., 1999). Selective and active solute acquisition via the parenchymatous interface may contribute significantly to the generation and maintenance of osmotic gradients between the host and parasite. However, observation of lumen-to-lumen tracheary/vessel continuities between host and parasite show that bulk intake of xylem fluids from host roots may also play a role in solute acquisition. A comparison of host- and parasite-derived xylem and phloem solutes combined with the application of tracers may indicate the relative quantities of solute movement/processing from hosts to *Hydnora*.

The placement of the Hydnoraceae in the Piperales with the

Aristolochiaceae provides an opportunity to search for synapomorphies within the group. Nickrent et al. (2002) summarizes some ancestral states, including floral merosity, placentation, embryo type, and perianth insertion. The Aristolochiaceae is a diverse group with some phylogenetic relationships still unresolved within the family and among the Piperales. Moreover, multiple character states are common within this group (Nickrent et al., 2002). Building on the work of Carlquist (1993) and Dickison (1996), our work presents mutual xylem features between the Aristolochiaceae, Lactoridaceae, Piperaceae, and now the Hydnoraceae. All of these groups have vessel elements with simple perforation plates and scalariform to alternate vessel pitting (scalariform in *Hydnora*).

The rhizome anatomy of *Hydnora* also has Piperalean features. The Piperales have several distinct arrangements of vascular bundles. Some genera of Piperaceae have scattered vascular bundles as in a typical monocotyledonous stem (Metcalfe and Chalk, 1954), which brings to mind Schimper's (1880) observation of a similar arrangement in *H. abyssinica*. Many Piperaceae and Aristolochiaceae have widely separated vascular bundles, radially arranged around a broad pith, without an interfascicular cambium as in *H. triceps* (Metcalfe and Chalk, 1954; Datta and Dasgupta, 1977; Dickison, 1996; Souza et al., 2004). "Aristolochia type" dichotomously branched vascular bundles were not observed (Metcalfe and Chalk, 1954), nor was a complete interfascicular cambium present as in some Aristolochiaceae (i.e., Dickison, 1996). Medullary vascular bundles noted from some Piperaceae (Souza et al., 2004) were not observed in our light microscopic investigations. However, cortical bundles and other cortical structures interpreted as mucilage ducts have been observed in *Prosopanche* (Cocucci and Cocucci, 1996) and *Hydnora* (Schimper, 1880). Moreover, in our hand sections of very large rhizomes (>3 cm diameter), some structures were observed that could be interpreted as either medullary bundles or, as previously discussed, vascular bundles separated into xylem- and phloem-specific bundles (resembling two concentric rings of vascular tissue). Alternatively, structures interpreted as medullary bundles and mucilage ducts by other authors may simply be vascular traces to the bumps. Unfortunately, our interpretations of these structures are limited because rhizome sections of very large diameter were not sampled for light microscopy.

As in the case of many intriguing root holoparasites, interpretations of our anatomical observations were made difficult by morphological reduction and homeotic feature transference. Our findings suggest that the vegetative body of *Hydnora* is a rhizome with a root-cap-like structure required for its subterranean habit. Its relationship to the Aristolochiaceae and general Piperalean stock is supported by our observations of xylem characters, the absence of an interfascicular cambium, and the collateral arrangement of its vascular bundles. Of course much remains to be examined among other members of the Hydnoraceae, and we regard findings in this paper as an incremental step in our understanding of morphological convergence and novelty in root holoparasites.

LITERATURE CITED

AREKAL, G. D., AND G. R. SHIVAMURTHI. 1976. Seed germination in *Balanophora abbreviata*. *Phytomorphology* 26: 135–138.

BAIRD, W. V., AND J. L. RIOPEL. 1986a. Life history studies of *Conopholis americana* (Orobanchaceae). *American Midland Naturalist* 116: 140–151.

BAIRD, W. V., AND J. L. RIOPEL. 1986b. The developmental anatomy of *Conopholis americana* (Orobanchaceae) seedlings and tubercles. *Canadian Journal of Botany* 64: 710–717.

BASKIN, C. C., AND J. M. BASKIN. 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, California, USA.

BEENTJE, H., AND Q. LUKE. 2001. Hydnoraceae. *In* H. Beentje and S. A. Ghazanfar [eds.], Flora of tropical East Africa, 1–7. A. A. Balkema, Rotterdam, Netherlands.

BERLYN, G. P., AND J. P. MIKSCHE. 1976. Botanical microtechnique and cytochemistry. Iowa University Press, Ames, Iowa, USA.

BEYER, C., W. FORSTREUTER, AND H. C. WEBER. 1989. Anatomical studies of the haustorium ontogeny and the remarkable mode of penetration of the haustorium in *Nuytsia floribunda* (Labill.) R. Br. *Botanica Acta* 102: 229–235.

CARLQUIST, S. 1993. Wood and bark anatomy of Aristolochiaceae; systematic and habital correlations. *IAWA Journal* 14: 341–357.

COCUCCI, A. E., AND A. A. COCUCCI. 1996. *Prosopanche* (Hydnoraceae): somatic and reproductive structures, biology, systematics, phylogeny and potentialities as a parasitic weed. *In* M. T. Moreno, J. I. Cubero, D. Berner, D. Joel, L. J. Musselman, and C. Parker [eds.], Advances in parasitic plant research, 179–193. Junta de Andalucía, Dirección General de Investigación Agraria, Cordoba, Spain.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York, USA.

DATTA, P. C., AND A. DASGUPTA. 1977. Comparison of vegetative anatomy of Piperales. I. Juvenile xylem of twigs. *Acta Biologica Academiae Scientiarum Hungaricae* 28: 81–96.

DICKISON, W. C. 1996. Stem and leaf anatomy of *Sauruma henryi* Oliv. including observations on raylessness in the Aristolochiaceae. *Bulletin of the Torrey Botanical Club* 123: 261–267.

DÖRR, I. 1997. How *Striga* parasitizes its host: a TEM and SEM study. *Annals of Botany* 79: 463–472.

ESAU, K. 1953. Plant anatomy. John Wiley, New York, New York, USA.

FINERAN, B. A. 1991. Root hemiparasitism in the Santalales. *Botanische Jahrbücher für systematik Pflanzengeschichte und Pflanzengeographie* 113: 227–308.

GEDALOVICH-SHEDLETZKY, E., AND J. KUIJT. 1990. An ultrastructural study of the tuber strands of *Balanophora* (Balanophoraceae). *Canadian Journal of Botany* 68: 1271–1279.

HARMS, H. 1935. Hydnoraceae. *In* A. Engler and H. Harms [eds.], Die natürlichen planzenfamilien, 282–295. Wilhelm Engelmann, Leipzig, Germany.

HAUPT, S., K. J. OPARKA, N. SAUER, AND S. NEUMANN. 2001. Macromolecular trafficking between *Nicotiana tabacum* and the holoprasaite *Cuscuta reflexa*. *Journal of Experimental Botany* 52: 173–177.

HIBBERD, J. M., W. P. QUICK, M. C. PRESS, J. D. SCHOLES, AND W. D. JESCHKE. 1999. Solute fluxes from tobacco to the parasitic angiosperm *Orobanche cernua* and the influence of infection on host carbon and nitrogen relations. *Plant Cell and Environment* 22: 937–947.

HSIAO, S. C., J. D. MAUSETH, AND L. D. GOMEZ. 1993. Growth and anatomy of the vegetative body of the parasite angiosperm *Helosis cayennensis* (Balanophoraceae). *Bulletin of the Torrey Botanical Club* 120: 295–309.

HSIAO, S. C., J. D. MAUSETH, AND L. D. GOMEZ. 1994. Growth and anatomy of the vegetative body of the parasite angiosperm *Langsdorffia hypogaea* (Balanophoraceae). *Bulletin of the Torrey Botanical Club* 121: 24–39.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York, New York, USA.

KUIJT, J. 1966. Parasitism in *Pholisma* (Lennoaceae). I. External morphology of subterranean organs. *American Journal of Botany* 53: 82–86.

KUIJT, J. 1967. Parasitism in *Pholisma* (Lennoaceae). II. Anatomical aspects. *Canadian Journal of Botany* 45: 1155–1162.

KUIJT, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, California, USA.

KUIJT, J. 1977. Haustoria of phanerogamic parasites. *Annual Review of Phytopathology* 17: 91–119.

LEEMANN, A. C. 1933. The anatomical and physiological relation between *Hydnora solmsiana* Dinter and its host. *Annals of Botany* 47: 159–162.

LEHMANN, N. L., AND R. SATTLER. 1992. Irregular floral development in *Calla palustris* (Araceae) and the concept of homeosis. *American Journal of Botany* 79: 1145–1157.

MAASS, E., AND L. J. MUSSELMAN. 2004. *Hydnora triceps* (Hydnoraceae)—first record in Namibia and first description of fruits. *Dinteria* 29: 1–10.

MAUSETH, J. D., S. C. HSIAO, AND G. MONTENEGRA. 1992. Vegetative body of the parasitic angiosperm *Ombrophytum subterraneum* (Balanophoraceae). *Bulletin of the Torrey Botanical Society* 119: 407–417.

METCALFE, C. R., AND L. CHALK. 1954. Anatomy of the dicotyledons—leaves, stem and wood in relation to taxonomy with notes on economic uses. Clarendon Press, Oxford, UK.

MUSSELMAN, L. J. 1991. The genus *Hydnora* (Hydnoraceae). *In* J. K. Ransom, L. J. Musselman, A. D. Worsham, and C. Parker [eds.], Proceedings of the fifth international symposium on parasitic weeds, 247–250. CIMMYT, Nairobi, Kenya.

MUSSELMAN, L. J., AND J. H. VISSER. 1987. *Hydnora johannis* in southern Africa. *Dinteria* 19: 77–82.

MUSSELMAN, L. J., AND J. H. VISSER. 1989. Taxonomy and natural history of *Hydnora* (Hydnoracecae). *Aliso* 12: 317–321.

NICKRENT, D. L., A. BLARER, Y. L. QIU, D. E. SOLTIS, P. S. SOLTIS, AND M. ZANIS. 2002. Molecular data place Hydnoraceae with Aristolochiaceae. *American Journal of Botany* 89: 1809–1817.

PAGEAU, K., P. SIMIER, B. LE BIZEC, R. J. ROBINS, AND A. FER. 2003. Characterization of nitrogen relationships between *Sorghum bicolor* and the root-hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth. using (KNO_3)-^{15}N as isotopic tracer. *Journal of Experimental Botany* 54: 789–799.

PATE, J. S., K. C. TRUE, AND E. RASINS. 1991. Xylem transport and storage of amino acids by southwest Australian mistletoes and their hosts. *Journal of Experimental Botany* 42: 427–439.

PRESS, M. C., J. D. SCHOLES, AND J. R. WATLING. 1999. Parasitic plants: physiological and ecological interactions with their hosts. *In* M. C. Press, J. D. Scholes, and M. G. Barker [eds.], Physiological plant ecology, The 39th symposium of the British Ecological Society, University of York, UK, 1998, 175–197. Blackwell Science, Oxford, UK.

RANGASWAMY, N. S. 1967. Morphogenesis of seed germination in angiosperms. *Phytomorphology* 17: 477–487.

RIOPEL, J. L., AND M. P. TIMKO. 1995. Haustorial initiation and differentiation. *In* M. C. Press and J. D. Graves [eds.], Parasitic plants, 39–79. Chapman & Hall, London, UK.

SATTLER, R. 1988. Homoeosis in plants. *American Journal of Botany* 75: 1606–1617.

SCHIMPER, A. F. W. 1880. Die vegetationsorgane von *Prosopanche burmeisteri*. *Bericht über die Sitzungen der Naturforschenden Gesellschaft zu Halle* 15: 21–47.

SEEL, W. E., AND W. D. JESCHKE. 1999. Simultaneous collection of xylem sap from *Rhinanthus minor* and the hosts *Hordeum* and *Trifolium*: hydraulic properties, xylem sap composition and effects of attachment. *New Phytologist* 143: 281–298.

SOUZA, L. A., I. S. MOSCHETA, AND J. H. G. OLIVEIRA. 2004. Comparative morphology and anatomy of the leaf and stem of *Peperomia dahlstedtii* C. DC., *Ottonia martiana* Miq. and *Piper diospyrifolium* Kunth (Piperaceae). *Gayana Botany* 6: 6–17.

TAKHTAJAN, A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, New York, USA.

TENNAKOON, K. U., AND J. S. PATE. 1997. Xylem fluxes of fixed N through nodules of the legume *Acacia littorea* and haustoria of an associated N-dependent root hemiparasite *Olax phyllanthi*. *Journal of Experimental Botany* 48: 1061–1069.

TENNAKOON, K. U., J. S. PATE, AND G. R. STEWART. 1997. Haustorium-related uptake and metabolism of host xylem solutes by the root hemiparasitic shrub *Santalum acuminatum* (R. Br.) A. DC. (Santalaceae). *Annals of Botany* 80: 257–264.

VACCANEO, R. 1934. Hydnoraceae Africae. *Atti Accademia Nazoinale Lincei Memorie* 5: 414–457.

VISSER, J. H. 1989. *Hydnora triceps*. *Flowering Plants of Africa* 50: 1992.

WEBER, H. C. 1980. Studies on Australian and New Zealand Loranthaceae/Viscaceae. 1. The morphology and anatomy of underground organs of *Nuytsia floribunda*. *Beiträge zue Biologie der Pflanzen* 55: 77–99.

WILLIAMSON, G. 1996. The succulent *Euphorbia* species of the Richtersveld and southern Namib desert (Sperrgebiet). *Euphorbia Journal* 10: 99–133.

WILLIAMSON, G. 2000. Richtersveld: the enchanted wilderness. Umdaus Press, Hatfield, South Africa.