Subterranean Morphology and Mycorrhizal Structures

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4.1 Introduction

For most, if not all plants, subterranean parts are less known than their aerial counterparts, due in part to the difficulty in extracting a complete root system (see Kutschera and Lichtenegger 1982, 1992; Kutschera et al. 2009) and the lack of morphological information in floras and taxonomic descriptions because many herbarium specimens do not include underground parts such as roots and rhizomes. Likewise, information from the fossil record is biased towards aerial structures (Peterson 1992) although there have been discoveries of fossils showing fungal associations with underground organs (e.g., Kidston and Lang 1921, Remy et al. 1994; Taylor et al. 1995; LePage et al. 1997; Stockey et al. 2001). To date, fossils of root-fungal associations of mycoheterotrophic plants are unknown.

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In autotrophic plants, many scientific questions can be dealt with using generalized concepts of root structure and function (e.g., Kutschera and Lichtenegger 1992; Polomski and Kuhn 1998; Gregory 2006). However, this certainly does not hold for mycoheterotrophic (MH) plants. The structure of roots, rhizomes, or subterranean scale leaves of MH plants intimately linked to the association with soil fungi is of critical ecological relevance because these plants essentially depend upon fungi for their carbon and perhaps other nutrient needs. Hence, the subterranean organs of MH plants often show remarkable morphological and anatomical adaptations to meet their specific requirements. This chapter, therefore, addresses the importance of morphology and anatomy to complement modern methods for understanding the fungal colonization patterns in MH plants and their relationships to function.

In the following, we summarize the current knowledge of structural aspects of the underground parts (for a peculiar exception, see *Afrothismia*) of MH plants ranging from bryophytes to angiosperms, the latter in systematical order following the Angiosperm Phylogeny Group (APG 2009), which has been regularly updated by Stevens (2001 onwards). We are aware of the gradual differences between species in terms of mycorrhizal dependence, however, due to space limitations, we focus on the visibly achlorophyllous species, and only include the partially mycoheterotrophs where they add to the common picture.

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The final section interprets the available information in terms of detecting phylogenetic trends of MH plants, in order to understand their evolutionary history, a subject that is receiving considerable attention in the mycorrhizal literature (see Brundrett 2002).

4.2 Nonvascular Plants

4.2.1 *Aneura*

Aneura mirabilis (Aneuraceae/Hepaticae) was described as *Cryptothallus mirabilis* by von Malmborg (1933, 1934), although it was noted earlier around 1914 (Schiffner 1934) and suggested to be either an *Aneura* or *Riccardia* species (e.g., Denis 1919; Schiffner 1934). Recently, *Cryptothallus* was formally transferred to *Aneura* by Wickett and Goffinet (2008) based on molecular and morphological characteristics. This decision is supported by the observation that the endophyte in *Aneura mirabilis* belongs to the same genus (*Tulasnella*) as that in *Aneura pinguis* (Bidartondo et al. 2003), and the mycorrhizal pattern in both species is very similar (Ligrone et al. 1993).

Aneura mirabilis mostly occurs in maritime climates (e.g., Sjörs 1949; Williams 1950; Petersen 1972; Wiehle et al. 1989; Sergio and Seneca 1997; Sergio and Garcia 1999; Boudier et al. 1999) in cool, humid, mostly peaty environments with large mats of bryophytes (Wiehle et al. 1989). Only a part of the seta and the sporangium is elevated above the surrounding mats consisting of several moss species (von Malmborg 1933; Wiehle et al. 1989; Sergio and Garcia 1999; Boudier et al. 1999). The whitish, vermiform to lobular-coralloid, brittle gametophytes are only a few centimeters in length and remain embedded within the mosses or litter. Male and female gametophytes differ in lobe shape (Williams 1950; Benson-Evans 1952; Wiehle et al. 1989).

The first structural work on the mycorrhiza in *A. mirabilis* by Denis (1919) revealed intracellular fungal colonization with hyphal coils in the ventral (lower) part of the thallus, although he considered the specimen as an albino of another chlorophyllous *Aneura* species. Von Malmborg (1933) observed hyphae growing through the seta into the sporangium and assumed that the fungus is distributed together with the spores. Williams (1950), unable to confirm this statement of von Malmborg (1933), published the first detailed investigations and provided drawings of the full life cycle, including the pattern of mycorrhizal colonization. The thallus lobes bearing antheridia or archegonia are devoid of hyphae; starch is deposited in the upper part of the thallus and around the gametangia. In an ultrastructural comparison of green hepatics and *Aneura mirabilis* (still called *Cryptothallus*), Pocock and Duckett (1984) confirmed the concentration of fungal colonization in the lower half of the thallus, but more recently, Ligrone et al. (1993) showed that the upper parts of the thallus can also become colonized in later stages. Rhizoids are also colonized, albeit in an uncoiled manner (Duckett et al. 1990). Most likely, these straight hyphae within rhizoids represent the connection to the external substrate. The carbon of this liverwort probably comes from surrounding beech (*Fagus sylvatica*) trees (Read et al. 2000; Bidartondo et al. 2003), with which it is connected via the mutual *Tulasnella* mycorrhizal fungus, although Ligrone et al. (1993) found dissimilar dolipore structures in the endophytes of birch (*Betula* spp.) and *Cryptothallus*. The fungal coils within the thallus cells eventually degenerate to dark masses (von Malmborg 1933; Williams 1950; Pocock and Duckett 1984), interpreted as digestion, and the cells can be reinfected (Ligrone et al. 1993). Williams (1950) and Pocock and Duckett (1984) stressed the difference in fungal identity between Aneuraceae hosting basidiomycetes and resembling orchid mycorrhiza, in contrast to other liverworts hosting "phycomycetous" (today considered as Glomeromycota, Schüßler et al. 2001) endophytes forming arbuscular mycorrhiza (AM) in higher plants. This fact, together with the identification of the fungi in *A. mirabilis* as *Tulasnella* spp. (Read et al. 2000; Bidartondo et al. 2003), has led to the hypothesis of a novel acquisition of *Tulasnella* spp. as associates in Aneuraceae. By attaining an epiphytic habit during phylogeny, liverworts may have lost the original

symbiotic relationship with Glomeromycota. Secondarily terrestrial Aneuraceae then could have associated with new fungal partners (Kottke and Nebel 2005; Bidartondo and Duckett 2009).

4.3 Seedless Vascular Plants

Mycoheterotrophy in the seedless vascular plants is restricted to their gametophytic phase (Read et al. 2000; Smith and Read 2008). Genera possessing acholorophyllous gametophytes (and photosynthetic sporophytes) belong to Lycopodiaceae (e.g., *Lycopodium*, *Huperzia*, Fig. 4.1a, b), Ophioglossaceae (*Ophioglosssum*, *Botrychium*, *Helminthostachys*, *Mankyua* Fig. 4.1d–f), Psilotaceae (*Psilotum*, *Tmesipteris*, Fig. 4.1g, h), some species of *Schizaea* and *Actinostachys* in the Schizaeaceae, and the monotypic species *Stromatopteris moniliformis* in the Gleicheniaceae.

4.3.1 Lycopodiaceae (Fig. 4.1a–c)

It was recognized very early that subterranean gametophytes of several *Lycopodium* species are associated with endophytic fungi (Treub 1885, 1890; Bruchmann 1885, 1910; Lang 1899; Burgeff 1938). Illustrations in Burgeff (1938) and Boullard (1979) clearly show that fungi colonize the basal region of gametophytes shortly after spore germination. Mature subterranean gametophytes show variations in form from disc-shaped with convoluted margins (*L. clavatum*, Fig. 4.1b, *L. obscurum*) to elongated, cylindrical structures (*L. complanatum* = *Diphasiastrum complanatum*, Bierhorst 1971; Gifford and Foster 1996). Gametophytes of all species have fungal colonization restricted to a zone underlying more surficial cells that give rise to antheridia and archegonia (Bierhorst 1971, Fig. 4.1c).

Although the identity of the fungus was unknown in these early studies, it was described as being aseptate and forming intracellular hyphal coils. An ultrastructural investigation of the fungal endophyte in association with achlorophyllous gametophytes of *L. clavatum* showed that complex hyphal coils and vesicles formed but arbuscules were absent (Schmid and Oberwinkler 1993a). Entrance of the fungus occurred either through rhizoids, degenerated epidermal cells, or between epidermal cells. Once within parenchyma cells of the gametophyte, host-derived plasma membrane and wall material was deposited around invading hyphae. Hyphae were multinucleate and contained bacterium-like organelles (BLOs). Hyphae became progressively more vacuolated and ultimately degenerated. The authors came to the conclusion, based on a number of unusual structural features, that this fungus-gametophyte interaction was unlike anything described in the literature and could not be attributed to a known mycorrhizal association. They therefore proposed a new term "lycopodioid mycothallus interaction" to describe the association.

More recently, based on structural features of the fungi within cells, the fungal symbionts in the gametophytes of all seedless vascular plants were suspected to be members of the Glomeromycota and to have the *Paris*-type arbuscular mycorrhiza association (Read et al. 2000). Molecular studies have confirmed this for the fungus associated with two subterranean gametophytes of *Huperzia hypogaea* collected in Ecuador: the fungus was identified as belonging to a specific clade of *Glomus*-Group A (Winther and Friedman 2008). Observations of these sectioned gametophytes confirmed earlier reports that hyphal coils are restricted to the basal region and that arbuscules are not formed.

4.3.2 Ophioglossaceae (Fig. 4.1d–f)

Gametophytes of *Ophioglossum* may be cylindrical (*O. nudicaule*, *O. vulgatum*, Boullard 1957; Gifford and Foster 1996), globose (*O. crotalophoroides*, Mesler 1976), or highly branched (*O. palmatum*, Mesler 1975). Fungal hyphae may be evenly distributed but avoiding the meristematic area and gametangia (Bierhorst 1971). Fungal colonization occurs immediately after spore germination (Campbell 1908) and gametophytes do not develop unless they are associated with the appropriate fungus. Hyphal coils, some of which

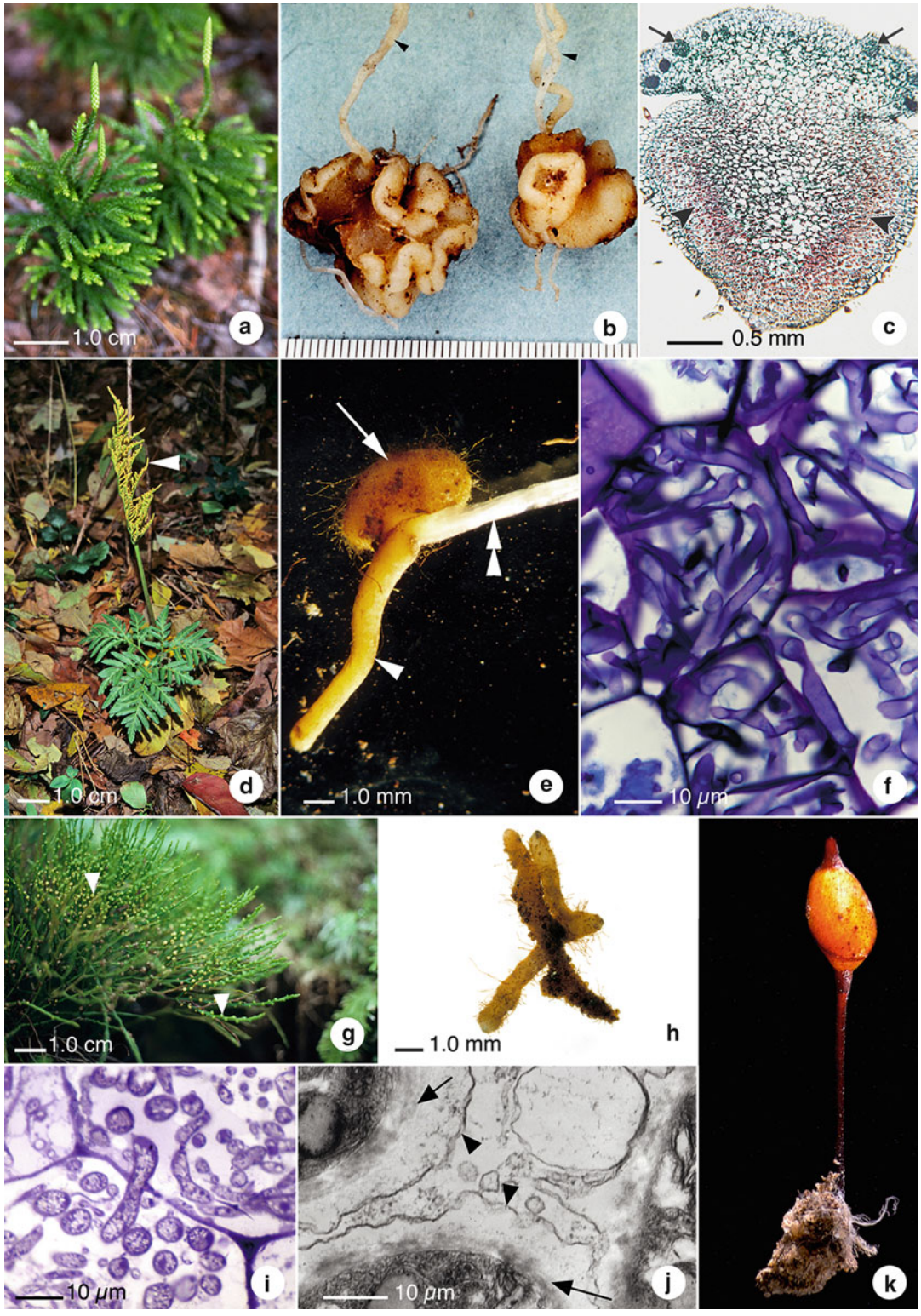

Fig. 4.1 (**a**–**c**) Lycopodiaceae, (**d**–**f**) Ophioglossaceae, (**g**–**j**) Psilotaceae. (**a**) *Lycopodium obscurum* sporophyte showing strobili. (**b**) *Lycopodium clavatum* mycoheterotrophic gametophytes with shoots (*arrowheads*). (**c**) Section of *L. obscurum* gametophyte showing zone of arbuscular mycorrhizal fungi (*arrowheads*) and antheridia (*arrows*).

have undergone degeneration, are illustrated in gametophyte cells of *O. pendulum* (Burgeff 1938). Mesler (1975) described the endophytic hyphae in gametophytes of *O. palmatum* as being non-septate and multi-nucleate. He also showed what he interpreted as vesicles in some gametophyte cells. Mesler (1976) gave a similar description of the fungal endophyte in *O. crotalophoroides*. Details at the ultrastructural level are lacking for gametophytes of *Ophioglossum* spp. and the identity of the fungus remains unknown.

Gametophytes of *Botrychium* also vary in their morphology from being tuber-like to disc-shaped (Bruchmann 1906; Burgeff 1943; Gifford and Foster 1996; Winther and Friedman 2007); endophytic fungi are restricted to a basal zone of parenchymatous cells (Bruchmann 1906; Bierhorst 1971). The fungus in *B. lunaria* has been described as forming aseptate intracellular coils and irregular vesicles (Bruchmann 1906). An ultrastructural study of the fungus-gametophyte interaction of this species (Schmid and Oberwinkler 1993b) has provided additional details. The intracellular hyphae contain vacuoles, endoplasmic reticulum, mitochondria, and lipid-like bodies. They are enclosed by host-derived plasma membrane and wall material that shows irregular outgrowths. Vesicles, some very irregular in shape, contain BLOs, and lipids; they can become very enlarged and then undergo degeneration. The identity of the fungal endophyte has been determined for the subterranean gametophytes of *B. crenulatum* (Fig. 4.1f) and *B. lanceolatum* based on DNA sequence data (Winther and Friedman 2007). The endophytes in both species belong to a major clade of glomalean fungi, *Glomus*-group A.

A third genus in the Ophioglossaceae, *Helminthostachys*, is monotypic (*H. zeylanica*) and native to the Indo-Malayan region (Gifford and Foster 1996). It also forms achlorophyllous mycoheterotrophic gametophytes (Lang 1902) but little is known of the fungal association.

A new genus and species (*Mankyua chejuense*) has been described from Cheju Island, Korea (Sun et al. 2001) based on differences in sporophyte morphological characters from the other genera in the family. Gametophytes have not been described but are presumed to be subterranean.

4.3.3 Psilotaceae (Fig. 4.1g–j)

The two genera, *Psilotum*, with two species and *Tmesipteris*, with ten species, have historically been of considerable interest because of the belief that they represented some of the most primitive extant seedless vascular plants (Bierhorst 1971; Gifford and Foster 1996). The lack of roots and the presence of much reduced leaf-like structures of the sporophyte strengthened this view. However, based on molecular evidence, Smith et al. (2006) include this family within the Psilotales, an order belonging to the extant ferns.

Subterranean gametophytes of *Psilotum* are highly variable cylindrical structures (Fig. 4.1h) sometimes showing repeated branching (Bierhorst 1971). Asexual reproductive propagules (gemmae) are frequently developed (Bierhorst 1971). Darnell-Smith (1917) was the first to succeed in achieving spore germination and to monitor early stages in gametophyte development. He reported that endophytic fungi appeared as dense "skeins" within interior cells of gametophytes and that hyphae entered rhizoids. Other authors have described an aseptate intracellular fungus thought to be a phycomycete in either field-collected gametophytes (Burgeff 1938; Boullard 1957) or

Fig. 4.1 (continued) (**d**) Shoot of *Botrychium virginianum* with fertile segment of leaf with sporangia (*arrowhead*). (**e**) Mycoheterotrophic gametophyte (*arrow*) of *B. virginianum* with a root (*arrowhead*) and base of a shoot (*double arrowhead*). (**f**) Intracellular hyphal coils of *Glomus*-Group A in a *Botrychium crenulatum* mycoheterotrophic gametophyte. Photo courtesy of Jennifer Winther. (**g**) Shoots of *Psilotum nudum* with synangia (*arrowheads*). (**h**) Branched mycoheterotrophic gametophyte of *P. nudum*. (**i**) Intracellular hyphal coils of an arbuscular mycorrhizal fungus in a sectioned *P. nudum* mycoheterotrophic gametophyte stained with Toluidine blue O. (**j**) Transmission electron micrograph of hyphae within a mycoheterotrophic gametophyte of *P. nudum* showing the interface consisting of host plasma membrane (perifungal membrane) (*arrowheads*) and host-derived intracellular matrix (*arrows*). (**k**) *Buxbaumia aphylla* sporophyte, 1.5 cm high

gametophytes growing in greenhouse pots containing various angiosperm species (Bierhorst 1953). Aspects of the ultrastructure of the gametophyte-fungus interaction have been described from gametophytes collected from greenhouse pots (Davis 1976; Peterson et al. 1981). The fungus in these gametophytes is aseptate and forms complex coils (Fig. 4.1i) that undergo degeneration; arbuscules are not formed. Intracellular hyphae are separated from the gametophyte cell cytoplasm by host-derived plasma membrane (perifungal membrane) and interfacial matrix material (Peterson et al. 1981, Fig. 4.1j), characteristics of arbuscular mycorrhizal associations (Bonfante and Perotto 1995). To date, the fungus has not been identified but the structural characteristics are typical of a *Paris*-type arbuscular mycorrhiza.

The fungal endophyte in subterranean gametophytes of *Tmesipteris tannensis* was described by Lawson (1917) and Holloay (1921) as consisting of intracellular fungal coils (pelotons). As with *Psilotum* gametophytes, the identity of the fungus has not been determined.

4.3.4 Schizaeaceae

The gametophytes of the genus (*Schizaea*) in this leptosporangiate fern family may either be surficial and green, subterranean and achlorophyllous, or a combination of both, depending on species and habitat (Bierhorst 1968, 1971). Gametophytes of all species are associated with endophytic fungi that have been described as aseptate and frequently associated with rhizoids (Bierhorst 1971; Swatzell et al. 1996).

Gametophytes of all species in the genus *Actinostachys* are axial structures that are subterranean with fungal hyphae confined to a distinctive zone (Bierhorst 1968). The identity of the fungi associated with achlorophyllous gametophytes in these two genera is unknown.

4.3.5 Gleicheniaceae

The monotypic genus *Stromatopteris moniliformis* (subfamily Stromatopteridaeae), has axial subterranean gametophytes with coiled fungal hyphae (Bierhorst 1971), reminiscent of *Paris*-type arbuscular mycorrhizas. Although Bierhorst (1971) concluded that the fungus present in the gametophyte is the same as that in the photosynthetic sporophyte, this needs to be confirmed with molecular methods.

Experimental evidence confirming transfer of nutrients from fungi to the subterranean gametophytes of all seedless vascular plants is lacking.

4.4 Gymnosperms

4.4.1 Podocarpaceae

The New Caledonian endemic *Parasitaxus usta* (not *P. ustus*, as many authors repeated the linguistically incorrect transfer from *Podocarpus* to the feminine genus *Parasitaxus* by de Laubenfels 1972) is a succulent shrub or small tree (up to 2 m high) with wine-red scale leaves (Cherrier et al. 1992; Schneckenburger 1999), unable to photosynthesize (Feild and Brodribb 2005) and only occurring closely associated with *Falcatifolium taxoides* (also Podocarpaceae, Sinclair et al. 2002). Root graft-like subterranean connections between the two species have led to the notion of parasitism in *P. usta* (de Laubenfels 1959; Köpke et al. 1981). However, Cherrier et al. (1992) and an English version of that paper adding a SEM micrograph (Woltz et al. 1994) found an endophytic mycelium (called "ectendomycelium") in both species, together with haustorial-like connections apparent at the cellular level developing in tissues up to the cambium of *F. taxoides*. The authors assume a symbiotic association of the three partners but, based on their anatomical observations, are convinced of parasitism in this case. The latest investigation on *P. usta* confirms the intimate vascular association of both species, but results from stable carbon isotope investigations suggest that most carbon is provided by the fungus (Feild and Brodribb 2005). With respect to water physiology however, *P. usta* has higher stomatal conductance and lower water potential values relative to its host, which is typical for parasitic angiosperms (Feild and Brodribb 2005). Hence, apart from being a

gymnosperm, woody, and relatively large, *Parasitaxus* is even more unique among heterotrophic organisms in possibly being a parasitic and mycoheterotrophic plant at the same time.

4.5 Monocots

4.5.1 Petrosaviaceae (*Petrosavia*)

The three species of *Petrosavia* are distributed from Japan to Java. The external morphology of the underground structures does not differ much among the species. Their subterranean rhizomes can be branched and thus, may bear several 10–15 cm high scapes with terminal racemes or corymbs of white flowers. Rhizomes measure up to 1.5 mm in diameter and are densely covered by sheathing scale leaves (Groom 1895a; Makino 1903; Stant 1970; Jessop 1979; Chen and Tamura 2000; Cameron et al. 2003). The filiform, hairless, approximately 0.5 mm thick and sparsely branched adventitious roots, are initiated from the rhizome, especially close to the base of the scape. They most likely originate from the axils of the scale leaves, as do the rhizome branches (Groom 1895a). In *Petrosavia sakuraii*, the roots predominantly grow horizontally through the substrate and can be up to 20 cm long (Watanabe 1944). This author also reports hyphae penetrating into the roots 2–5 mm proximal from the root tip.

The epidermis is either ephemeral (Groom 1895a) or partly persistent (Stant 1970). The cortex consists of a suberized exodermis, 4–6 layers of parenchyma cells, and an endodermis with particularly strong u-shaped tertiary thickenings surrounding the tetrarch central cylinder (Groom 1895a; Watanabe 1944; Stant 1970). This is similar to many mycoheterotrophic Burmanniaceae (Johow 1889; Uphof 1929) with *Dictyostega orobanchoides* as an extreme example (Imhof 2001, Fig. 4.4f). Watanabe (1944) mentions that segments of older roots lose the cortex parenchyma but remain connected to the rhizome by the central cylinder that is surrounded by the fortified endodermis. The maintenance of connectivity between roots and rhizomes bearing inflorescences is particularly important for MH plants having filiform roots, since not only water and nutrients but also carbohydrates must be transported through these comparatively long structures. A tertiary endodermis, a synapomorphy of monocots (Esau 1965), seems to be less costly than the production of layers of lignified tissue, which is the equivalent option for non-monocots in order to protect the connectivity. This economical advantage of monocots may be part of the explanation as to why monocots include disproportionately so many MH plants (Imhof 2010).

Previous investigations on *Petrosavia* (Groom 1895a; Watanabe 1944; Stant 1970) report coiled mycorrhizal hyphae within the cortex parenchyma cells. The figures and descriptions of Watanabe (1944) resemble a *Paris*-type AM but without the typical lateral arbuscules, which is similar to the mycorrhiza found in *Voyria truncata* (Gentianaceae, Imhof and Weber 1997). The advantage of the frequent feature of MH plants of having a specialized mycorrhizal colonization pattern allowing a selective digestion of hyphae while keeping the fungus alive (see further), is not apparent. Petrosaviaceae are a rather basal clade of the monocots (Cameron et al. 2003; APG 2009), which might explain its plesiomorphic, i.e., basic mycorrhizal pattern. Most recently, Yamato et al. (2011a) confirmed the structural descriptions of Watanabe (1944), and revealed this mycorrhiza as an association with a highly specific clade of *Glomus*-group A.

4.5.2 Thismiaceae (Figs. 4.2–4.4 and 4.10)

Thismiaceae are either considered to be a tribe, Thismieae, in the Burmanniaceae (e.g., Jonker 1938, Cronquist 1988) or a separate family (e.g., Agardh 1858; Thorne 1992; Takhtajan 1997; Stevens 2001 onwards). APG (2009) is still reluctant to separate them from Burmanniaceae but acknowledge the arguments for separation given by Merckx et al. (2006). We regard them as a family based on floral morphology (e.g., Maas et al. 1986; Caddick et al. 2000) and molecular evidence (Merckx et al. 2006).

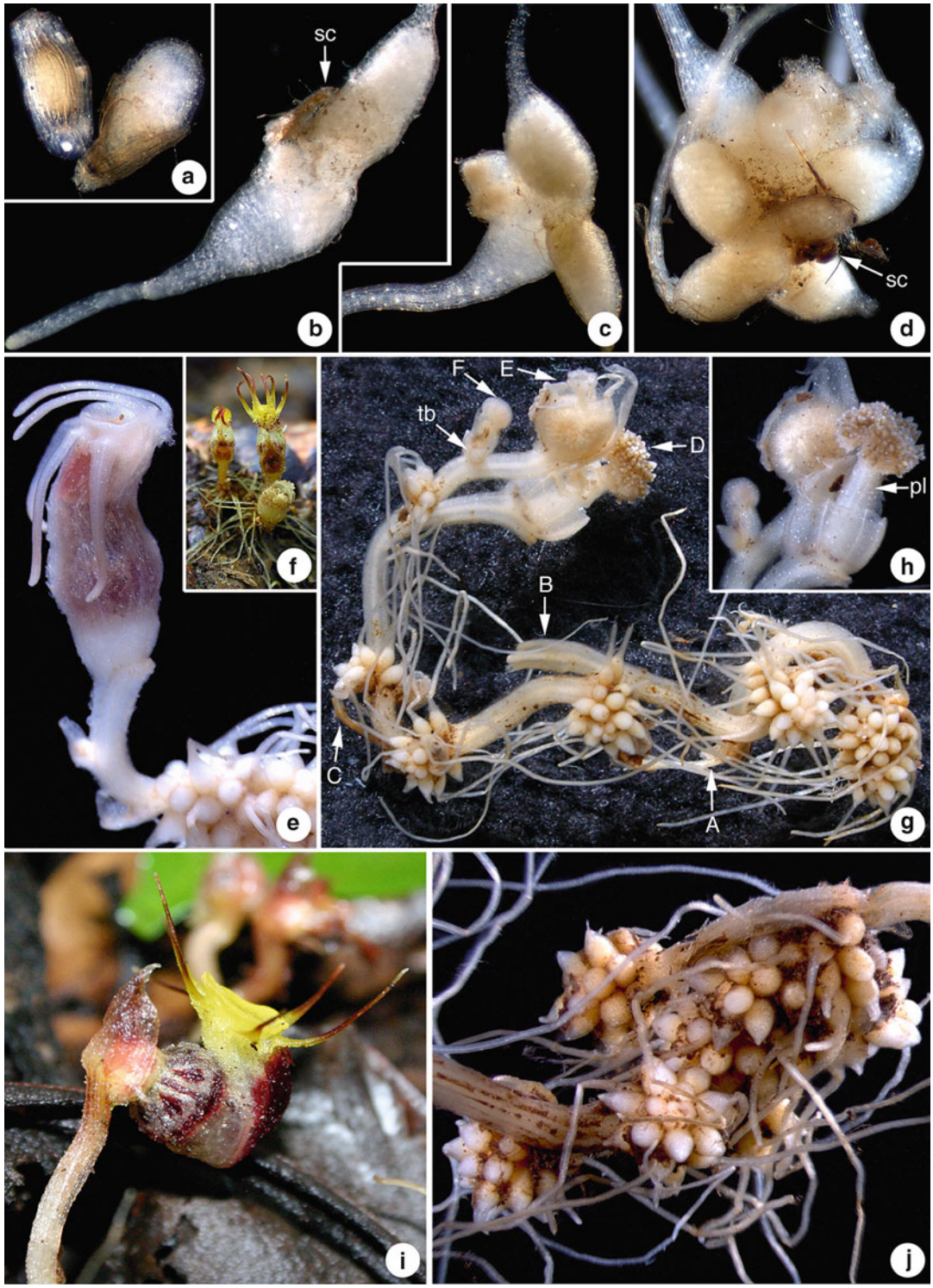

Fig. 4.2 (**a**–**h**) *Afrothismia hydra*, (**i**, **j**) *Afrothismia winkleri* (Thismiaceae). (**a**) Seed (*left*, 0.6 mm long) and an early germination stage (*right*) of *A. hydra* with disrupted seed coat (sc), giving rise to a first root tubercle. (**b**–**d**) More tubercles develop successively at the base of the initial one and the root extensions elongate. The seed coat (sc)

4.5.2.1 *Haplothismia, Oxygyne, Tiputinia*

The extremely rare *Haplothismia exannulata* from India has vermiform to tuberous, up to 3.5 cm long, roots radiating from the shoot base (Airy Shaw 1952; Sasidharan and Sujanapal 2000). For *Oxygyne triandra* from Cameroon (probably extinct, Yokoyama et al. 2008), the subterranean organs are unknown. The Japanese species, *O. shinzatoi* and *O. yamashitae*, only known from their type localities, have vermiform roots (Yokoyama et al. 2008; Yahara and Tsukaya 2008), and the original description of *O. hyodoi*, also from Japan, states "rhizoma repens" (Abe and Akasawa 1989). *Tiputinia foetida* is represented by a single specimen from Ecuador (Woodward et al. 2007), measuring about 9 cm in length. The largest part of it is an orthotropous, vermiform, 4 mm thick root, giving rise to two subterranean shoots, with only the terminal flower being epiterrestrial. The root cortex contains "intracellular, looped, septate" hyphae (Woodward et al. 2007).

4.5.2.2 *Thismia* (Fig. 4.10g, h)

This genus is by far the largest of the family, with a worldwide, although mostly tropical, distribution. The underground structures are quite variable. Most species have horizontal runner-like, vermiform roots of 1–2 mm in diameter which bear root-borne shoots (e.g., Groom 1895b; Warming 1901; Pfeiffer 1914; Chantanaorrapint 2008; Chiang and Hsieh 2011) and give rise to additional similar roots where the shoots emerge, thus forming star-like clusters (e.g., Groom 1895b; Bernard and Ernst 1910; Pfeiffer 1914; Larsen 1965; Saunders 1996; Yang et al. 2002; Wapstra et al. 2005). This indicates the trend towards a star-like radiating root system, typical for MH plants. The runner-like parts of the roots can be short (e.g., *Thismia appendiculata*, Schlechter 1919), and in this case, the shoots emerge in nest-like tufts above the soil surface. In other species, the root system is reduced to a coralloid structure, e.g., *Thismia yorkensis* (Cribb 1995), *T. goodii* (Kiew 1999), or *T. clandestina* and *T. versteegii* (Bernard and Ernst 1911). *Thismia versteegii* shows similarities to the unique fan-shaped roots of *Thismia clavigera* (Stone 1980), which probably develop through short, dichotomously branched and congenitally merged roots. *Thismia annamensis* and *T. tentaculata* have short rhizomes bearing a dense covering of vermiform roots (Larsen and Averyanov 2007), also resulting in a star-like root system. This is morphologically similar but ontogenetically quite different from the other species mentioned above. The decision whether a condensed root system is developed by root-borne shoots or shoot-borne roots is often difficult to make and sometimes requires anatomical investigations (see Imhof 2004). Finally, some neotropical species have globose tubers (see Fig. 4.10g), from which a shoot as well as numerous filiform roots arise (e.g., *Thismia hyalina*, Miers 1866, *T. glaziovii*, Poulsen 1890a, *T. janeirensis*, Warming 1901, *T. panamensis*, Maas et al. 1986, Fig. 4.8j, *T. saülensis*, Maas and Maas 1987). Inferred from *T. luetzelburgii*, these tubers are roots, giving rise to up to four endogenous flowering shoots. The filiform roots can develop new tubers at their apices (Goebel and Süssenguth 1924).

The fungal colonization of *Thismia* spp. has been investigated quite early and in great detail. Like many other MH plants, *Thismia* also shows different fungal morphologies in distinct tissue

Fig. 4.2 (continued) is still attached. The whitish content is the fungal colonization. (**e**) The rhizome has developed into a shoot terminated by a 1 cm long flower. (**f**) *A. hydra* in its natural habitat with the filiform root elongations superficially clinging to the substrate. (**g**) *A. hydra* showing the strictly sympodial flowering mode with clusters of root tubercles at the base of each pedicel. Three basal flowers (A, B, C) have already detached, the following are in dissemination stage with placentophore developed (D), in fruit (E) and in bud (F). Note early tubercle (tb) development at the base of the flower bud. (**h**) Close up of the placentophore (pl). (**i**) Flower of *A. winkleri*, measuring 1.5 cm from the subtending scale leaf to the bending of the tube. (**j**) Root/rhizome system of *A. winkleri*

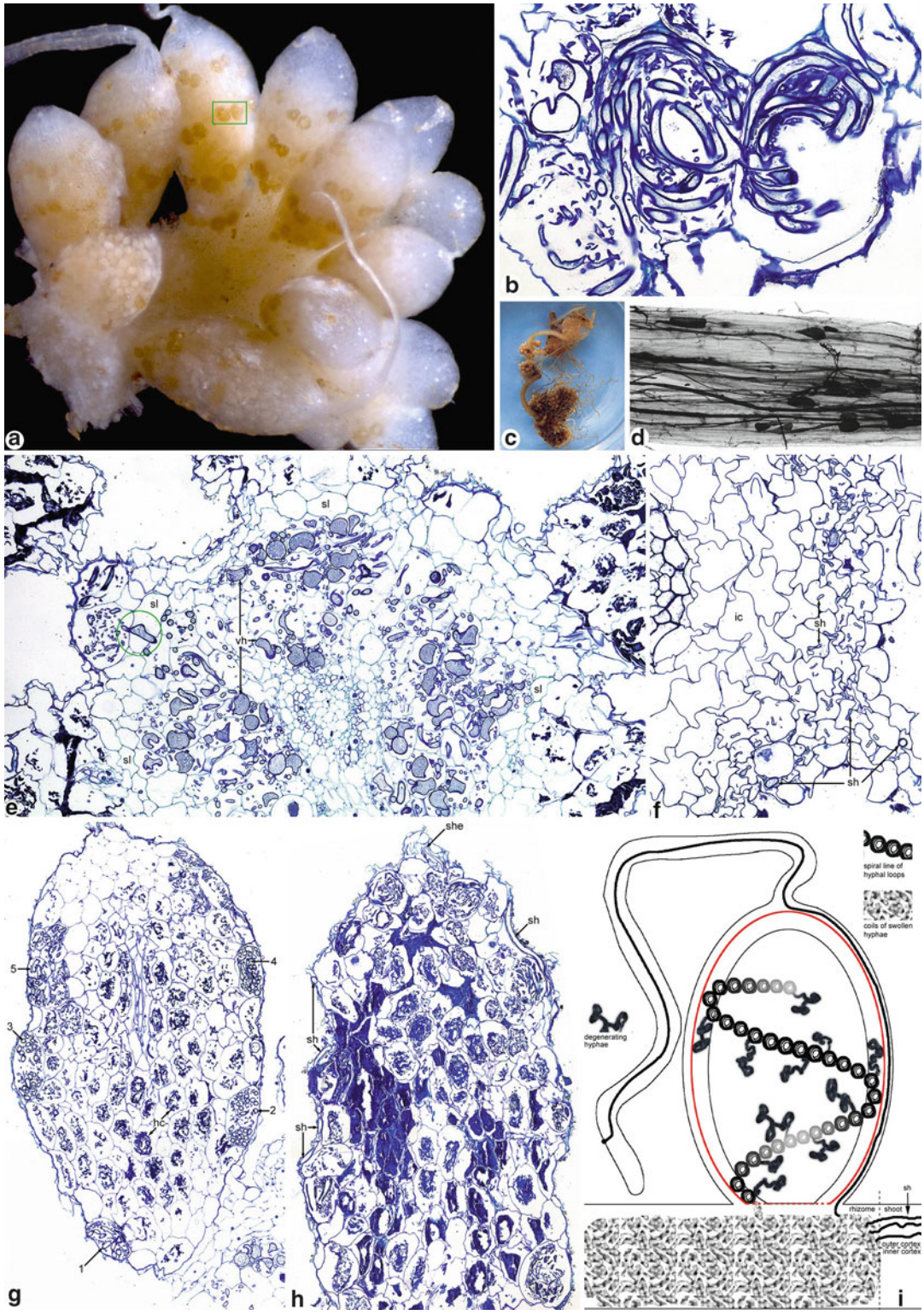

Fig. 4.3 *Afrothismia saingei* (Thismiaceae). (**a**) Rhizome tip with many root tubercles; some tubercles have been detached. The characteristic hyphal loops (*green rectangle*), developed in spiral lines within the tubercles, are

compartments, which sometimes are anatomically different. In *T. clandestina* (coralloid root system) and *T. aseroe* (vermiform roots), the outer cortex parenchyma layers contain straight hyphae with only a few coils; in the middle cortex layers, the hyphae are coiled but not digested; the inner layers show amorphous fungal material (Groom 1895b; Janse 1896; Meyer 1909; Bernard and Ernst 1911). In *T. americana*, *T. rodwayi*, and *T. javanica* (vermiform roots), straight hyphae are missing, instead, the outer cortex layer is occupied by coiled hyphae which do not degenerate. Digestion takes place in the inner cortex (Bernard and Ernst 1910; Pfeiffer 1914; Coleman 1936; McLennan 1958; Campbell 1968). Of the species having root tubers, *T. luetzelburgii* (Goebel and Süssenguth 1924) and T. *glaziovii* (Poulsen 1890a) have been investigated. They also show compartmentation of digested and undigested fungal material, whereas the digestion is more prominent in the proximal and central part of the tuber. The filiform roots connecting the mother tuber with smaller daughter tubers bear straight undigested hyphae linking the two tubers (Goebel and Süssenguth 1924). This is partly reminiscent of structures found in *Afrothismia* spp. (see next paragraph).

Due to the structural characteristics typical of a *Paris*-type AM, the fungus colonizing *Thismia* spp. almost certainly belongs to the Glomeromycota. This has recently been confirmed for *T. rodwayi* using molecular identification methods (Merckx et al. 2012).

4.5.2.3 *Afrothismia* (Figs. 4.2–4.4)

The genus *Afrothismia* from tropical Africa is characterized by its dense aggregates of small tuberous roots elongated by a filiform extension of various lengths between the species (Figs. 4.2g + j, 4.3a + c, and 4.4b). Although our chapter deals with subterranean organs, this is not entirely correct for some *Afrothismia* spp. In fact, the peculiar root/rhizome/shoot systems often grow entirely epiterrestrially (Fig. 4.2f), the filiform part of the roots clinging to rotten wood, leaf litter, or bare soil (e.g., *A. foertheriana*, Franke et al. 2004, *A. hydra*, Sainge and Franke 2005, *A. winkleri*, Imhof pers. observ., Fig. 4.2i–j). Only *A. baerae* (Cheek 2003a) and *A. gesnerioides* (Imhof pers. observ., Fig. 4.4a) are known to be rooted in the soil. The latter two species also differ by their conspicuously short filiform parts of the roots (Fig. 4.4b, Cheek 2003a; Maas-van de Kamer 2003). *Afrothismia zambesiaca*, described from a herbarium specimen collected in 1955, is only inferred to have an underground stem with bulbils (Cheek 2009). The ontogeny of *Afrothismia hydra* from seed to the open fruit has been

Fig. 4.3 (continued) visible from the outside. (**b**) Close up of the green rectangle in (**a**). Tangential section through two hypodermal cells colonized by looped hyphae that are connected to each other. (**c**) Specimen (Wilks No. 1179) of *A. saingei* under investigation from the Herbarium in Utrecht, labeled as *Afrothismia winkleri*. (**d**) Cleared preparation of a filiform root extension showing straight growing hyphae and vesicles. (**e**) Transverse section through a rhizome of *A. saingei* with coils of enlarged hyphae (vh) in the cortex. Mostly only once per tubercle these hyphae transit (*green circle*) over an inconspicuous separating layer (sl) into the hypodermis of a tubercle to start the spiral line of hyphal loops (see (**a**, **b**)). (**f**) Transverse section through a shoot/pedicel of *A. saingei*. The enlarged hyphae in the rhizome cortex (**e**) are continuous with the straight growing, also quite large hyphae (sh) in the outer shoot cortex, thus connecting the spatially separated tubercle clusters along the plant (**c**). The inner cortex (ic) is free of hyphae. (**g**) Longitudinal section through a young root tubercle showing the looped coils in an alternating pattern in the hypodermis as to be expected from its spiral arrangement (1–5) whereas all other cortex cells contain degenerated hyphal coils (hc–). (**h**) Longitudinal section through an old root tubercle where the digestion of hyphal coils has advanced but the epidermis now contains straight growing, nondegenerated hyphae (sh) linking those in the filiform root extension (she, see (**d**)) with the enlarged hyphae in the rhizome cortex (see (**e**)). (**i**) Schematic view of the mycorrhizal colonization pattern in *A. saingei*: Straight hyphae grow through root extension and tubercle epidermis, enter the rhizome cortex becoming enlarged and coiled, transit once per tubercle into the hypodermis of the tubercle starting a spiral line of looped interconnected hyphae around it (hl), and from there send hyphal branches into the rest of the cortex parenchyma for digestion (dh). The red line signifies an impenetrable barrier to the fungus. The spatially separated clusters of tubercles share the fungus via straight hyphae growing along the shoot axis in its outer cortex parenchyma

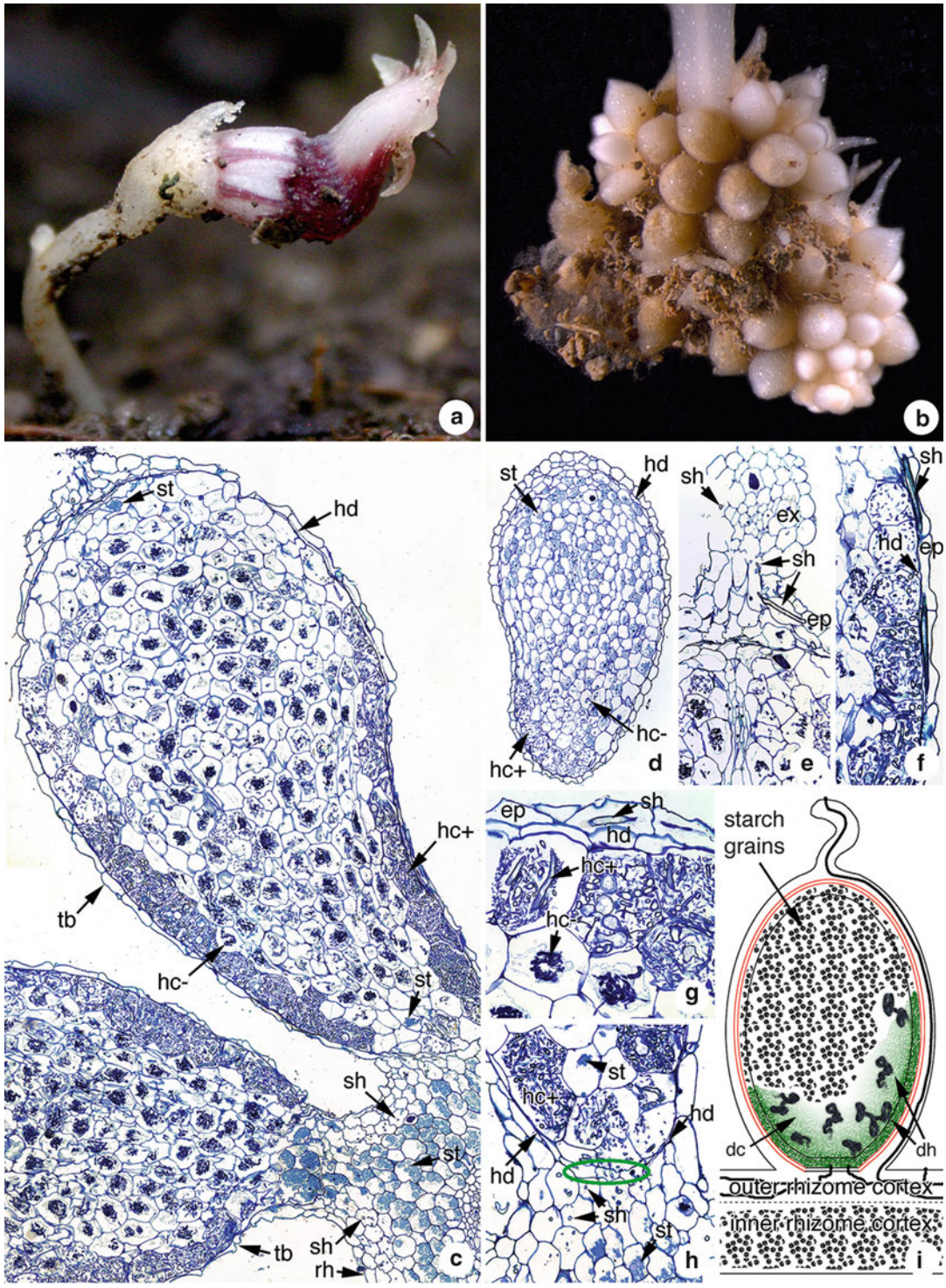

Fig. 4.4 *Afrothismia gesnerioides* (Thismiaceae). (**a**) *A. gesnerioides* emerging about 1.5 cm above the soil. In contrast to many other *Afrothismia* spp., roots and rhizome are subterranean. (**b**) Root/rhizome system of

described (Imhof and Sainge 2008), and since structurally the genus is quite consistent, this example will be detailed here to represent the whole genus. With germination, a root tubercle without a filiform extension is generated (Fig. 4.2a). Successively, more tubercles develop on a rhizome that is gradually increasing in size and the root extensions elongate (Fig. 4.2b–d). This creates a globose to ovate, coarsely echinate structure due to the characteristic roots. At some point, the rhizome proceeds to grow without root development. This axis, now more accurately called a shoot, will terminate with a flower (Fig. 4.2e), and a side shoot appears in the uppermost scale leaf of the shoot. The base of this side shoot also bears a cluster of tubercles with extensions, and this shoot will also end in a flower. This sympodial pattern is repeated several times (Fig. 4.2g). The fruit is a pyxidium, opening by means of a placentophore (Fig. 4.2h, see details in Imhof and Sainge 2008).

The fungal colonization of *Afrothismia saingei* is an extreme example of mycorrhizal complexity (Imhof 1999a, treated as *A. winkleri*[1]). Briefly, the pattern of colonization is as follows (schematic view on Fig. 4.3i). The filiform root extension bears straight hyphae, continuous with those in the epidermis of the tubercle (Fig. 4.3d+h). These hyphae never pass from the epidermis into the cortex of the tubercle but proceed around it towards the rhizome. As soon as the fungus reaches the rhizome at the tubercle base, it colonizes the rhizome cortex tissue with coiled, swollen, vesicle-like structures, but still does not show signs of degeneration (Fig. 4.3e). From there, few hyphae re-enter the tubercle from the rhizome cortex, and grow towards the subepidermal layer of the tubercle (Fig. 4.3e). Characteristic loops of hyphae are developed in the subepidermal cells (Fig. 4.3b+g), and an upward spiral line of cells containing such looped hyphae proceed around the tubercle (Fig. 4.3a). No digestion of hyphae occurs to this stage. Side branches from these hyphal loops enter the other cells of the tubercle cortex, where they degenerate to amorphous clumps (Fig. 4.3g, h). Connections to more distant tubercle clusters along the plant are provided by straight growing hyphae in the outer cortex of the shoot internodes (Fig. 4.3f, see details in Imhof 1999a). This complicated plant structure and colonization pattern represent a sophisticated and ecologically functional system. The filiform root extensions increase the surface for contact with and invasion by hyphae, the root tubercle increases the number of cells for colonization by

[1]According to Maas-van de Kamer and Maas (2010), the material under investigation in Imhof 1999a (=Wilks no. 1179, received from the herbarium of Utrecht, labeled as *A. winkleri*) turned out to be *A. saingei* (Franke 2004), synonymous to *A. gabonensis* (Dauby et al. 2008).

Fig. 4.4 (continued) *A. gesnerioides*, about 1 cm wide. (**c**) Transverse section through a rhizome (rh) and longitudinal sections of root tubercles (tb) showing starch grains (st) in the inner rhizome cortex and uncolonized root cortex, straight hyphae (sh) in the outer rhizome cortex, nondegenerated dense hyphal coils in the third cell layer of the root tubercle (hc+) and degenerated hyphal coils in the tubercle parenchyma (hc−). The hypodermis (hd) is largely collapsed. (**d**) Longitudinal section through a young tubercle of *A. gesnerioides* where the hypodermis (hd) is still visible. Fungal colonization has just started from the tubercle base in the third cell layer (hc+) and starch depositions (st) are still present in the parenchyma, which will disappear when fungal colonization proceeds. First degenerated hyphal coils (hc−) are also present in the inner parenchyma. The root extension has not yet developed. (**e**) Longitudinal section through a root tip of an older tubercle showing the root extension (ex) partly in transverse view, colonized by straight hyphae (sh) which proceed into the root epidermis (ep, see (**f**)). (**f**) Root tubercle epidermis (ep) only contains straight growing hyphae (sh) which never penetrate the hypodermis (hd, collapsed) but are continuous with those in the outer rhizome cortex. (**g**) Four neighboring tissues of the root tubercle hosting distinct morphotypes of fungal colonization: epidermis (ep) with straight hyphae (sh), hypodermis (hd) as a barrier to the fungus, the third root layer with nondegenerated dense hyphal coils (hc+) and the multilayered root parenchyma containing degenerated hyphal coils (hc−). (**h**) Transition of colonization (*green oval*) between the straight hyphae (sh) in the outer rhizome cortex and the nondegenerating hyphal coils (hc+) in the third root layer of the tubercle across a layer continuous with the otherwise impenetrable hypodermis (hd). Uncolonized parenchyma cells and the inner rhizome cortex contain starch grains (st). (**i**) Schematic view of the mycorrhizal colonization pattern in *A. gesnerioides*: straight hyphae grow through root extension, tubercle epidermis and outer rhizome cortex, transit at the base of the tubercle into its third layer to form dense coils (dc, green texture), and branches from there colonize the inner tubercle parenchyma to become digested (dh). The red marked hypodermis is impenetrable for the fungus

hyphae and eventual digestion, representing the locations of the beginning and end of the mycorrhizal colonization pattern. Between these events, the different hyphal forms serve three fundamental functions: (1) transportation and distribution of carbohydrates and nutrients within the root-rhizome-complex, (2) storage, and finally (3) as a carbon source for the plant following digestion. The straight hyphae in the filiform root extension and the epidermis allow for rapid transport of nutrients and carbohydrates towards the rhizome. The swollen hyphae in the rhizome cortex store these substances, eventually for the benefit of the plant. The spiral line of hyphal loops is the geometrically and economically optimal distribution mode around the parenchyma of the tubercle. With a minimum of living hyphae, this provides short distances and limits the number of cell passages for side branches to penetrate into all parenchyma cells, necessary due to the quick degeneration process therein. The fungus in *Afrothismia gesnerioides* shows a similar colonization pattern with straight hyphae in the short root extension (Fig. 4.4e) and the root epidermis (Fig. 4.4f, g), as well as digestive tissue in the inner root parenchyma (Fig. 4.4c, see details in Imhof 2006). However, it does not develop a spiral line of hyphae around the tubercle parenchyma. Instead, dense coils of living irregular hyphae develop in the third root layer, encompassing the parenchyma in a collar-like pattern (Fig. 4.4c + f–h). Economically speaking, this pattern is less efficient than that in *A. saingei*, considering the amount of living fungal biomass necessary to supply the digesting cells with hyphal branches. Moreover, the rhizome of *A. gesnerioides* contains straight growing hyphae in its outer cortex, whereas the inner cortex cells contain starch deposits (Fig. 4.4c), as does the uncolonized tubercle cortex (Fig. 4.4d + h, schematic view on Fig. 4.4i). This means that *A. gesnerioides*, in contrast to *A. saingei*, converts the carbon delivered by the fungus into starch grains. In the case of *Afrothismia* spp. however, this appears as a unnecessary metabolic step, since the carbon source is permanently present. Therefore, although the mycorrhizal patterns in *Afrothismia* spp. are highly complex, they still show signs for an ongoing evolutionary progression of mycorrhizal structures within the genus, whereas *A. gesnerioides* can be considered to be less advanced than *A. saingei*. More of the 12 *Afrothismia* species described so far should be investigated to determine if intermediate structures exist (see 4.8 Trends, Conclusions, and Future Directions).

The fungal species associated with *Afrothismia* spp., as identified by molecular methods, all belong to *Glomus*-group A (Franke et al. 2006), and are species-specific (Merckx and Bidartondo 2008).

4.5.3 Burmanniaceae (Figs. 4.5–4.7)

Of the ten genera in this family, only *Burmannia* contains green representatives. *Burmannia tenella* is the only entirely achlorophyllous neotropical species, others occur in Africa (e.g., *B. hexapterella*) and Asia (e.g., *B. championii*, *B. candida*). However, many species with intermediate mycoheterotrophic status, between

Fig. 4.5 (continued) structures (a), and vesicles (v). (**f**) Transverse section through a root of *D. orobanchoides* showing the central cylinder (cc) consisting of a central tracheary element surrounded by one ring of smaller tracheary elements and the pericycle, a thickened endodermis (en), two layers of small parenchyma layers, and epidermal cells (ep). The epidermal cells contain hyphal coils (hc+) and arbuscules (a), the latter partly degraded. (**g**) SEM micrograph of a rhizome of *D. orobanchoides* imbricately covered by peltate scale leaves with fringed margins. The leaf interstitials contain fungal hyphae (hy). (**h**) Transverse section through a rhizome covered with imbricate scale leaves (le) showing fungal colonization including vesicles (v) within (ihy) and in between (ohy) the scale leaves. The rhizome axis (rh) is not colonized but contains starch grains (st). (**i**) Tangential section through a rhizome (rh) including the imbricate scale leaves (le) showing dense hyphal masses in the leaf interstitials (li). (**j**) Schematic view of the mycorrhizal colonization pattern of *D. orobanchoides*: the peltate scale leaves and their interstitials are colonized by hyphal coils and vesicles. The root is colonized only in the epidermis by hyphal coils, arbuscules, and vesicles; the arbuscules are the first to degenerate

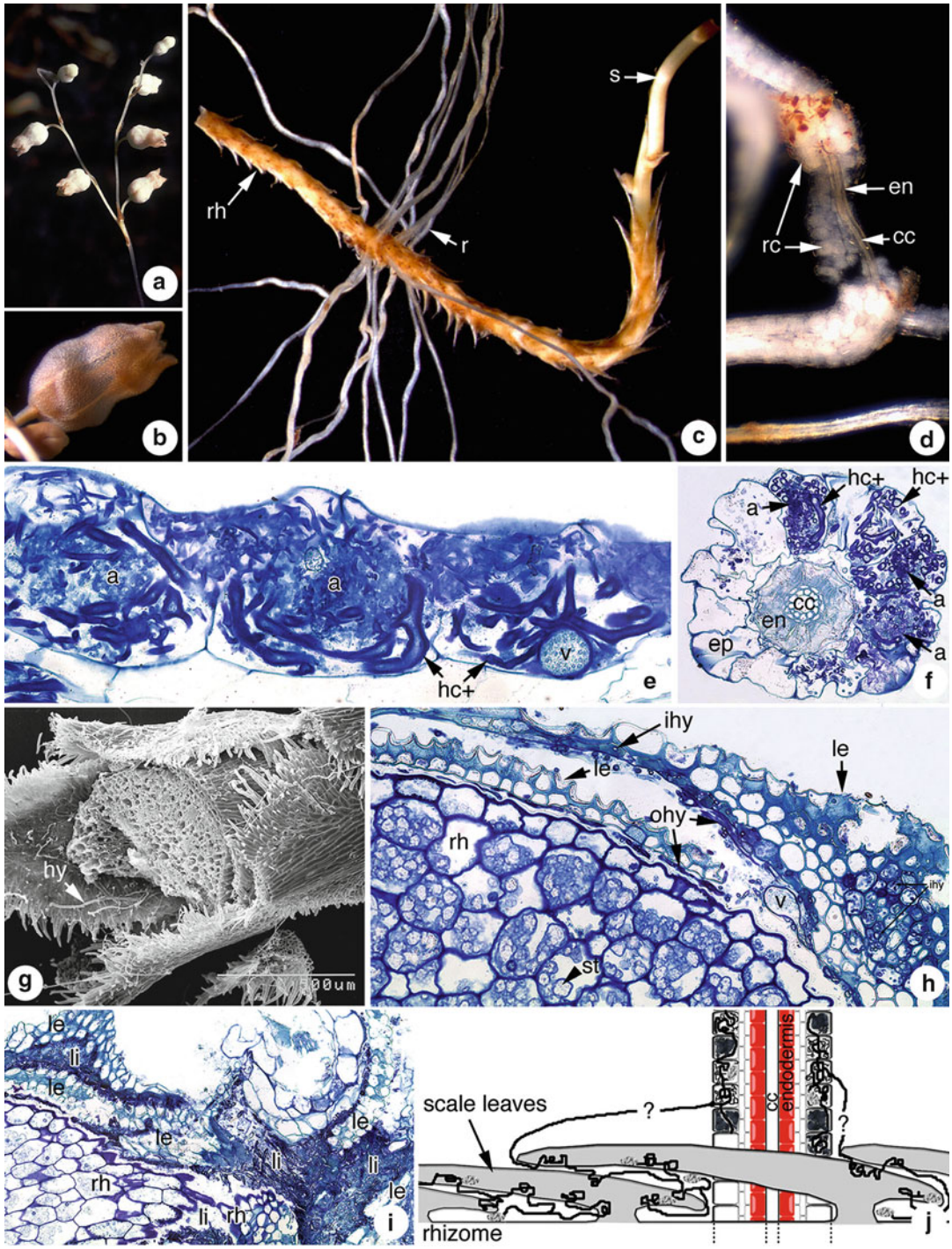

Fig. 4.5 *Dictyostega orobanchoides* (Burmanniaceae). (**a**) Inflorescence of *D. orobanchoides*, composed of a bifurcate cincinnus. (**b**) Single preserved flower of *D. orobanchoides*, 2.5 mm long. (**c**) Rhizome (rh) of *D. orobanchoides* about 1.5 mm thick with a tuft of filiform roots (r). Apically the rhizome turns into a shoot (s). (**d**) Root of *D. orobanchoides*, cortex (rc) partly detached, exposing the thickened endodermis (en) which encloses the central cylinder (cc). The whitish cell contents are fungal coils. (**e**) Longitudinal section through a root epidermis of *D. orobanchoides*. Fungal colonization consists of coiled hyphae (hc+), partly decomposed arbusculate

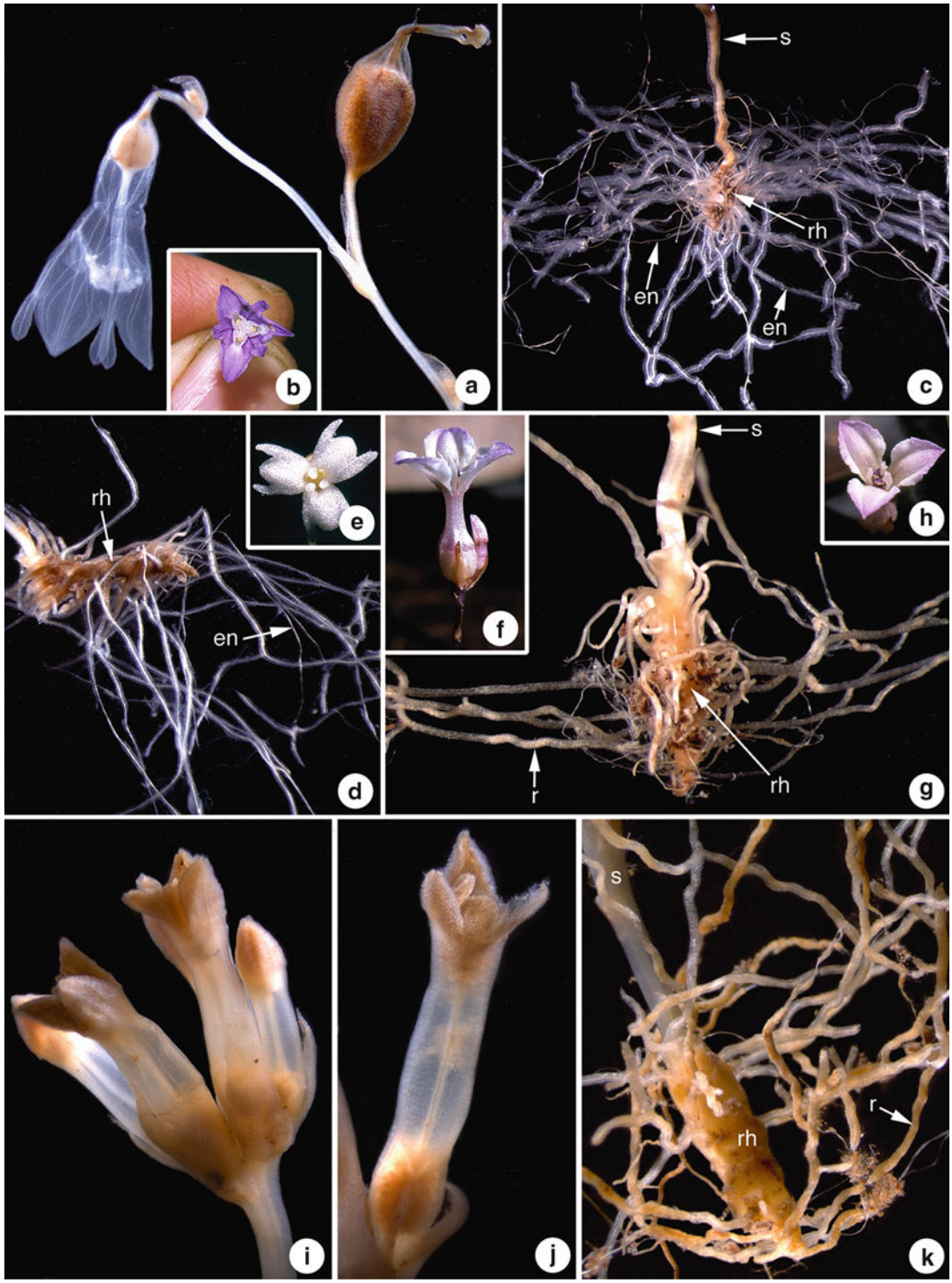

Fig. 4.6 (**a**–**c**) *Apteria aphylla*, (**d**–**e**) *Gymnosiphon divaricatus*, (**f**–**h**) *Hexapterella gentianoides*, (**i**–**k**) *Campylosiphon congestus* (Burmanniaceae). (**a**) Preserved flower (9 mm long) and fruit of *A. aphylla*. (**b**) Top view of a flower of *A. aphylla* (courtesy of H and PJM Maas). (**c**) Subterranean system of *A. aphylla*. The shoot (s) is continuous with the short (3 mm) orthotropous rhizome (rh) bearing numerous filiform roots. The root cortex parenchyma is often disrupted, leaving the thickened endodermis (en) with central cylinder enclosed as the only connection with the rhizome.

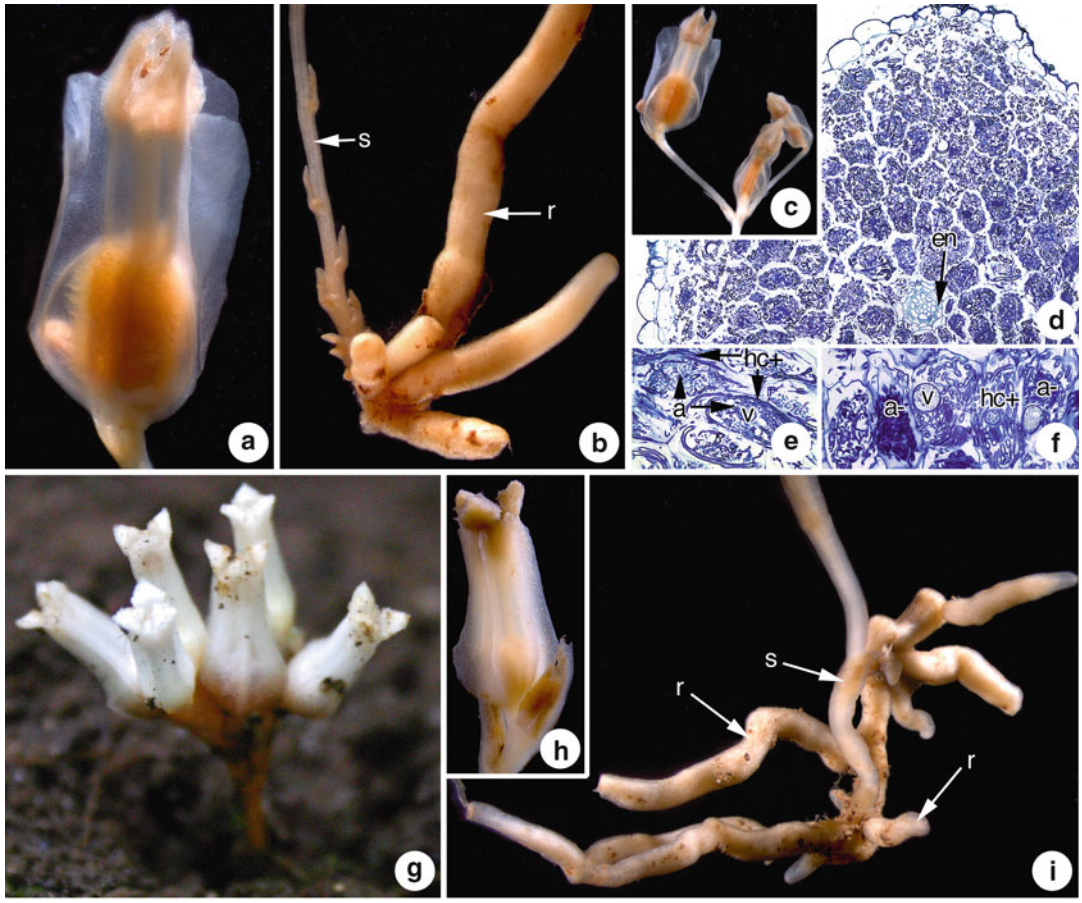

Fig. 4.7 (**a**–**f**) *Burmannia tenella*, (**g**–**i**) *Burmannia hexaptera* (Burmanniaceae). (**a**) Preserved flower (6 mm long) of *B. tenella*. (**b**) Root system of *B. tenella* with several star-like radiating vermiform roots (r), about 1.2 mm thick, at the base of the shoot (s). (**c**) Inflorescence of *B. tenella*, the bifurcate cincinnus usually consists of a few flowers. (**d**) Transverse section through a root of *B. tenella* with extensive fungal colonization of the multilayered cortex parenchyma. The central cylinder is reduced and surrounded by a tertiary endodermis (en). (**e**, **f**) The fungus in the root parenchyma cells of *B. tenella* forms heteromorphic coils of hyphae of various width (hc+), arbusculate structures (a) as well as vesicles (v), often within one cortex cell. Degeneration begins with the arbusculate structures (a–); the thicker hyphae tend to persist longer. (**g**) Flowers of *B. hexaptera* emerging only a few centimeters above the soil surface. (**h**) Preserved flower (1 cm long) of *B. hexaptera*. (**i**) Root system of *B. hexaptera* comprised of vermiform roots (r), about 1.2 mm thick, also with the tendency to radiate at the base of the shoot (s), resulting in a coralloid appearance

Fig. 4.6 (continued) (**d**) Subterranean system of *G. divaricatus*, very similar to that of *A. aphylla* (see (**c**)) with short rhizome (rh) and exposed endodermis (en). (**e**) Top view of a flower of *G. divaricatus* (courtesy of H and PJM Maas). (**f**) Flower of *H. gentianoides* (courtesy of H and PJM Maas). (**g**) Subterranean system of *H. gentianoides*, also with a short rhizome (rh, 4 mm long) continuous with the shoot (s). The rhizome bears filiform roots (r); light coloration indicates fungal colonization. (**h**) Top view of a flower of *H. gentianoides* (courtesy of H and PJM Maas). (**i**) Preserved inflorescence of *C. congestus*. (**j**) Preserved single flower (9 mm long) of C. *congestus*. (**k**) Subterranean system of *C. congestus* with the shoot (s) continuous with a slightly tuberous rhizome (rh), 9 mm long and 2.5 mm thick, bearing filiform roots (r)

leafy *Burmannias* and achlorophyllous, scale-leaved species exist (Jonker 1938; Maas et al. 1986; Leake 1994), suggesting an evolutionary trend towards mycoheterotrophy. All other genera are fully mycoheterotrophic. The monotypic genus *Desmogymnosiphon* (Guinea Lopez 1946) is most probably a *Gymnosiphon* species (compare to Maas et al. 1986).

4.5.3.1 *Apteria, Campylosiphon, Dictyostega, Gymnosiphon, Hexapterella, Marthella, Miersiella* (Figs. 4.5 and 4.6)

Except for *Campylosiphon congestus* and the pantropical *Gymnosiphon*, all these genera are exclusively neotropical (Jonker 1938; Maas et al. 1986). All species have the same basic architecture for their underground parts. The aerial shoots are continuous with rhizomes, densely covered by scale leaves. These scale leaves are conspicuously fringed in *Dictyostega* (Imhof 2001, Fig. 4.5g), which has led to the hypothesis they might ecologically replace the missing root hairs (Goebel and Süssenguth 1924; Maas et al. 1986). The rhizomes can be longer (up to 7.5 cm in e.g., *Miersiella umbellata*, Maas et al. 1986, up to 4 cm in *Dictyostega orobanchoides*, Imhof 2001, Fig. 4.5c) or rather short (e.g., *Apteria aphylla* (Fig. 4.6c), Uphof 1929, *Gymnosiphon longistylus*, Hepper 1968, *G. divaricatus* (Fig. 4.6d), Maas et al. 1986, *Hexapterella gentianoides* (Fig. 4.6g)), and can be slightly tuberous (e.g., *Campylosiphon purpurascens*, Maas et al. 1986, *Campylosiphon congestus*, Fig. 4.6i–k). Many filiform, less than 0.5 mm thick, sparsely branched roots arise from the axils of the scales. Species with short rhizomes, therefore, have a star-like root system (Fig. 4.6c, d+g), but roots also emerge as tufts on longer rhizomes (Imhof 2001, Fig. 4.5c). As a peculiar exception in this group of species, *Gymnosiphon afro-orientalis* develops little tubers of unknown origin beside scale leaves and filiform roots at the short rhizome (Cheek 2009), superficially reminiscent of those found in *Afrothismia* (e.g., Fig. 4.4b), but fundamentally differing in being distinct from the filiform roots.

Anatomically, these roots are characterized by a much reduced central cylinder with one central enlarged tracheary element surrounded by a ring of much smaller tracheary elements, and a pericycle (e.g., Fig. 4.5f). The tertiary endodermis is conspicuously reinforced (e.g., *Marthella trinitatis*, erroneously called *Burmannia capitata* by Johow 1885, *Gymnosiphon refractus* (formerly *Cymbocarpa refracta*, Merckx 2008), treated under two different synonyms by Johow 1889 and Goebel and Süssenguth 1924, *Apteria aphylla*, Uphof 1929). In transverse sections of a *Dictyostega orobanchoides* root, the fortification of a single endodermal cell may even be wider than the entire central cylinder (Imhof 2001, Fig. 4.5f). This reinforcement protects the essential connection to the shoot. In fact, the thin-walled cortex tissue is often found to be disrupted (Figs. 4.5d and 4.6c, d+g) whereas the central strand is even hard to disconnect using forceps (Imhof 2001, see section on *Petrosavia* for interpretation).

The two to three parenchyma layers and, in particular, the often large-celled persistent epidermis (Johow 1889; Imhof 2001 and unpublished observations) are colonized by coils of hyphae (Uphof 1929), vesicles, as well as arbuscular-like structures, often all together within a single cell (Imhof 2001). The fungal material often appears amorphous, suggesting a digestion process (Fig. 4.5e, f).

Dictyostega orobanchoides also has fungal colonization in the scale leaves (Fig. 4.5h) as well as in the interstitials of their imbricate arangement along the rhizome (Fig. 4.5g–i), but not in the rhizome axis. These hyphae and vesicles do not show signs of degeneration, and it has been hypothesized that they serve as a refugium for the fungus, which in turn enhances the rhizomosphere with the appropriate mycobiont (Imhof 2001, see Fig. 4.5j for a schematic view). It can be interpreted as a strategy for a sustained use from the fungus, analogous to the often complex colonization pattern in other MH plants (e.g., *Voyria*, *Afrothismia*, *Triuris*, *Sciaphila*). More investigations might clarify the possible general relevance of rhizomes and their scale leaves for the mycorrhiza in other Burmanniaceae.

Franke et al. (2006) found several *Glomus*-group A species and an Acaulosporaceae in *Campylosiphon congestus* (treated as *Burmannia congesta*). Also, *Dictyostega orobanchoides* is associated with *Glomus*-group A species (Merckx et al. 2010), as are *Apteria* (Courty et al. 2011) and *Gymnosiphon* spp. (Dirk Redecker, pers. comm. cited in Leake 2005; Courty et al. 2011).

4.5.3.2 *Burmannia* (Fig. 4.7)

Burmannia species are more diverse with respect to their subterranean structures than their sister genera. Although they are sometimes similar to the latter (e.g., *B. championii*, Ernst and Bernard 1911), they also can have thicker roots also arising from rhizomes (e.g., *B. larseniana*, Zhang and Saunders 1999) or even vermiform, up to 2.6 mm thick roots and no (visible) rhizomes (e.g., *Burmannia candida*, Smith 1911, *B. liukiuensis*, Terashita and Kawakami 1991, *B. tenella*, Imhof 1999b, Fig. 4.7b, *B. hexaptera*, Imhof unpublished, Fig. 4.7i). Others have tuberous organs of uncertain nature (*Burmannia hunanensis*, Liu et al. 2001), with filiform roots. However, more taxonomic investigation may result in new classifications resolving some of this subterranean diversity, as in fact, *Burmannia congesta*, having a tuberous rhizome, only recently was attributed to *Campylosiphon* (Fig. 4.6i–k) by molecular and morphological data (Merckx 2008; Maas-van de Kamer and Maas 2010).

Root anatomy is also diverse. Epidermal cells may be conspicuously enlarged (Johow 1889; Ernst and Bernard 1911, 1912; Bernard and Ernst 1914) or not (Colozza 1910; Ernst and Bernard 1911; Imhof 1999b, Fig. 4.7d). Depending on the variability of root thickness, the cortex parenchyma layers can be from three to many (Janse 1896; Ernst and Bernard 1911; Larsen 1963; Terashita and Kawakami 1991; Imhof 1999b, Fig. 4.7d), and can be uniform (Imhof 1999b) or heteromorphic (Ernst and Bernard 1911) or with lacunae (Johow 1889; Malme 1896a; Colozza 1910). Similar to the other genera of the family, the endodermis has obvious tertiary reinforcements and the central cylinder is much reduced (e.g., Malme 1896a; Ernst and Bernard 1911; Terashita and Kawakami 1991; Imhof 1999b, Fig. 4.7d).

In species with filiform roots, the mycorrhizal fungus colonizes epidermal cells (Johow 1889; Ernst and Bernard 1911), whereas in the species with thick roots, the cortex parenchyma cells are colonized (Meyer 1909; Ernst and Bernard 1911; Terashita and Kawakami 1991; Imhof 1999b, Fig. 4.7d). In the thick roots of *Burmannia tenella*, hyphal coils, vesicles and arbuscular-like structures may occur together in a single cell (Fig. 4.7e, f). A colonization pattern with compartmentation of root tissue similar to other MH plants is not obvious (Fig. 4.7d). However, a selective digestion of the thinner, arbusculate hyphae but not the thicker hyphae within cells (Imhof 1999b, Fig. 4.7e, f), seems to allow a sufficient spread of the colonization within the cortex parenchyma by the latter, while carbon and nutrients are obtained through digestion of the former.

The only *Burmannia* species which has been investigated for the identity of its mycorrhizal fungus is *B. hexaptera* (Fig. 4.7g–i). It is mycorrhizal with *Glomus*-group A species (Franke et al. 2006; Merckx and Bidartondo 2008).

4.5.4 Triuridaceae (Figs. 4.8–4.10)

Fossil specimens of this exclusively achlorophyllous family from the Upper Cretaceous (ca. 90 mya) are the oldest unequivocal monocotyledonous remnants known (Gandolfo et al. 2002). Eleven genera are grouped in three tribes, the Sciaphilae are pantropical, Triurideae neotropical (Maas-van de Kamer and Weustenfeld 1998), and Kupeaeae only occur in tropical Africa (Cheek 2003b). All genera except for *Sciaphila* and *Andruris* (included in *Sciaphila* by van de Meerendonk 1984) contain only one to three species. The affiliation of the family was long uncertain (Rübsamen-Weustenfeld 1991; Maas-van de Kamer 1995; Maas-van de Kamer and Weustenfeld 1998). Today, molecular methods have assigned them to the Pandanales, which is supported by structural features (Furness et al. 2002; Rudall and Bateman 2006).

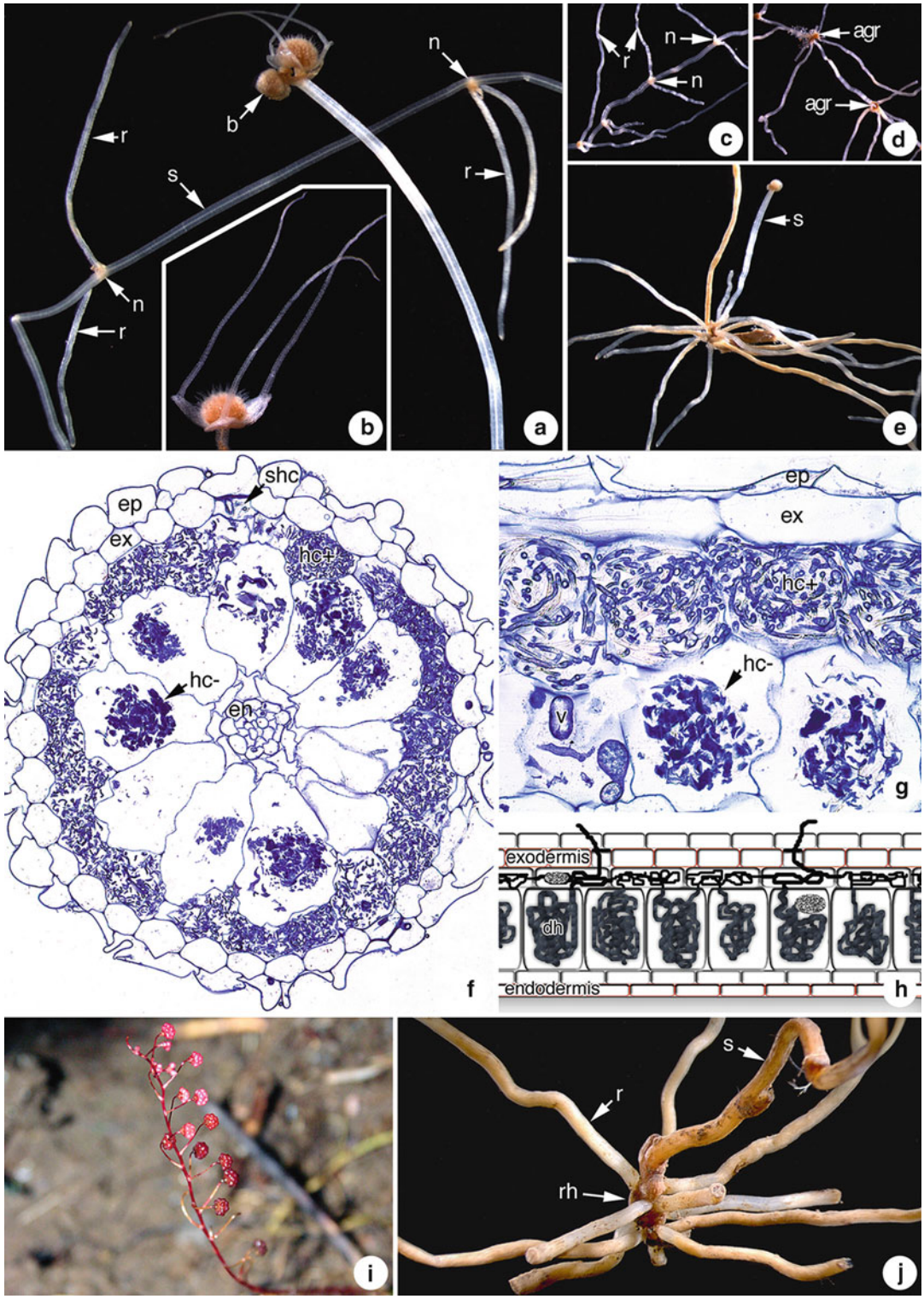

Fig. 4.8 (**a**–**i**) *Triuris hyalina*, (**j**–**k**) *Sciaphila ledermannii* (Triuridaceae). (**a**) Subterranean shoot (s) of *T. hyalina* with two nodes (n) bearing paired roots (r) in the axils of the scale leaves. The female inflorescence has one mature flower and one bud (b). (**b**) Female flower of *T. hyalina* with the characteristic tail-like tepal appendages.

With only few exceptions, the subterranean organs of Triuridaceae are rather uniform. The epiterrestrial shoots are continuous plagiotropically to orthotropically with subterranean shoot segments with various internode lengths without increasing in diameter (Fig. 4.8a+c, d). In addition to occasional side shoots, the axils of the nodal scale leaves bear pairs (sometimes solitary) of long filiform roots about as thick as the shoots (e.g., van de Meerendonk 1984; Maas and Rübsamen 1986; Maas and Maas-van de Kamer 1989), which can be glabrous (e.g., *Triuris hyalina*, Imhof 1998, *Triuridopsis intermedia*, Franke et al. 2000, Fig. 4.8a+c–e), sparsely hairy (e.g., several *Sciaphila* spp., Schlechter 1913, *Lacandonia schismatica*, Martinez and Ramos 1989) to conspicuously pilose (e.g., *Soridium spruceanum*, Miers 1852. several *Sciaphila* spp., Johow 1889; Hemsley 1907; Larsen 1972, *Andruris* spp., Schlechter 1913, *Seychellaria madagascariensis*, Fig. 4.10c). Usually the scale leaves and, consequently, the root pairs are spaced along the subterranean shoot (Fig. 4.8a+c), but there can also be dense clumps of filiform roots seemingly radiating from a single origin (e.g., several *Sciaphila*, *Triuris*, and *Peltophyllum* spp., Larsen 1972; Maas and Rübsamen 1986, Fig. 4.8d, e+j, *Seychellaria madagascariensis*, Fig. 4.10c), sometimes occurring in two or three tiers along the subterranean shoot (Fig. 4.8d). There are also a few species with more stout roots but also showing a star-like arrangement at the base of the shoot, namely the three species of the Kupeaeae (Cheek et al. 2003; Cheek 2003b, Fig. 4.10d–f), but also *Sciaphila polygyna* (Imhof 2004, Fig. 4.9a–d). *Sciaphila ledermannii* (Fig. 4.8i) has an intermediate root thickness (Fig. 4.8j). The star-like root aggregations by filiform or stout roots, even if they appear superficially very different, all follow the same developmental pattern, that is maximally one pair of shoot-borne roots per node, but are formed by the initiation of a side shoot from the scale leaf axil directly bearing a next node with scale leaf, giving rise to another pair of roots and a side shoot and so forth. The side shoots often do not elongate, which explains the abundance of roots (see details in Imhof 1998, 2004).

The tendency towards aggregations of thick and short roots seems to be characteristic for mycoheterotrophic plant families (Leake 1994, Imhof 2010, this chapter). Hence, the quite recent discovery of this feature in the Triuridaceae (Cheek et al. 2003; Imhof 2003, 2004, see Figs. 4.9d and 4.10f) was not too surprising.

The root anatomy of Triuridaceae is quite uniform also. Internal to the epidermis, there is a

Fig. 4.8 (continued) The apocarpous gynoecium is about 1.5 mm wide. (**c**) Subterranean shoots of *T. hyalina* with spaced nodes (n) where paired roots (r) arise from each scale leaf axil giving it a ladder-like appearance. The roots are uniformly 0.4 mm thick. (**d**) Each node seen in (**c**) may develop aggregations of paired roots (agr) as explained in text. (**e**) An aggregation of roots seen in (**d**) results in a star-like root system. At this stage, it may have already borne several flowering shoots (detached). A new shoot (s), 2 cm long, bearing a flower bud has developed. (**f**) Transverse section through a *T. hyalina* root measuring 0.4 mm in diameter. The epidermis (ep) is mostly free of hyphae and the exodermis (ex) is a barrier to the fungus except for the short cells (shc) with thickend outer tangential walls serving as passage cells. The outer cortex parenchyma layer bears dense hyphal coils (hc+) which do not become digested but may collapse when older. The middle parenchyma layer consists of enlarged cells containing mostly amorphous clumps of hyphal masses (hc−). The inner cortex layer of much smaller cells is free of hyphae. The endodermis (en) is only slightly suberized. (**g**) Longitudinal section through a *T. hyalina* root showing epidermis (ep), exodermis (ex), the dense hyphal coils (hc+) in the outer and the degenerated ones (hc−) in the middle cortex parenchyma layer. Occasionally vesicles (v) may occur in both layers. (**h**) Schematic view of the colonization pattern in *T. hyalina*: after penetration of epidermis and a short cell of the exodermis the hyphae start to coil and decrease their diameter while spread longitudinally and tangentially within the outer cortex parenchyma. The dense coils of narrow hyphae (see (**g**)) send branches into the middle parenchyma layer where they degenerate to amorphous clumps. Vesicles may occur in both layers. The red marked cells are impenetrable to the fungus. (**i**) Inflorescence of *S. ledermannii* showing female flowers. (**j**) Subterranean system of *S. ledermannii* consisting of a short rhizome (rh) continuous with the epiterrestrial shoot (s). The rhizome bears filiform roots (r) in this specimen up to 9 cm long and 0.8 mm thick

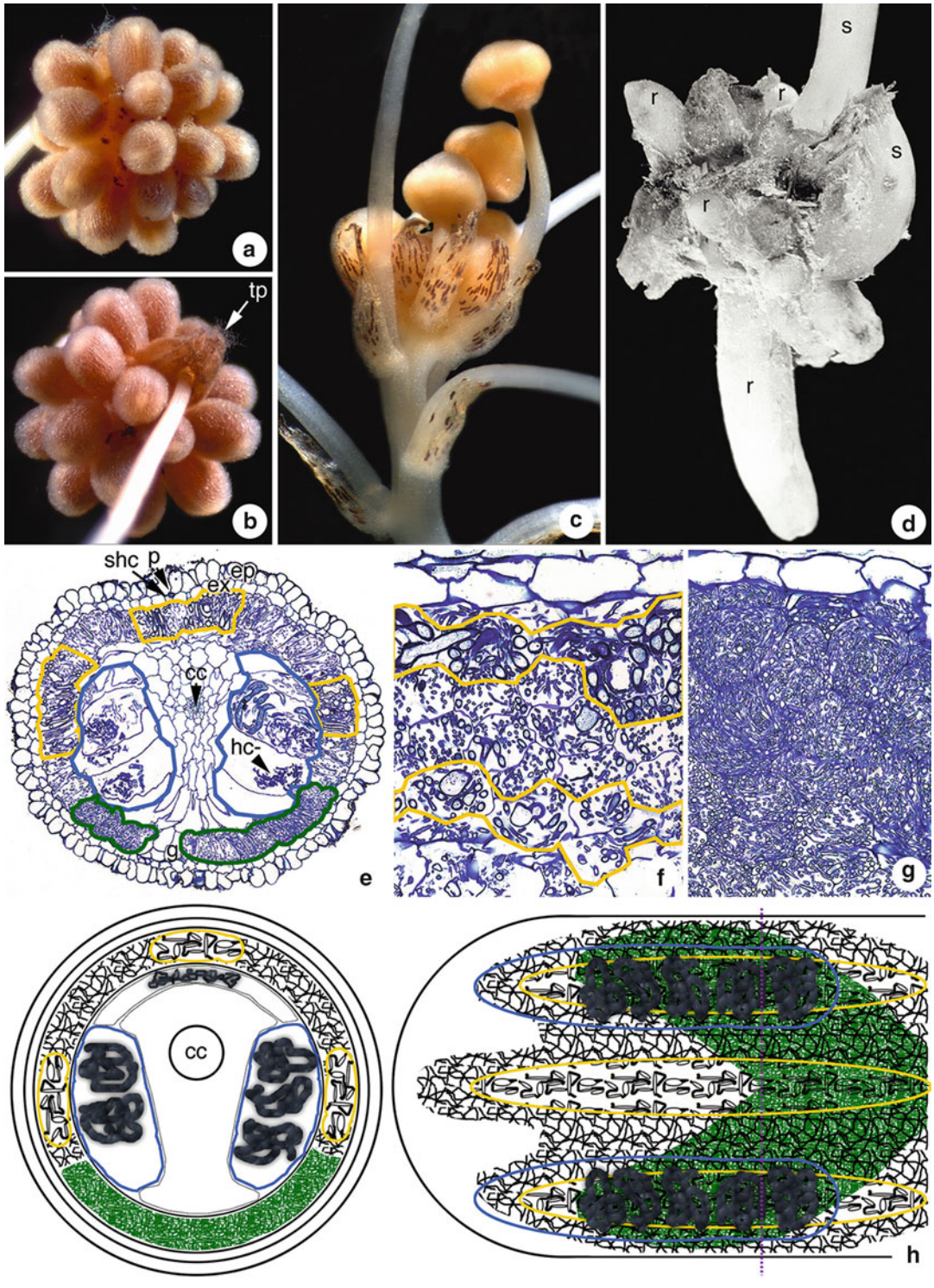

Fig. 4.9 *Sciaphila polygyna* (Triuridaceae). (**a**) Top view of a female flower of *S. polygyna* with its numerous carpels in fruiting stage (about 3 mm wide). (**b**) Same flower from the lower side showing the tepals (tp) with hair tufts. (**c**) Apex of an inflorescence of *S. polygyna* with numerous flower buds. (**d**) Subterranean system of *S. polygyna* with

suberized exodermis (Fiebrig 1921; Imhof 1998, 2003) and two (Johow 1889; Tomlinson 1982), three (Fiebrig 1921; Imhof 1998), to several (Imhof 2003) cortical parenchyma layers. The endodermis and/or pericycle may be reinforced (Poulsen 1886, 1890b; Johow 1889, Milanez and Meira 1943; Larsen 1963; Tomlinson 1982) or not (Malme 1896b; Imhof 1998, 2003). The central cylinder is much reduced. Very characteristic is the second cortex parenchyma layer, which mostly consists of conspicuously enlarged cells (Poulsen 1886, 1890b; Johow 1889; Fiebrig 1921; Tomlinson 1982; Imhof 1998, 2003), with the exception of *Sciaphila thaidanica* according to Larsen (1963, Figs. 4.8f, g and 4.9e).

Mycorrhizal colonization was recognized very early (e.g., Poulsen 1886, 1890b; Johow 1889; Janse 1896), with additional information added later (Fiebrig 1921; Ohga and Sinoto 1932; Milanez and Meira 1943; Palacios-Mayorga and Pérez-Silva 1993), but details of the colonization pattern were described rather recently (Imhof 1998, 2003; Franke 1999). *Triuris hyalina* attains a sustained benefit from the endophytic fungus by maintaining the hyphae in a functional state in the first cortical parenchyma layer and digesting them only in the enlarged cells of the second parenchyma layer (Fig. 4.8f–h, see details in Imhof 1998). An unpublished diploma thesis on *Sciaphila purpurea* (Franke 1999) not only yielded detailed information on morphology, anatomy, and ecology of the reproductive parts, but also confirmed the distinction of undigested hyphae in the outer vs. the digestion of hyphae in the inner enlarged cells of the root cortex. Beyond that, the structural diversity of the mycorrhizal colonization pattern in *Sciaphila polygyna* (Fig. 4.9a–d) is much more complicated and certainly belongs to the most complex mycorrhizas known (Fig. 4.9e–h). It includes four different morphologies of hyphae occurring in four distinct root tissue compartments. Moreover, it shows a disparate colonization at the tip compared to the base as well as the dorsal vs. the ventral side of the root, creating a monosymmetrical (only one plane of symmetry) root in transverse and longitudinal sections (Fig. 4.9h, see details in Imhof 2003). The purpose of these complex structures, except for the strictly localized digestion in the "giant cells" for a sustained carbon influx (Imhof 2003), is not yet understood.

The fungus in *Sciaphila secundiflora* (Yamato 2001, treated as *S. tosaensis*, the two treated as being synonymous by Ohashi 2000) was determined by DNA sequencing to be a *Glomus* species (Glomeromycota). More recently, *S. secundiflora* (still called *S. tosaensis*) and *Andruris japonica* (treated as *Sciaphila japonica*) were described to associate with *Glomus*-group A fungi (Yamato et al. 2011b), the phylotypes extracted from each species being closely related to another but quite distant when compared between the two species. *Sciaphila ledermannii* was also found to be colonized by a species from

Fig. 4.9 (continued) thick roots (r, several have detached) radiating from the base of two shoots (s, one is detached). For the architecture of this root system, see Imhof (2004). (**e**) Transverse section through a central part of a 1.2 mm thick root of *S. polygyna* surrounded by epidermis (ep) and exodermis (ex) with short cells (shc) serving as the only passage cells for fungal penetrations (p). Root anatomy and mycorrhizal pattern are highly heteromorphic with cells of the fourth root layer being much larger ("giant cells," *blue border*) than others dislocating the central cylinder (cc) out of its central position to create a dorsiventral architecture of the root. Fungal material degenerates (hc–) only in the fourth layer. The third layer has loose hyphal coils with swellings (*yellow border*), coils without swellings (not marked) and very dense coils of thin hyphae (*green border*, *ventral side*). Colonization by dense coils follows a v-shaped pattern leaving a gap of colonization (g) in some parts of the roots (see *right hand side* of (**h**)). (**f**) Tangential section through the dorsal side of a *S. polygyna* root showing areas colonized by coils with many (*yellow border*) and with less swellings. (**g**) Tangential section through the ventral side of the third root layer at the same magnification as (**f**) indicating the differences of the three types of coils in the third layer. (**h**) Schematic view of the mycorrhizal colonization pattern in *S. polygyna* in transverse (*left*) and longitudinal view (*right*). The coloration corresponds to those in (**e**, **f**). Note that the different colonization morphotypes, the appearance of giant cells, the digestion of hyphae therein is also heteromorphic in the longitudinal view as it is in transverse view (see details in Imhof 2003)

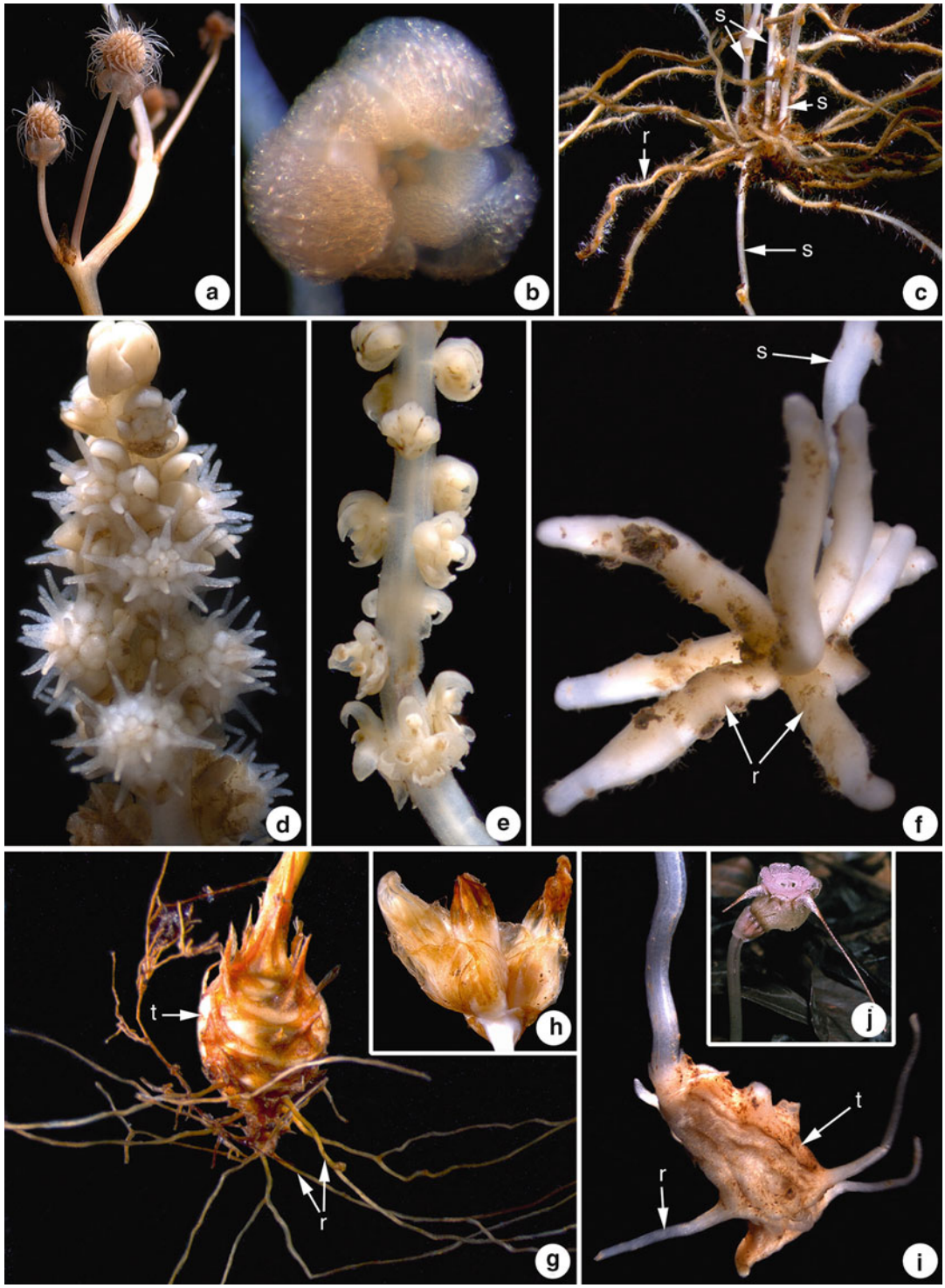

Fig. 4.10 (**a**–**c**) *Seychellaria madagascariensis*, (**b**–**f**) *Kupea martinetugei*, (Triuridaceae) (**g**, **h**) *Geosiris aphylla*, (Iridaceae) (**i**, **j**) *Thismia panamensis* (Thismiaceae). (**a**) Female flowers of *S. madagascariensis* about 2 mm wide with the basal filiform styles projecting above the carpels. (**b**) Young male flower of *S. madagascariensis*.

Glomus-group A as well as by an *Acaulospora* sp. (Franke et al. 2006), whereas Merckx and Bidartondo (2008) detected only a *Glomus*-group A fungi in a specimen of *S. ledermannii* from Mount Cameroon. *Kupea martinetugei* was associated with two closely related fungi of *Glomus*-group A (Franke et al. 2006), confirmed by Merckx and Bidartondo (2008).

4.5.5 Corsiaceae (Fig. 4.11)

4.5.5.1 *Arachnitis*

Roots of the inconspicuous plant *Arachnitis uniflora* (Corsiaceae, Fig. 4.11a), one of two unusual mycoheterotrophs in the genus confined to a few locations in the southern hemisphere (Dimitri 1972; Cribb et al. 1994; Ibisch et al. 1996; Domínguez and Sérsic 2004), are short and fleshy, radiating from the shoot base, and lack root hairs. Reiche (1907) described colonization of peripheral parenchyma cells in roots by endotrophic mycorrhizal fungi whereas Colozza (1910) called the plant "parassita," adding "fors'anche saprofita?" (= perhaps saprophytic?) in brackets. More recently, Minoletti (1986) referred to the colonization pattern as an ectendomycorrhiza because both intercellular and intracellular hyphae were present in the outer cortex of roots. Molecular methods have proven that roots are colonized by an AM fungus belonging to *Glomus*-Group A (Bidartondo et al. 2002). However, details of the structural characteristics of the plant-fungus interaction are unlike other plant associations with *Glomus* spp. (Domínguez et al. 2006, 2009). Unusual branched structures with inflated ends (Fig. 4.11c–g) form in addition to hyphal coils (Fig. 4.11d) in the cortical cells of the plant's fleshy roots. Arbuscules do not form and vesicles (Fig. 4.11g) rarely occur. The function of the branched structures is unknown but they, along with the hyphal coils, may be involved in the transfer of sugars from fungus to root cells (Domínguez et al. 2009). *Arachnitis uniflora* also develops unusual asexual propagules (Fig. 4.11b) on its fleshy roots (Domínguez et al. 2006) that are colonized by fungi from the parent root before they detach. The propagules develop a shoot apical meristem and adventitious roots and ultimately new plants that presumably link to neighboring photosynthetic plants for their source of carbon (Domínguez et al. 2006, 2009)

4.5.5.2 *Corsia*

Only a general description of roots in *Corsia* species is given by van Royen (1972); no further specific information can be found in the taxonomic section of his monograph. The roots are filiform, unbranched, white to cream-colored, growing horizontally through the humus layer. Compared to the entire plant, they are "quite sizable" and "extend over considerable distance in many directions" (van Royen 1972). They arise from short, creeping rhizomes, with sheathing scale leaves (Williams 1946; van Royen 1972). Beccari's (1877) and Schlechter's (1905) drawings, however, show some root branches in *Corsia ornata* and *C. unguiculata*, respectively. Similarly, Cribb (1985), without discussing them, depicts branching roots in *C. pyramidata*, also arising from branching rhizomes a few centimeters below the soil surface. Jones and Gray (2008), describing the only Australian species *C. dispar*, explained this discrepancy as an oversight by van Royen, since many herbarium sheets of *Corsia* spp. in the herbarium of Canberra have branched roots as well. According to Jones and Gray (2008), the rhizome in *C. dispar* is about 4 mm

Fig. 4.10 (continued) (**c**) Root aggregation of *S. madagascariensis* (compare Fig. 4.8e) showing four shoots (s) and numerous pilose roots (r) up to 10 cm long and 0.6 mm thick. (**d**) Female inflorescence of *K. martinetugei*. (**e**) Male inflorescence of *K. martinetugei*. (**f**) Root system of *K. martinetugei* with several radiating, 1.5 mm thick and up to 7 mm long, roots (r) at the base of the shoot (s). (**g**) Subterranean parts of *G. aphylla* with a rhizomatous tuber (t) and filiform roots (r) at its base. (**h**) Preserved capitulum of *G. aphylla*. (**i**) Subterranean tuber (t) of *T. panamensis* with filiform roots (r) radiating from it. (**j**) Flower of *T. panamensis* (courtesy of H and PJM Maas)

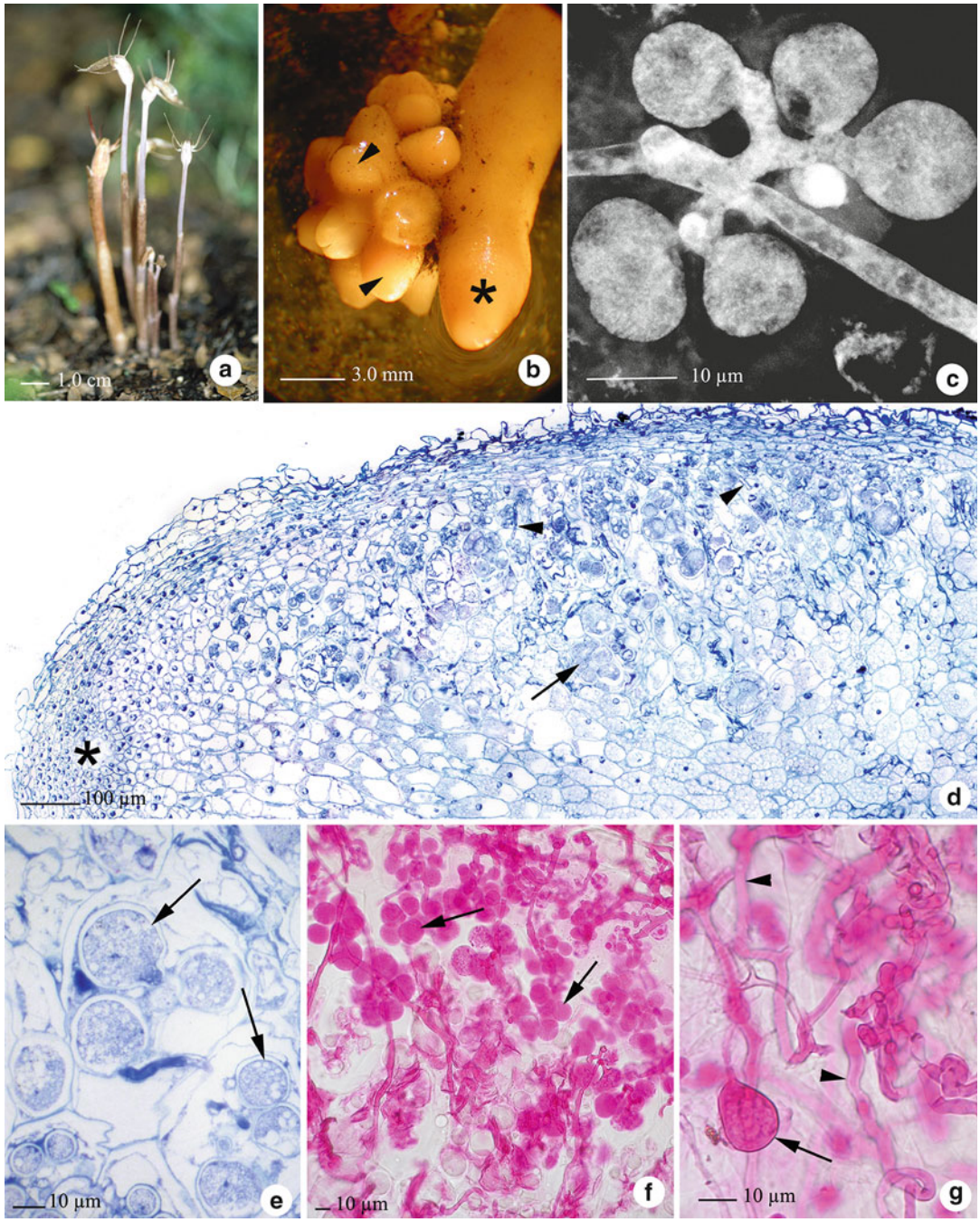

Fig. 4.11 *Arachnitis uniflora* (Corsiaceae). (**a**) Flowering stems of *Arachnitis uniflora*, each with a single flower. Image courtesy of Laura Domínguez. (**b**) Propagules (*arrowheads*) on a fleshy root (*asterisk*) of *A. uniflora*. (**c**) Confocal microscopy of intracellular branched hyphal structures of *Glomus*-Group A in a root of *A. uniflora*. (**d**) Longitudinal section of resin-embedded root of *A. uniflora* stained with toluidine blue O showing the apical meristem (*asterisk*), intracellular hyphae of *Glomus*-Group A (*arrowheads*), and intracellular branched hyphal structures (*arrow*). (**e**) Enlarged portion of a similar section of a *A. uniflora* root showing intracellular branched hyphal structures of *Glomus*-Group A (*arrows*). (**f**) Clearings of root cells of *A. uniflora* stained with acid fuchsin showing numerous intracellular branched hyphal structures of *Glomus*-Group A (*arrows*). (**g**) Clearings of root cells of *A. uniflora* stained with acid fuchsin showing intracellular hyphae (*arrowheads*) and a vesicle (*arrow*) of *Glomus*-Group A

thick and grows in annual increments. In contrast to the information given by van Royen (1972), Cribb (1985) and Jones and Gray (2008), a new variety *C. purpurata var. wiakabui* (Takeuchi and Pipoly 1998), later considered to be a separate species (Jones and Gray 2008), has a conspicuously tuberous rhizome bearing the roots (interpreted from the drawing in Takeuchi and Pipoly 1998). This is partly reminiscent of the third genus of the family, *Corsiopsis*, discussed below. No anatomical studies exist on this genus which could elucidate its mycorrhiza.

4.5.5.3 *Corsiopsis*

The monotypic *Corsiopsis chinensis* is only known from a single herbarium specimen (Zhang et al. 1999). The original description is of an ellipsoid rhizome 12–15 mm long and 5 mm in width, the drawing showing it in an orthotropous orientation. Roots were not seen.

4.5.6 Orchidaceae (Fig. 4.12)

The family Orchidaceae has the largest number of mycoheterotrophic genera of any plant family, with approximately 35 % of more than 500 fully mycoheterotrophic angiosperm species recognized (Leake 1994; Merckx et al. 2009; Imhof 2010). It is impossible to characterize the subterranean structures of all mycoheterotrophic orchid species (see Rasmussen 1995 for a thorough discussion) but a few examples will demonstrate the variability. Some species (e.g., *Cyrtosia javanica*) have rhizomes bearing fleshy adventitious roots, others (e.g., *Epipogium aphyllum*; *Corallorhiza* spp., *Rhizanthella garderi*) with rhizomes only, and others (e.g., *Wullschlaegelia calcarata*) with roots, some of which are modified as tubers.

Regardless of the nature of the underground structures, the majority of achlorophyllous orchid species are associated with fungi that form intracellular hyphal coils (pelotons) similar to those in photosynthetic orchids. These can develop within the majority of root or rhizome cortical cells and sometimes even in scale leaf tissue (Groom 1895c) and have an ephemeral existence since they undergo digestion by host cells (Smith and Read 2008). This process, termed tolypophagy (Burgeff 1932), can be repeated with recolonization by pelotons of cells containing hyphal remnants and subsequent digestion of these. Often, the cortex parenchyma is divided into an outer "Pilzwirtsschicht" (fungus host layer), where the coils do not degenerate, and an inner "Pilzverdauungsschicht" (fungus digestion layer), where digestion takes place (Magnus 1900; Burgeff 1932). Moreover, in some mycoheterotrophic species (e.g., *Gastrodia* spp.), a process called ptyophagy occurs (Burgeff 1932; Wang et al. 1997; Rasmussen 2002). While keeping the fungus host cell layers, this is characterized by only short hyphae penetrating the single-layered and particularly voluminous digestion cells and releasing their contents into it without coiling (Janse 1896; Burgeff 1932; Campbell 1962, 1963, 1964). As such, it very much resembles the "hyphal pegs" in monotropoid mycorrhizas (Lutz and Sjolund 1973; Duddridge and Read 1982). It is open to speculation if this can be interpreted as an evolutionary progression within orchid mycorrhiza, from non-differentiated colonization pattern (see e.g., Peterson et al. 2004), over the tissue compartmentation in host and digestion layers, to the ptyophagy as a special type of the latter in few MH orchids. More structural work is needed to elucidate this, but since arbuscular mycorrhizas and ectomycorrhizas seem to have undergone evolutionary progression (Imhof 2009), it would be surprising if this is not the case in orchid mycorrhizas.

Because of their "dust seeds," consisting of a rudimentary embryo and limited storage reserves, all orchid species (Fig. 4.12a) growing in native habitats require a suitable fungal partner to germinate and for the subsequent development of the protocorm (Peterson et al. 1998, 2004). The intracellular fungal hyphal coils (pelotons) are essential features for metabolite transfer into developing protocorms (Fig. 4.12b) and roots (Fig. 4.12c). All orchid species can, therefore, be considered to be mycoheterotrophic during this early stage of their life cycle (Leake 2004). The fungi involved are basidiomycete anamorphs such as *Ceratorhiza*, *Epulorhiza* and *Moniliopsis* which are capable of enzymatically reducing

Fig. 4.12 Orchidaceae. (**a**) Flowering stems of *Corallorhiza trifida*. (**b**) Diagram of a developing orchid protocorm with intracellular fungal hyphal coils (pelotons) (*arrowheads*). (**c**) Section of an orchid root showing intact pelotons (*arrowheads*) and degraded hyphae (*arrows*). Image courtesy of Carla Zelmer. (**d**) *C. trifida* root cells (*arrow*) showing peloton stained with chlorazol black E (*arrowheads*). Scale bar = 10 μm. Image courtesy of Carla

complex carbohydrates to simple sugars that are not only used for fungal growth but are also transferred to developing protocorms to enable seedling establishment to occur.

Although the majority of orchid species develop photosynthetic adult plants, a considerable number of genera remain dependent on mycorrhizal fungi for carbon compounds throughout their life cycle and therefore continue to be mycoheterotrophs. In these situations, developing seedlings link to photosynthetic plant species via fungal mycelium, mostly belonging to members of the Basidiomycota (Taylor and Bruns 1997). Zelmer and Currah (1995) demonstrated that the fungus isolated from roots of *Corallorhiza trifida*, although not identified, formed pelotons in *Corallorhiza trifida* root cells (Fig. 4.12d) and typical ectomycorrhizas with lodgepole pine (*Pinus contorta*, Fig. 4.12e). It was recently demonstrated by Zimmer et al. (2008) that the fungal symbiont associated with *C. trifida* is a *Tomentella* sp. (Thelephoraceae). Another example in which seeds of *Neottia nidus-avis* (also a non-photosynthetic orchid) were enclosed in seed packets and placed either near adult plants or at some distance from them in a beech (*Fagus sylvatica*) woodland, McKendrick et al. (2002) were able to show that the genus *Sebacina* (anamorph, *Epulorhiza*) was the symbiont involved in the stimulation of seed germination. Adult plants of the mycoheterotroph *Neottia nidus-avis* remain associated primarily with the basidiomycete family Sebacinaceae (Selosse et al. 2002) whereas *Cephalanthera austinae* (another mycoheterotroph) associates with members of the Thelephoraceae (Taylor and Bruns 1997). Recently, Ogura-Tsujita and Yukawa (2008) reported the extreme specificity of the mycoheterotrophic orchid *Eulophia zollingeri* with the fungal symbiont, *Psathyrella candolleana* in the Agaricales (Basidiomycetes). In contrast, mycoheterotrophic species within the genus *Epipactis* have been reported to associate not only with members of the Basidiomycota but also with members of the Ascomycota, including *Tuber* (truffle) species (Selosse et al. 2004).

4.5.7 Iridaceae (*Geosiris*, Fig. 4.10g, h)

Unlike the other larger families comprising both autotrophic and mycoheterotrophic species (Orchidaceae, Burmanniaceae, Gentianaceae, Polygalaceae, Ericaceae), Iridaceae do not comprise morphologically intermediate species with reduced photosynthetic surface or amount of chlorophyll. *Geosiris aphylla* (Fig. 4.10g, h) and the recently described *G. albiflora* (Goldblatt and Mannings 2010) are the only mycoheterotrophic exceptions in the entire family. Systematically, *Geosiris* has been treated as a member of Iridaceae, Burmanniaceae or a family of its own (see Rübsamen-Weustenfeld et al. 1994). Within the Iridaceae, it has been considered as Nivenioideae (Goldblatt 1990; Goldblatt et al. 1987, 1998), but recently a position in its own subfamily Geosiridoideae, as suggested earlier (e.g., Thorne 1983), has been confirmed (Goldblatt et al. 2008).

Geosiris aphylla has an orthotropous, corm-like, oval to elongate rhizome with numerous scale leaves. The flowering shoots arise from the apical tip of this tuber-like organ, whereas at its base numerous filiform roots develop (Fig. 4.10g), similar to the base of onion bulbs. Anatomically, the rhizome consists of a wide cortex parenchyma surrounding a vascular cylinder with occasional gaps due to leaf and bud traces. A thin-walled epidermis and a fortified endodermis

Fig. 4.12 (continued) Zelmer and Randy Currah. (**e**) The same fungus isolated from *C. trifida* root cells and inoculated on *Pinus contorta* roots formed typical ectomycorrhizas with a mantle (*arrow*) and Hartig net (*arrowheads*). Scale bar = 25 μm. Image courtesy of Carla Zelmer and Randy Currah. (**f**) Subterranean system of *Neottia nidus-avis*, consisting of numerous roots, 1–2.5 cm long and 2 mm thick, emerging from a short orthotropous rhizome. (**g**) Subterranean system of *Wullschlaegelia calcarata*, with spindle-shaped root tubers (max. 2 cm long and 2 mm thick) at a short rhizome

around the vascular cylinder are present and some isolated amphivasal (xylem around phloem) bundles are found in the ground tissue of the central pith. The parenchyma cells, particularly in the pith, contain starch grains (Goldblatt et al. 1987). The roots also have a thin-walled epidermis, the cortex has four layers of parenchyma cells and a strongly fortified tertiary endodermis (Goldblatt et al. 1987). As pointed out previously (see *Petrosavia*), this again corroborates the view of Imhof (2010), who considers a strong tertiary endodermis as one of the common adaptations of monocotyledonous MH plants in order to secure the essential linkage of roots and shoots.

The only anatomical work on *G. aphylla* (Goldblatt et al. 1987), aside from Rübsamen-Weustenfeld et al. (1994) studying embryology, does not mention any fungal colonization of roots or rhizomes. This is rather curious, since a carbon source for this non-photosynthetic plant is mandatory. A parasitic mode of life (*sensu* Weber 1993) is highly unlikely because, in contrast to the roughly 4,500 eudicotyledonous parasitic plants, monocots have never been found to be parasitic (Raynal-Roques and Paré 1998; Heide-Jørgensen 2008). Possibly, Goldblatt et al. (1987) may have overlooked the mycorrhizal structures. Therefore, further anatomical investigations focusing on the putative mycorrhiza of this species are necessary.

4.6 Eudicots

4.6.1 Polygalaceae (*Epirixanthes*, Fig. 4.13)

Epirixanthes from Southeast Asia is the only genus in the Polygalaceae entirely devoid of chlorophyll, although there are other species in *Polygala* and *Salomonia* (e.g., *Polygala setacea* (southeast USA) and *Salomonia ciliata* (Southeast Asia and northern Australia) that also show reductions in photosynthetic surface). The taxonomic accounts (e.g., Smith 1912; Ridley 1922; Backer and van den Brink 1963; van der Meijden 1988; Hsieh et al. 1995; Pendry 2010) of the six species of *Epirixanthes* do not yield information on the subterranean organs. However, there are two older and one contemporary study on the mycorrhizal roots of *E. papuana*, *E. elongata*, and *E. cylindrica* (Penzig 1901; van der Pijl 1934; Imhof 2007).

The rhizome of *E. papuana* and *E. elongata* is only a few millimeters long and continuous with the aerial shoot. The scale leaf axils give rise to sparsely branched filiform roots that are up to 12 cm long and have a maximum diameter of 0.65 mm (Imhof 2007, Fig. 4.13a+d). A primary root was never found. Since the rhizome is short, the roots seem to be radiating from the shoot

Fig. 4.13 (continued) (**c**) Inflorescence of *E. papuana*. (**d**) Subterranean system of *E. elongata* similar to that of *E. papuana* seen in (**a**). This specimen has basal shoot ramifications. Labels as in (**a**). (**e**) Longitudinal section through the cortex of an *E. papuana* root showing a part of the straight, cascading hyphae (ch) in the outer parenchyma, coiled hyphae (hc+) in layer 2 (l2) and degenerated coils (hc−) in layer 1 (layers counted from the endodermis). (**f**) Longitudinal section through parenchyma layers 1 (l1) and 2 (l2) of a *E. elongata* root. Layer 2 contains initially straight hyphae sending hyphal branches (hb1) into layer 1 where they immediately degenerate to amorphous clumps (hc−). (**g**) Tangential section external to the central cylinder through an *E. papuana* root showing 2 cell rows of each layer 1 (l1), 2 (l2) and 3 (l3). Hyphae in layer 2 remain functional (hc+) and send branches centripetally into layer 1 (hb1) as well as centrifugally into layer 3 (hb3), both of which digest the fungal material (hc−). A part of the cascading hyphae coming from the outer cortex layers is also visible (ch). (**h**) Transverse section through an *E. papuana* root. The epidermis (ep) as well as the outer three cortex parenchyma layers are not colonized by hyphal coils, layer 1 (l1) and 3 (l3) contain degenerated coils whereas layer 2 (l2 and *dotted line*) contains functional hyphae. The central cylinder inside the endodermis (en) is largely composed of lignified fibers. (**i**) Schematic view of the mycorrhizal colonization pattern in *Epirixanthes* spp.. After penetration, the hyphae grow straight in a cascading manner through the outer cortex (1), retain the straight growth when reaching layer 2 but send branches into layer 1 for digestion (2), start to coil hyphae in layer 2 when the mycorrhization proceeds (3) and then also send branches in layer 3 for digestion (4)

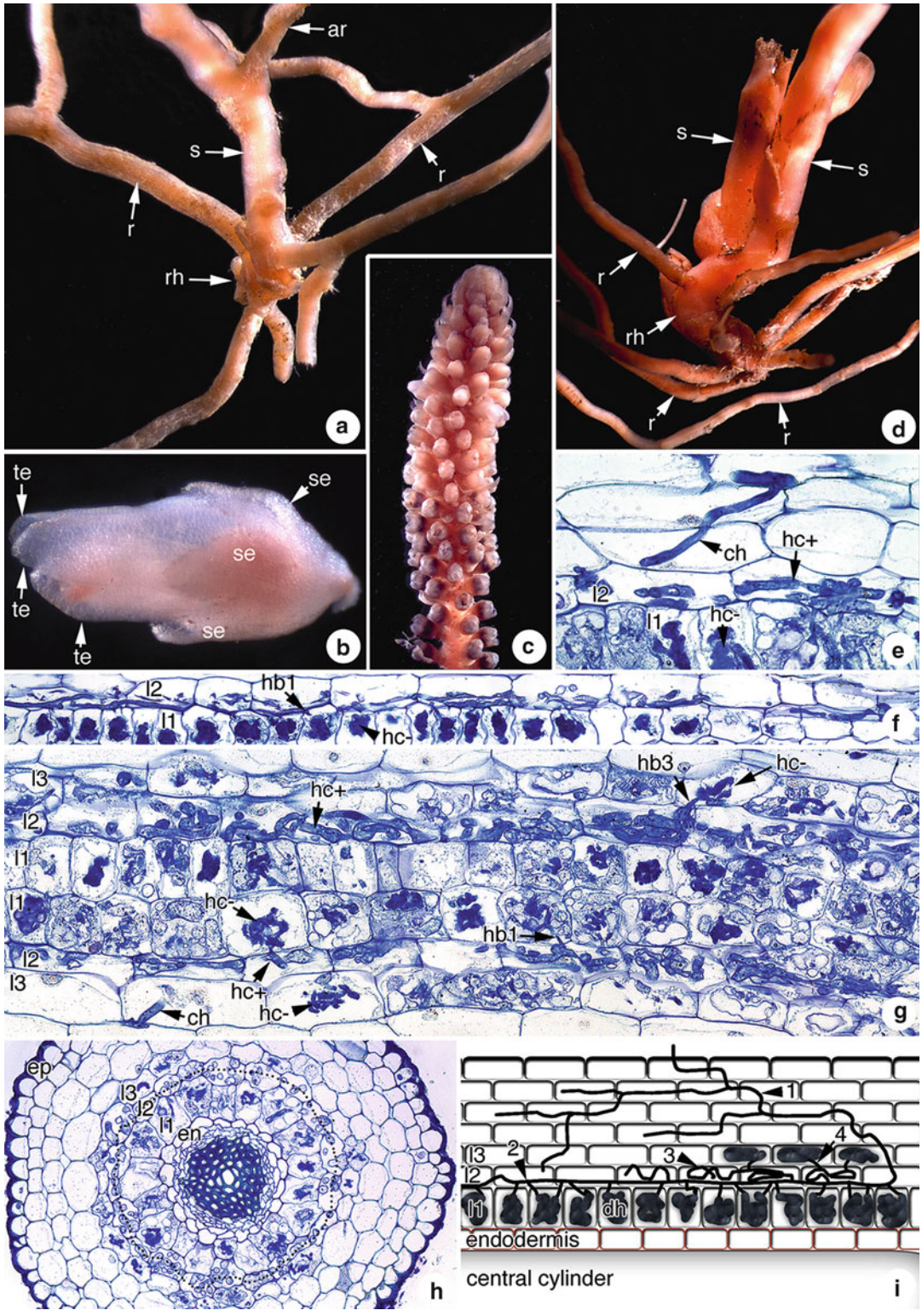

Fig. 4.13 *Epirixanthes* spp. (Polygalaceae). (**a**) Subterranean system of *E. papuana* with approximately 0.6 mm thick roots (r) arising from a short rhizome (rh) continuous with the epiterrestrial shoot (s). Additional roots (ar) may develop along the shoot where it is connected to the soil. (**b**) Isolated flower of *E. papuana* (spirit material), little more than 2 mm long, with three tepals (te). Not all of the five sepals (se) are visible.

base, but additional roots may develop along the shoot when it is still covered by soil or litter substrate (Imhof 2007, Fig. 4.13a). *Epirixanthes cylindrica* has thicker roots (up to 0.75 mm diameter) and the rhizome bearing the roots is longer (Penzig 1901). Roots of *Epirixanthes* have a triarch central cylinder with many lignified fibers, a pericycle, a suberized endodermis, up to seven cell layers of cortex parenchyma, and an epidermis (Fig. 4.13h). The cells of the innermost cortex parenchyma layer are larger in radial but shorter in longitudinal direction than the other cells (Penzig 1901; van der Pijl 1934; Imhof 2007, Fig. 4.13e, f+h).

Penzig (1901) recognized the coiled intracellular hyphae, their degeneration stages, especially in parenchyma layers 1 and 3 when counted outwards from the endodermis, and the nearly fungus-free outer cortex layers. Van der Pijl (1934) added the observation that the hyphae in layer two grow in longitudinal direction and send hyphal branches into the inner layer for digestion. The whole colonization pattern, however, is more complicated and only perceivable when considering sequential sections. After penetration, the hyphae grow straight through the cells of the outer cortex, branch repeatedly and spread coarsely in this root segment until they reach layer 2 (Fig. 4.13e, layer 1 being the innermost cortex parenchyma layer). There the hyphae keep growing straight (Fig. 4.13f), but later develop hyphal branches that coil within the cells (Fig. 4.13f, g). Directly after having reached layer 2, lateral hyphae enter the anatomically distinct layer 1 where they immediately swell and degenerate (Fig. 4.13e, f). In a later stage, layer 3 is also colonized from coiled hyphae in layer 2, again with immediate degeneration in layer 3 (Fig. 4.13g, h). In contrast, the hyphae in layer 2 as well as the straight hyphae in the outer cortex, remain alive for nearly the lifetime of the root (Fig. 4.13g, h). This rather complicated colonization pattern is interpreted as a reasonable strategy in order to have maximum as well as sustained benefit from the few fungal penetration events. It includes a coarse (outer cortex colonization) as well as a fine scale distribution mode (colonization in layer 2), specialized cells for digestion, and tissue to keep the fungus alive (schematic view on Fig. 4.13i, details see Imhof 2007).

4.6.2 Ericaceae (Monotropoideae, Figs. 4.14–4.17)

Eleven genera of mycoheterotrophic species are now recognized in the Monotropoideae: *Allotropa*, *Cheilotheca*, *Hemitomes*, *Hypopitys*, *Monotropa*, *Monotropastrum*, *Monotropsis*, *Pityopus*, *Pleuricospora*, *Pterospora* and *Sarcodes*, with several species endemic to a particular continent (Wallace 1975). Molecular phylogeny work has revealed that *Monotropa uniflora* is more closely related to *Monotropastrum humile*, whereas *Monotropa hypopitys* seem to be sister of *Pityopus californicus* (Bidartondo and Bruns 2001; Tsukaya et al. 2008). Therefore, some standard taxonomies (Stevens et al. 2004; Seybold 2011) have erected *Hypopitys* as a separate genus from *Monotropa*, and now use *Hypopitys monotropa* coined by Crantz (1766).

The minute seeds of members of the Monotropoideae have underdeveloped embryos and minimal nutritive tissue and therefore depend

Fig. 4.14 (continued) mycorrhiza with mantle (*asterisk*), fungal peg (*arrow*) and flask-shaped cystidia (*arrowheads*). (**g**) Scanning electron micrograph of large calcium oxalate crystals (*arrows*) among flask-shaped cystidia. (**h**) Freehand transverse section of root showing mantle (*asterisk*) and labyrinthine branching of Hartig net (*arrowheads*). (**i**) Longitudinal section of resin-embedded root stained with Toluidine blue O showing the apical meristem (*asterisk*), and the mantle covering the root apex. (**j**) Paradermal section of resin-embedded root stained with Toluidine blue O showing labyrinthine branching of Hartig net hyphae and fungal pegs in transverse section (*arrows*). (**k**) Higher magnification of a longitudinal section of resin-embedded root stained with Toluidine blue O showing mantle (*asterisk*), Hartig net (*arrowheads*) and fungal peg (*arrow*). (**l**) Transmission electron micrograph showing detail of the fungal peg with finger-like wall depositions (*arrows*)

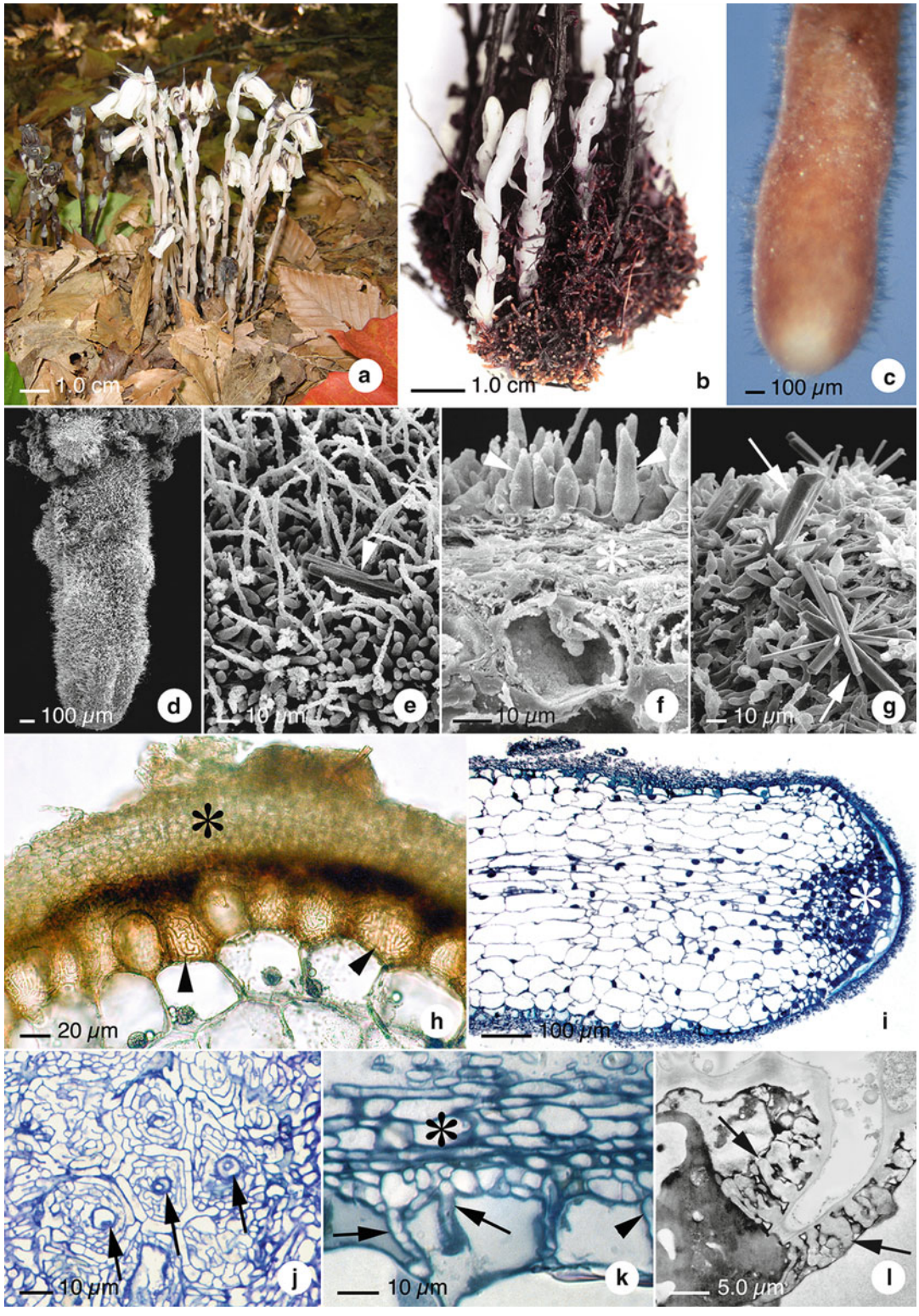

Fig. 4.14 *Monotropa uniflora* (Ericaceae/Monotropoideae). (**a**) Cluster of flowering stems in a hardwood forest in southern Ontario, Canada. (**b**) Young shoots and associated root ball. (**c**) Mycorrhizal root tip showing compact mantle with cystidia (*arrowheads*). Photo courtesy of Brent Young. (**d**) Scanning electron micrograph of a root tip showing cystidia. (**e**) Higher magnification scanning electron micrograph of portion of a mycorrhiza with a calcium-oxalate crystal (*arrowhead*) among cystidia. (**f**) Scanning electron micrograph of a fracture of a

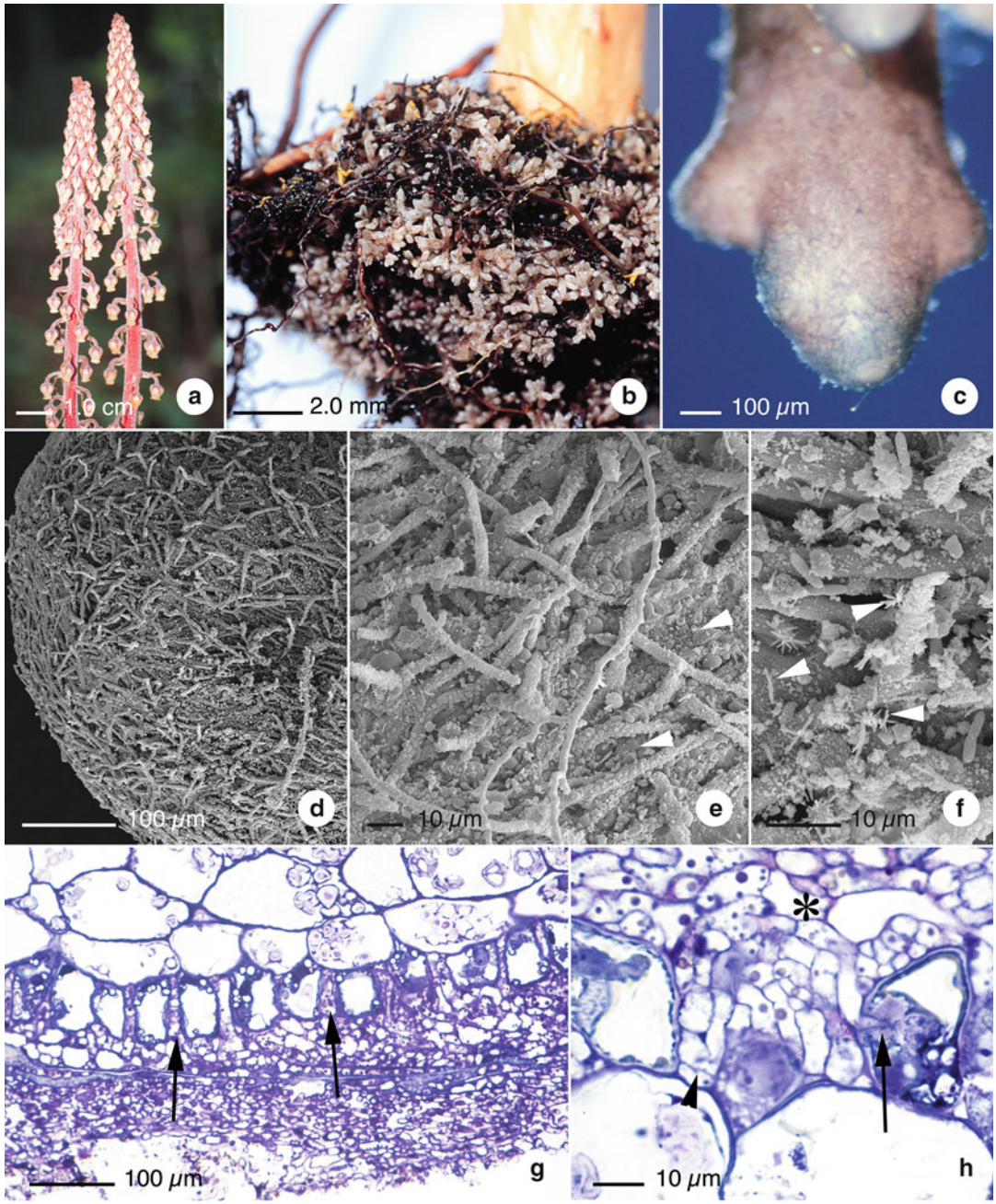

Fig. 4.15 *Pterospora andromedea* (Ericaceae/Monotropoideae). (**a**) Two flowering stems in the boreal forest in British Columbia, Canada. (**b**) Root ball showing mycorrhizal root tips. (**c**) Branched root tip showing colored mantle characteristic of a *Rhizopogon* sp. (**d**) Scanning electron micrograph of a portion of mantle showing compact hyphae. (**e**) Higher magnification scanning electron micrograph showing irregular hyphae and abundant small crystals (*arrowheads*). (**f**) Higher magnification scanning electron micrograph showing details of various crystals (*arrowheads*). (**g**) Longitudinal section of a root showing the thick mantle (*asterisk*) and Hartig net (*arrows*). (**h**) Longitudinal section of a root showing the inner mantle (*asterisk*), Hartig net (*arrowhead*) and fungal peg (*arrow*) penetrating the radial epidermal cell wall

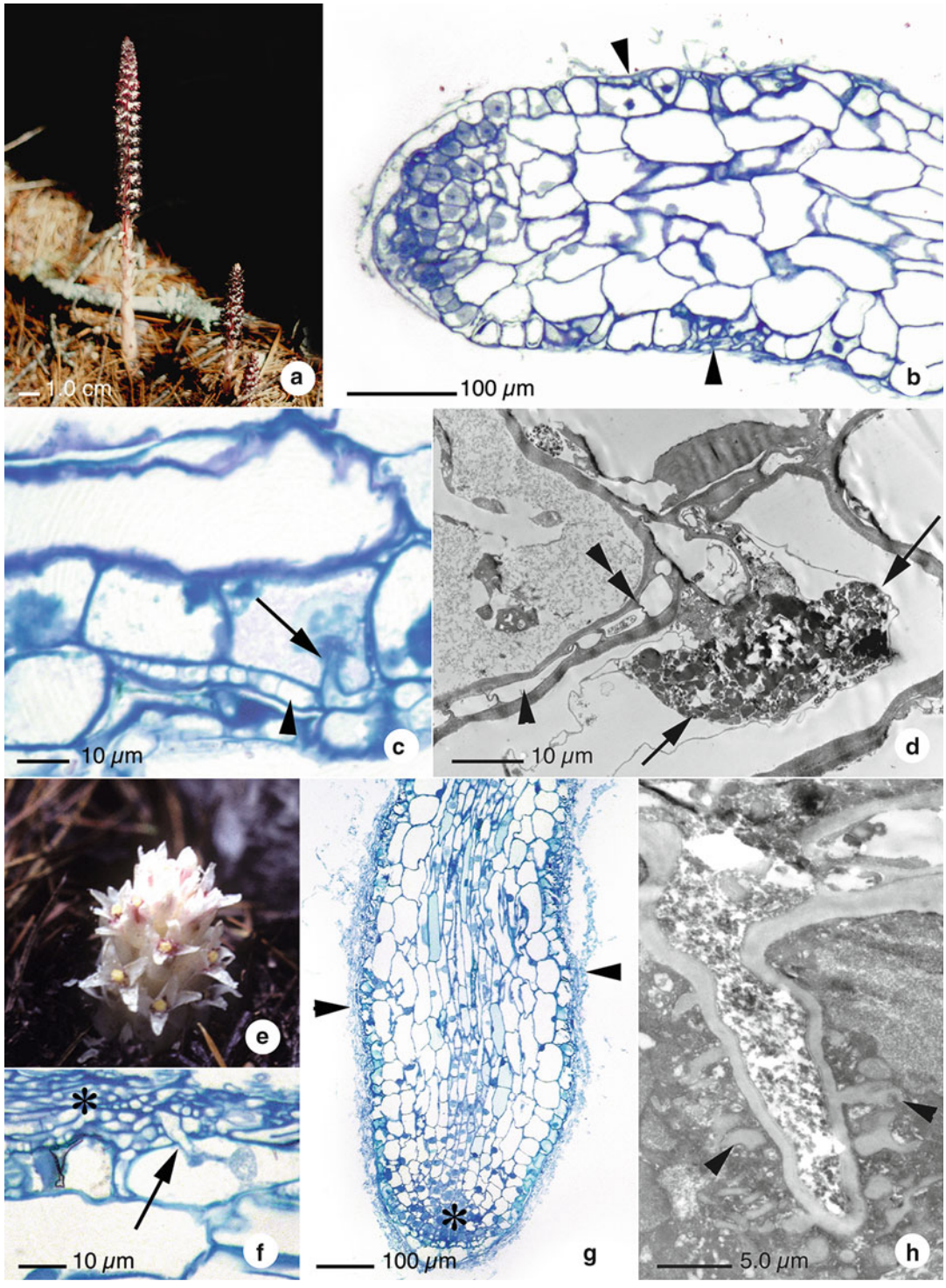

Fig. 4.16 (**a**, **b**) *Allotropa virgata*, (**e**–**h**) *Pleuricospora fimbriolata*, (Ericaceae/Monotropoideae). (**a**) Flowering shoots of *Allotropa virgata*. (**b**) Longitudinal section of a root showing a small apical meristem (*asterisk*) and some fungal colonization (*arrowheads*). (**c**) Higher magnification showing Hartig net (*arrowhead*) and fungal peg (*arrow*). (**d**) Transmission electron micrograph showing Hartig net (*arrowhead*) and detail of the fungal peg with finger-like wall depositions (*arrows*). (**e**) Emerged shoot with flowers of *Pleuricospora fimbriolata*. Photo courtesy of Dan Luoma. (**f**) Longitudinal section of a root showing the apical meristem (*asterisk*) and well-developed mantle (*arrowheads*). (**g**) Mantle (*asterisk*) and fungal peg (*arrow*) penetrating the outer tangential wall of an epidermal cell. (**h**) Transmission electron micrograph showing a fungal peg with finger-like wall depositions (*arrowheads*)

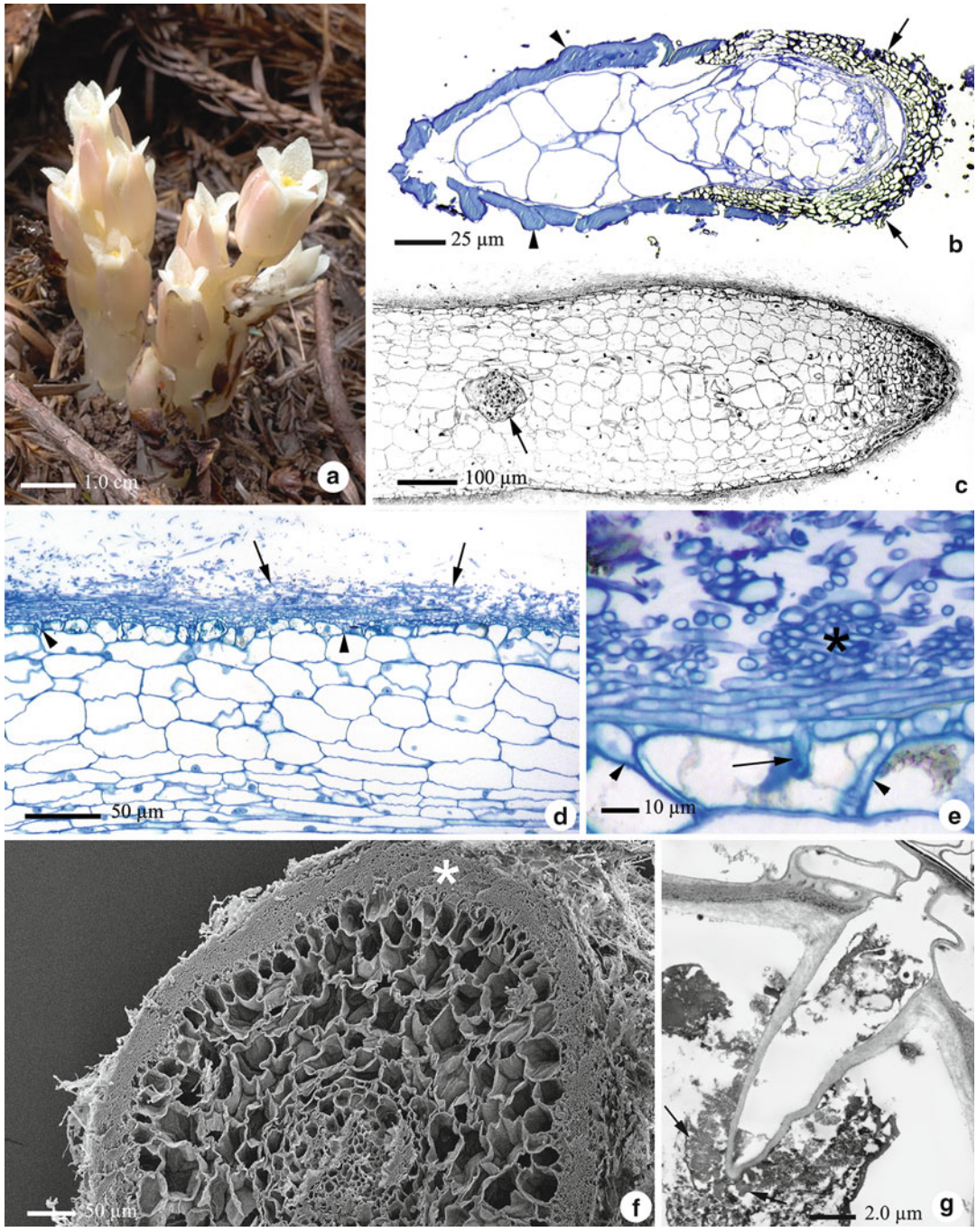

Fig. 4.17 *Pityopus californicus* (Ericaceae/Monotropoideae). (**a**) Flowering stems of *Pityopus californicus*. Photo courtesy of Barry Rice. (**b**) Developing embryo with multilayered mantle (*arrows*) on developing root. Remnants of seed coat are obvious (*arrowheads*). (**c**) Longitudinal section of an older root with mantle covering the apex. An emerging lateral root (*arrow*) is evident. (**d**) Higher magnification showing mantle (*arrows*) and Hartig net (*arrowheads*). (**e**) Detail of mantle (*asterisk*), Hartig net (*arrowheads*), and fungal peg (*arrow*) penetrating the tangential wall of an epidermal cell. (**f**) Scanning electron micrograph of fractured root showing a thick mantle (*asterisk*). (**g**) Transmission electron micrograph showing a fungal peg with finger-like wall depositions (*arrows*)

on the presence of a suitable fungus to provide sugars and perhaps other nutrients needed for germination and seedling establishment (Bruns and Read 2000; Leake et al. 2004; Smith and Read 2008). Massicotte et al. (2007) have shown that, in *Pityopus californicus*, fungal hyphae become associated with germinating seeds and form a mantle as the embryo begins to elongate. Later, a mantle, Hartig net, and fungal pegs form in the developing root. Mycoheterotrophy is therefore established very early in the life cycle of these plant species. Their root systems vary among species, ranging from large root balls comprised of numerous mycorrhizal roots (e.g., *Monotropa*, *Pterospora*), to more diffuse root systems with mycorrhizal roots distributed more randomly (e.g., *Pleuricospora*, *Monotropsis*, *Allotropa*). Hirce and Finocchio (1972) described in detail the remarkably compact root system and anatomy of *M. uniflora* and concluded that it represents a variation of the normal dicotyledonous condition. They documented a decrease in anatomical complexity of first order (hexarch stelar configuration of vascular tissue, several centimeters long and up to 1.4 mm thick, linking adjacent plants) over second order (up to 8 mm long and 0.85 mm thick) to the third order roots (protostelic arrangement, max. 4 mm long and 0.5 mm thick), even if all roots are densely covered with a mantle (and are presumably active). At the other extreme, *Allotropa* exhibits a more loose system of elongated rhizomes with first and second order adventitious roots (Massicotte et al. 2010), and likewise in *Monotropsis odorata* (treated as *Cryptophila pudica*) "the root system resembles a slender, mostly repeated many-branched, creeping rhizome" (Wolf 1922). Compared to *Allotropa*, *Pleuricospora* seems to have a slightly more condensed subterranean system (Massicotte et al. 2010).

In the Monotropoideae (formerly Pyrolaceae), a progressive compaction of the root system, from fibrous roots (e.g., *Allotropa*) to coralloid roots (e.g., *Pleuricospora*) to tight rootballs (e.g., *Monotropa*) has been hypothesized as reflecting a progressive dependence on epiparasitic mycotrophy (Furman and Trappe 1971), although this remains to be tested physiologically.

Structurally, monotropoid mycorrhizas resemble ectomycorrhizas in that a mantle and a Hartig net in this case confined to the epidermis, form (Peterson et al. 2004). However, they possess a unique feature, the invasion of epidermal cells by short hyphae originating from the Hartig net or inner mantle. These structures, referred to as fungal pegs (Lutz and Sjolund 1973; Duddridge and Read 1982; Robertson and Robertson 1982; Peterson and Massicotte 2004; Peterson et al. 2004) form either along the outer tangential wall of epidermal cells, or at the base of the radial wall of epidermal cells. Host cells respond by depositing additional cell wall material, in finger-like projections, around each peg. It has been hypothesized (Lutz and Sjolund 1973; Duddridge and Read 1982; Massicotte et al. 2005) that these structures, resembling "transfer cells" in other plant species, may be involved in nutrient transfer between the fungus and root cells although there is no experimental evidence to support this. In these systems, the Hartig net likely also plays a role in nutrient transfer but this needs to be confirmed. Kuga-Uetake et al. (2004) have shown the close association of microtubules with the fungal pegs in *M. uniflora*.

All of these species form monotropoid mycorrhizas with various fungal genera. The vast majority of fungi colonizing monotropoid roots are basidiomycetes and most of them have been identified using molecular approaches (Cullings et al. 1996; Lefevre 2002; Kretzer et al. 2000; Bidartondo and Bruns 2001, 2002). In the following paragraphs, we explore these critical features for five genera of Monotropoideae.

4.6.2.1 *Monotropa uniflora* (Fig. 4.14)

Monotropa uniflora, a northern hemisphere species (Fig. 4.14a), along with the Asian *Monotropastrum humile* (Yokoyama et al. 2005; Yamada et al. 2008; Matsuda et al. 2011) have a strong affinity for fungi in the family Russulaceae, including many species of *Russula* such as *R. brevipes*, *R. decolorans*, *R. nitida*, as well as *Lactarius* spp. (Young et al. 2002, Bidartondo 2005; Bidartondo and Bruns 2005; Yang and Pfister 2006). *Hypopitys monotropa* (=*Monotropa hypopitys*), in contrast, forms mycorrhizas mostly

with fungal species in the Tricholomataceae. Excavated plants of *M. uniflora* reveal well-developed root balls (Fig. 4.14b), packed with mycorrhizal tips of Russulaceae (Fig. 4.14c), most forming numerous cystidia in the outer mantle (Fig. 4.14d). Scanning electron microscopy of the mantle surface has shown that cystidia can take various forms including, awl-shaped (Fig. 4.14e) and flask-shaped (Fig. 4.14f). As well, frequent calcium-oxalate crystals may be present (Fig. 4.14g). The mantle (Fig. 4.14h, i) and Hartig net (Fig. 4.14h) are easily observed but sectioning of mycorrhizal roots is required to show the presence and structure of fungal pegs (Fig. 4.14j–l).

4.6.2.2 *Pterospora andromedea* (Fig. 4.15)

Pterospora andromedea (Fig. 4.15a) and *Sarcodes sanguinea* (not shown), are confined to western North America and appear to associate almost exclusively with the section of *Rhizopogon* (Rhizopogonaceae) encompassing *R. ellenae*, *R. salebrosus* and *R. arctostaphyli* (Kretzer et al. 2000; Bidartondo and Bruns 2001, 2002; Taylor et al. 2002; Dowie et al., 2011). Large root balls (Fig. 4.15b) dominated with *Rhizopogon* mycorrhizas (Fig. 4.15c) are evident on excavated plants. A compact mantle with crystal inclusions of variable dimensions and shapes (Fig. 4.15d–f) is characteristic of the mycorrhizas of this species when viewed by scanning electron microscopy. Light microscopy shows a thick mantle, a well-developed Hartig net (Fig. 4.15g) and a fungal peg apparatus penetrating the radial epidermal cell wall (Fig. 4.15h).

4.6.2.3 *Allotropa virgata* and *Pleuricospora fimbriolata* (Fig. 4.16)

Allotropa virgata (Fig. 4.16a) forms mycorrhizas exclusively with *Tricholoma magnivelare* (Lefevre 2002; Taylor et al. 2002), and is one of the most specific host-fungal monotropoid symbiosis documented so far. Light microscopy reveals sporadic colonization at the root surface with a thin mantle (Fig. 4.16b) and a fungal peg penetrating radial walls of epidermal cells (Fig. 4.16c, d). *Pleuricospora fimbriolata* (Fig. 4.16e) parasitizes the fungal species *Gautieria monticola* (Bidartondo and Bruns 2001, 2002), a truffle forming species belonging to the Gomphaceae (Humpert et al. 2001). Typically, a well-developed mantle envelops the root (Fig. 4.16f) and fungal pegs, penetrating the outer tangential walls of the epidermis (Fig. 4.16g), are obvious. Characteristic finger-like wall depositions are found on the fungal peg (Fig. 4.16h).

4.6.2.4 *Pityopus californicus* (Fig. 4.17)

Pityopus californicus (Fig. 4.17a) also forms mycorrhizas mostly with fungal species in the Tricholomataceae, in this case *Tricholoma myomyces* (Bidartondo and Bruns 2005). However, a developmental study on young mycorrhizal embryos of *P. californicus* suggests other fungi are present in earlier stages (Fig. 4.17b) that are presumably replaced at later stages by *T. myomyces* (Fig. 4.17c, Massicotte et al. 2007). Mature mycorrhizal roots typically show a thick mantle (Fig. 4.17d + f), a well-developed Hartig net and a fungal peg, penetrating the outer tangential wall of epidermal cells (Fig. 4.17e). Small finger-like projections can be seen on the fungal peg (Fig. 4.17g).

4.6.3 Gentianaceae (Figs. 4.18–4.20)

In the Gentianaceae, 25 species in four genera are mycoheterotrophic. Additional to the genera covered here, two others are also considered to be at least partially "saprophytic" (Johow 1889; Knoblauch 1894; Gilg 1895; Holm 1897, 1906; Perrot 1898; Wood and Weaver 1982). The monotypic *Obolaria virginica* has scale-like leaves along the lower stem with larger spatulate-obdeltoid leaves towards the inflorescence. The fleshy stem and leaves are purplish-green. The roots are coralloid and mycorrhizal (Holm 1897; Gillett 1959; Wood and Weaver 1982), like many of the *Voyria* species described below. *Bartonia* comprises four species, *B. virginica*, *B. verna*, *B. paniculata*, the latter of which has two subspecies (Gillett 1959), and *B. texana* (Correll 1966). All species have only scale leaves but an overall greenish appearance. Compared to *Obolaria*

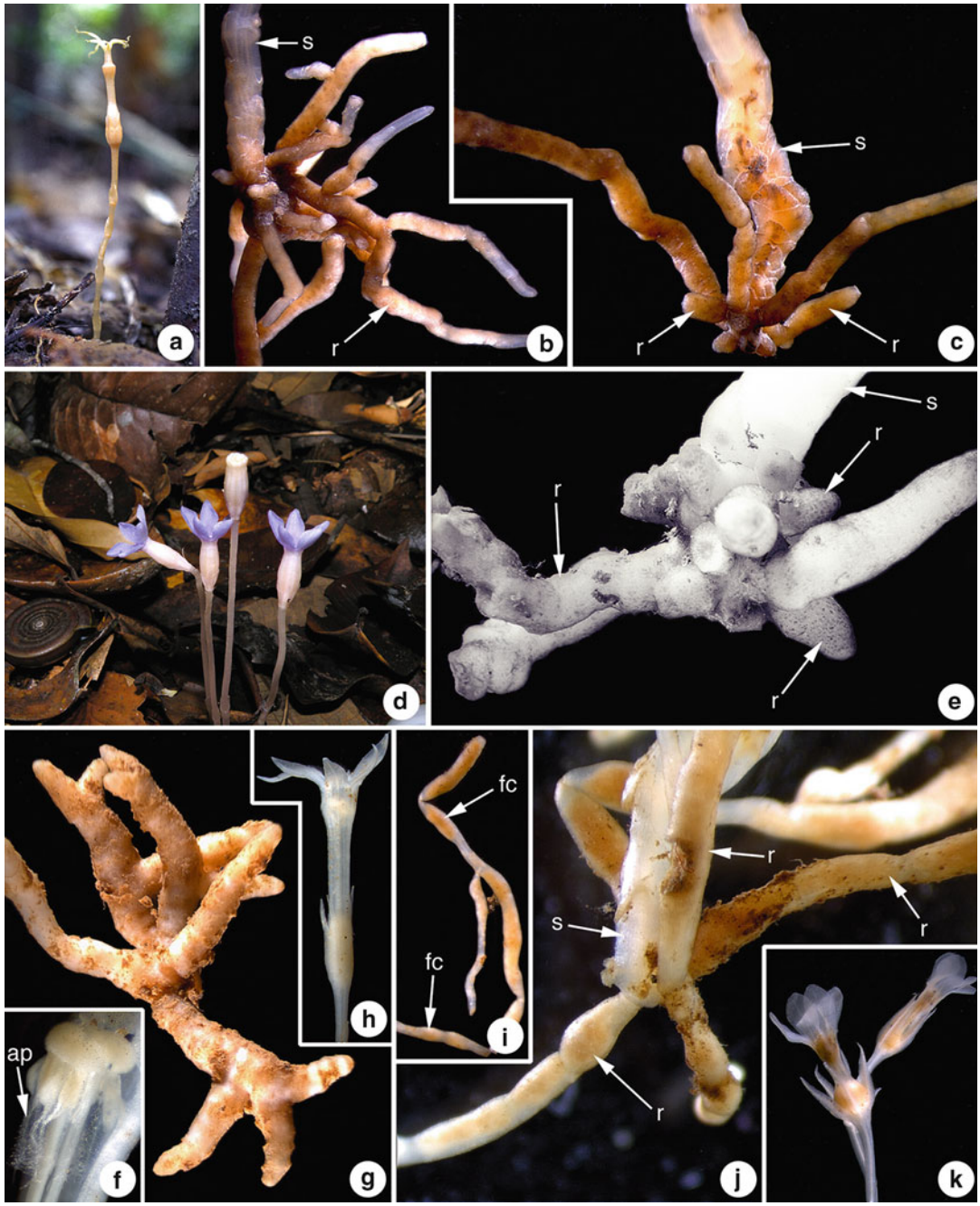

Fig. 4.18 (**a**–**c**) *Voyria tenuiflora*, (**d**, **e**) *Voyria obconica*, (**f**–**h**) *Voyria spruceana*, (**i**–**k**) *Exochaenium oliganthum* (Gentianaceae). (**a**) *V. tenuiflora* in its natural habitat. (**b**) Coralloid shaped root system of *V. tenuiflora* with branched roots (r) clumped at the base of a shoot (s). (**c**) Subterranean organs of *V. tenuiflora* showing the tendency to radiating roots (r) at the base of a shoot (s). The roots can be up to 1 mm thick and several centimeters long. (**d**) *V. obconica* in its natural habitat (courtesy of H and PJM Maas). (**e**) Subterranean system of *V. obconica* with stout, up to 1.5 mm thick and 1 cm long roots (r) at the base of a shoot (s). (**f**) Characteristic fringed, tail-like thecae appendages (ap) of *V. spruceana*. (**g**) Coralloid shaped root system of *V. spruceana*, also having the tendency for a star-like structure (this specimen measuring 14 mm in maximal extension). (**h**) Preserved flower (1.2 cm long) of *V. spruceana*. (**i**) Vermiform to filiform root of *E. oliganthum* with thickenings up to 0.8 mm where a light brown coloration indicates fungal colonization (fc). (**j**) *E. oliganthum* tends to develop radiating roots (r) at the base of a shoot (s). (**k**) Two preserved flowers (7 mm long) of *E. oliganthum*

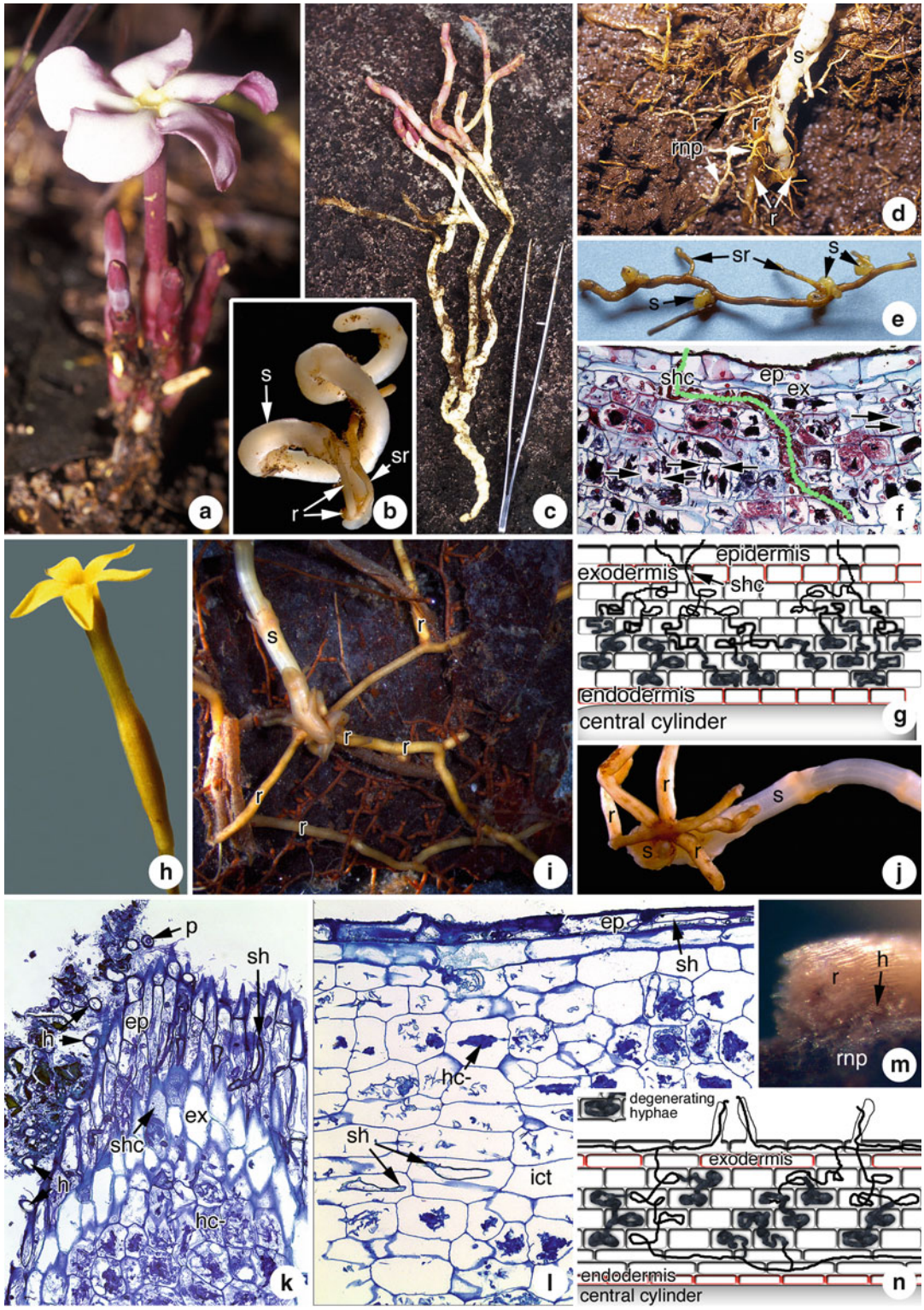

Fig. 4.19 (**a**–**g**) *Voyria truncata*, (**h**–**n**) *Voyria aphylla* (Gentianaceae). (**a**) Epiterrestrial part of *V. truncata*. (**b**) Subterranean shoot (s) of *V. truncata*, spirally bent due to soil obstructions. The shoot arises from the axil between a main root (r) and a side root (sr). (**c**) Complete specimen of *V. truncata* extracted from the soil, basally arising from

virginica, the *Bartonia* species are more delicate, but also have fleshy, sparsely branched mycorrhizal root systems (Holm 1906). Recent physiological investigations using a stable isotope distribution approach found strong indications for a partial mycoheterotrophy in *Obolaria virginica* and *Bartonia virginica* (Cameron and Bolin 2010).

4.6.3.1 *Exacum*

Cotylanthera was originally a genus comprising four achlorophyllous species, which was suspected to be closely related to the large genus *Exacum* (e.g., Raynal 1967a, Klackenberg 1985, 2002, Yuan et al. 2003); the genus has now been formally transferred into *Exacum* by Klackenberg (2006). The taxonomic accounts (e.g., Miquel 1856; Gray 1871; Clarke 1885; Gilg 1895; Lace 1914; Hara 1975) mostly do not refer to the subterranean parts, but there are two rather old but quite detailed morphological descriptions of the roots for *Exacum tenue* (Janse 1896; Figdor 1897, treated as *Cotylanthera tenuis*). Janse (1896) described the roots as tufted around the stem base. Such star-like root systems are a common feature of most MH plants and interpreted as a strategy to decentralize carbohydrate and nutrient transport: a root system with many but hierarchically equivalent roots can better compensate for the failure of some of its elements than few but high-capacity roots, as is the case in most allorhizic root systems (Imhof 2010). Combining the information given by Figdor (1897) and Imhof et al. (1994), the former showing a seedling of *E. tenue*, the latter describing the ontogeny of another gentian (*Voyria tenella*), the star-like root system of *E. tenue* is probably generated by a primary root, developing ray-like lateral roots, and only then does this structure give rise to a root-borne shoot. Later, further root-borne shoots are a means of vegetative propagation. Root hairs, if present, are much reduced (Figdor 1897). A considerable portion of the stem, up to half its length, may also be subterranean, often bent due to

Fig. 4.19 (continued) a plagiotropous root (detached). Only the upper, reddish branches were epiterrestrial and, hence, appeared superficially as clustered but distinct individuals. (**d**) Subterranean shoot (s) of *V. truncata* arising from a runner-like, plagiotropic root (r), intermingled with roots of neighboring plants (rnp). (**e**) Runner-like root (7 cm long and up to 2 mm thick) of *V. truncata* extracted from the soil with several side roots (sr) in the axils of which one or at most two root-borne shoots (s) develop. (**f**) Longitudinal section through a *V. truncata* root showing the epidermis (ep) and multilayered cortex with intracellular hyphal coils in various stages of degradation. The *green dotted line* indicates a course of colonization from penetration to the inner cortex. The passage through a short cell (shc) of the exodermis (ex) is not visible on this section but present on the subsequent one (not shown). The pattern of newly inserted cell walls (*arrows*) indicate an ongoing primary thickening. (**g**) Schematic view of the mycorrhizal colonization pattern in *V. truncata*. After penetration of the epidermis and a short cell (shc) of the exodermis as the only passage cells, the hyphae grow in a coiling manner from cell to cell deeper into the cortex. Extent of hyphal degradation increases with cortex depth. (**h**) Flower of *V. aphylla*. (**i**) Shoot (s) of *V. aphylla* arising from a net of runner-like, up to 0.5 mm thick roots (r), much smaller than in *V. truncata*. (**j**) Radiating roots (r) at the base of a shoot (s) of *V. aphylla*. (**k**) Tangential section through the cortex of a *V. aphylla* root showing the epidermis (ep) with straight, nondegenerated hyphae (sh), the exodermis (ex) with only the short cells (shc, anatomically not particular distinct) being used as passage cells for the hyphae, and the cortex parenchyma with often degenerated hyphal coils (hc−). Root hairs (h) only occur where organic material is attached to the root. Fungal penetrations (p) mostly happen via the root hairs. (**l**) Tangential section just external to the central cylinder through a *V. aphylla* root, showing the epidermis (ep) with straight hyphae (sh) and mostly degenerated hyphal coils (hc−) in the cortex parenchyma. However, straight hyphae (sh) also occur in the innermost parenchyma layers (ic), linked to the hyphae in the epidermis by nondegenerated coiled hyphae (not shown). (**m**) Root of *V. aphylla* (r) attached to a root of a neighboring plant (rnp). Root hairs (h) develop only at such root to root connections. (**n**) Schematic view of the mycorrhizal colonization pattern in *V. aphylla*. After penetration of root hairs, the hyphae grow straight in the epidermis, cross the exodermis via passage cells (miss the red coloration), build coils in the outer cortex parenchyma which partly degenerate but also reach the innermost cortex parenchyma layers, where they again grow in a straight manner along the root axis. From these inner straight hyphae which do not become digested, branches grow back into the outer cortex to build coils for digestion

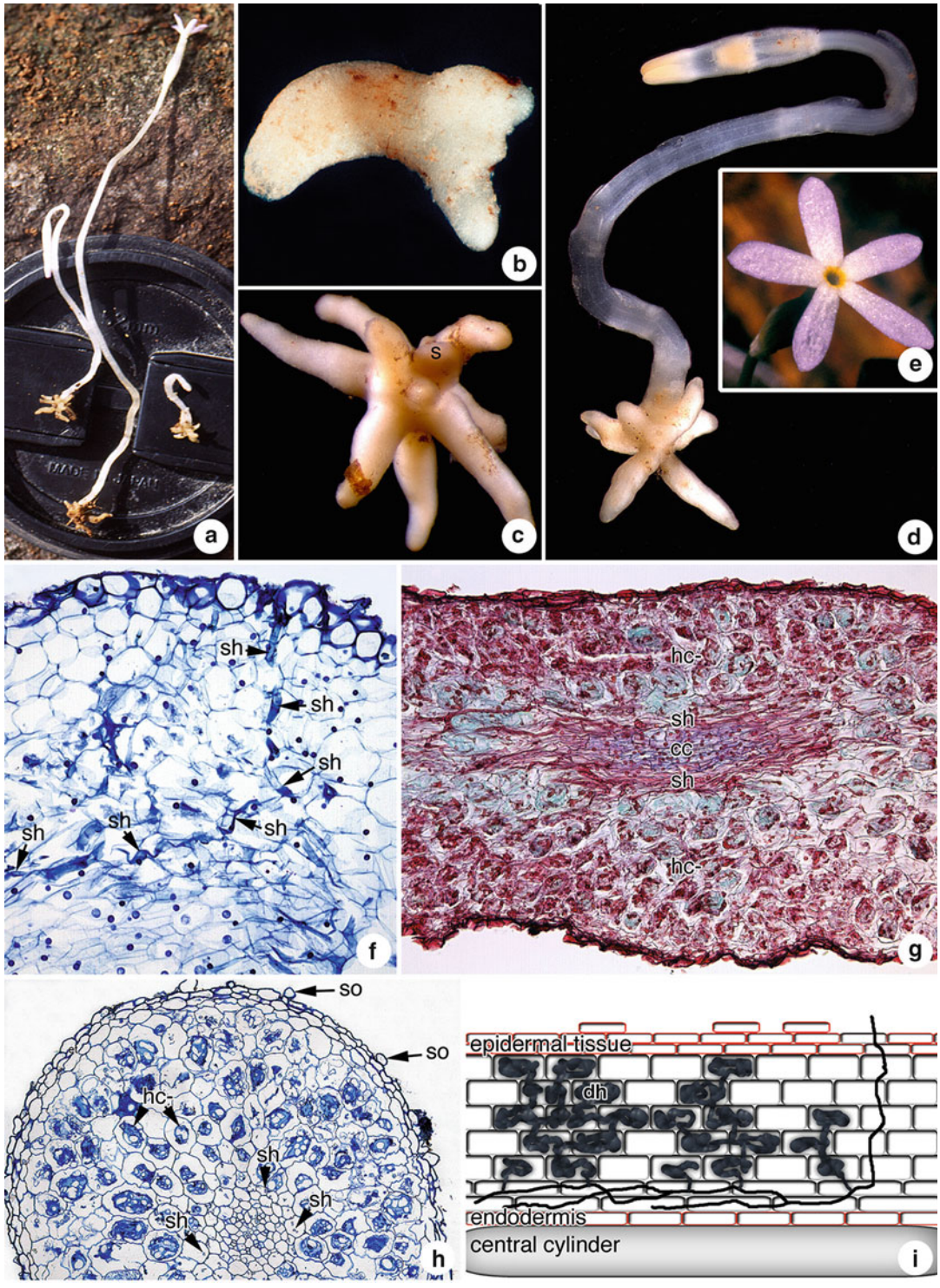

Fig. 4.20 *Voyria tenella* (Gentianaceae). (**a**) Three specimens of *V. tenella* in various stages of development. The younger specimens still show the nodding flower bud. (**b**) Youngest specimen of *V. tenella* found, measuring 2 mm in length. The primary root formed during germination (the arched part on the *left hand side*) has initiated three root primordia (on the *right hand side*). A shoot bud has not formed at this stage. (**c**) The first shoot primordium (s)

obstacles in the soil (Figdor 1897). Similar observations were made in *Voyria truncata* (Imhof and Weber 1997) and *Triuris hyalina* (Imhof 1998). The roots are several centimeters long and irregularly thickened, with the thicker parts three (Figdor 1897) to four times (Janse 1896) wider than the thinner ones. Tangential cell divisions in the outer cortex increase the number of parenchyma cell layers in those parts where fungal hyphae are found within the inner cortex cells. Uncolonized root segments, thus, have only four parenchyma layers whereas the mycorrhizal parts can have up to eight (Janse 1896). Cells in the course of division do not become colonized, neither does the epidermis, exodermis nor the first cortical parenchyma layer. The hyphae within the cells are coiled (Figdor 1897) and show local swellings. They form "sporangioles," a term used by Janse (1896) for degenerating hyphae. Figdor (1897) also speaks of clumped masses of dead hyphae. A prolonged primary thickening of the root as described by Janse (1896) has also been seen in *Voyria truncata*, although there the cell divisions happen throughout the cortex, irrespective of being colonized or not (Imhof and Weber 1997).

No information is given as to whether the subterranean part of the stem is colonized by the fungus. For comparison, more recent studies in *Voyria* species did not find hyphae in shoot tissues (Imhof and Weber 1997, 2000; Imhof 1997, 1999c), although Svedelius (1902) reports hyphae in aerial stems of *V. tenella* (treated as *Leiphaimos azurea*).

4.6.3.2 *Exochaenium* (Fig. 4.18i–k)

Exochaenium oliganthum (previously *Sebaea oligantha* Kissling et al. 2009; Kissling 2012) from Central Africa is the only species in this genus that is achlorophyllous (Raynal 1967a, Kissling et al. 2009). However, representatives with little photosynthetic surface such as *E. debilis*, *E. rara* and *E. pulsilla* (Marais and Verdoorn 1963) suggest that partial mycoheterotrophy may also be present in other species of the genus. As is often the case, the subterranean organs are neglected in taxonomic accounts (Gilg 1899; Robyns 1962). Raynal (1967a), recognizing this lack, described the roots as radiating from the stem base, being few (her drawing shows five at the shoot base), sparsely branched, terete, carnose, and up to 0.5 mm thick (Fig. 4.18i, j). This description is very similar to that for achlorophyllous *Exacum* species discussed above. More exciting is Raynal's (1967a) finding of additional, almost subterranean cleistogamous flowers covered by the leaf litter of the soil surface. In contrast to the straight epiterrestrial shoots, the stems and pedicels of these "subterranean" flowers are mostly positively geotropic, coiled, and intermingled with each other, but basically develop the same flowers and fruits as the aerial counterparts, except for some reductions in floral structures. The fruits, due to positive geotropism, are geocarpic (Raynal 1967a).

Information on the roots additional to that mentioned above is very scarce. Professor Mangenot did investigate the mycorrhiza of

Fig. 4.20 (continued) appears only after the development of a characteristically radiating root system (this specimen is 4 mm wide in its maximal extension). (**d**) Young specimen of *V. tenella*, the shoot is 1 mm thick. (**e**) Flower of *V. tenella*. (**f**) Longitudinal section through a young root of *V. tenella* showing the penetration and the subsequent direct growth of the straight hypha (sh) towards the inner root cortex where it proceeds along the central cylinder. (**g**) Longitudinal section through a mature root of *V. tenella* showing the straight hyphae (sh) in the inner cortex parenchyma around the central cylinder (cc) and the degenerated coils of hyphae (hc−) in the outer cortex. (**h**) Transverse section through a root of *V. tenella* (0.8 mm in diameter). The straight hyphae (sh) in the inner cortex parenchyma are visible as small circles. The epidermal tissue (et) consist of 2–3 layers of smaller cells never colonized by the fungus except for penetration points. Their outermost cells slough off (so) and are replaced by derivates of the layers underneath. The obvious digestion of hyphal coils (hc−) takes place in the majority of the cortex parenchyma. (**i**) Schematic view of the mycorrhizal colonization pattern in *V. tenella*. After penetration, the hyphae grows straighly towards the inner cortex layers which are longitudinally elongated and proceed therein along the central cylinder. Branches of these inner hyphae grow back into the outer cortex and degenerate (dh)

Exochaenium oliganthum, but never published it. Raynal (1967a) reports his findings communicated to her, which revealed fungal coils within the cells very similar to the conditions in *Neottia nidus-avis* (Orchidaceae). However, we know today that orchid mycorrhizas (e.g., Smith and Read 2008) and the AMs in gentians (e.g., Imhof 1999c) are only superficially alike. Recently, molecular methods have detected a *Glomus*-group A endophyte in *Exochaenium oliganthum*, which seems to be highly specific for this species (Franke et al. 2006).

4.6.3.3 *Voyriella*

There are roughly 30 publications mentioning the monotypic *Voyriella*, many of them only as part of an enumeration of gentianaceous genera. The approximately dozen of taxonomic or geographic accounts mostly lack information on subterranean organs. In fact, there are only five statements on the roots of *Voyriella parviflora*: "radice fibrosa" (Miquel 1851), "roots on the transition from filiform to coralloid shape" (Johow 1889, translated from German), "filiform" (Jonker 1936), and "30 mm long and 0.3 mm thick" (Maas and Ruyters 1986). The latter statement is simply restated by Pires O'Brien (1997) in a plant checklist of the Jari river in Brazil. There is no figure showing the roots of *V. parviflora*, but its mycorrhizal fungus has been identified by 18S rDNA sequencing. *V. parviflora* seem to be highly specific to a basal clade of *Glomus*-group A of the Glomeromycota (Bidartondo et al. 2002).

4.6.3.4 *Voyria* (Figs. 4.18–4.20)

There is considerable information available for the roots of several of the 19 *Voyria* species. The earliest observations by Aublet (1775) on *Voyria rosea* indicated that it has irregularly tuberous roots of the size of a fist and become eaten by the indigenous people (Garipons of the Guianas) after being cooked in a coal fire, tasting similar to potatoes. However, although *V. rosea* has roots up to 40 mm long and 15 mm (!) thick, it appears more as a loosely coralloid root system rather than a tuberculous one (Maas and Ruyters 1986). Either the specimen seen by Aublet had densely intermingled roots only appearing like a tuber of that size, or Aublet confused it with another plant. Aublet (1775) called the achlorophyllous genus after the Garipon name of that edible plant, *Voyria*. There is still some confusion on the nature of the subterranean parts of *Voyria* spp. as they are sometimes erroneously called rhizomes (e.g., Süssenguth 1937; Jonker 1936; Fukarek et al. 1994; Pringle 1995). Possibly, since the root-borne shoots (Imhof et al. 1994; Imhof and Weber 1997; Imhof 1997) often have to grow through a considerable layer of soil before they reach the surface (Fig. 4.19b–d, Imhof and Weber 1997, see before under *Exacum*), they might have been misinterpreted as orthotropous rhizomes.

Considerable differences, most probably representing evolutionary steps, occur within this genus. Paralleled by the reduction of floral (Oehler 1927; Maas and Ruyters 1986) and shoot anatomical features (Johow 1885; Solereder 1908; Oehler 1927; ter Welle 1986), the root systems can also be arranged according to reduction particularly in root length. Roots of *Voyria truncata* (a primitive member of the genus), can presumably be several meters long, growing horizontally and runner-like (Fig. 4.19e) as deep as 20 cm beneath the soil surface (Fig. 4.19c, d), and up to 2 mm thick, frequently branched, and give rise to two root-borne shoots in the axils of side roots (Imhof et al. 1994; Imhof and Weber 1997, Fig. 4.19e). Hence, seemingly distinct specimens above soil can well belong to the same individual, either by shoot ramifications already in the soil (Fig. 4.19c), or because of different root sprouts originating from the same root (Fig. 4.19e). Field observations recognized *V. truncata* shoots (Fig. 4.19a–d) emerging like a chain of beads for several meters, denoting the root course in the soil (Imhof et al. 1994). Aublet (1775) also reports on roots of *V. rosea* being a foot deep in the soil.

In contrast, the most advanced representative of the genus, *Voyria tenella*, has a small, star-like root system, shallowly rooted or only loosely connected to the litter substrate (Imhof et al.

1994, Fig. 4.20a–e). Ontogenetic studies revealed a root to appear first after germination (Fig. 4.19b) and only after a small star-like root system form does a first shoot arise (Imhof et al. 1994, Fig. 4.20c). In spite of the ray-like appearance, the roots do not grow evenly in all directions. In fact, there is a single pole of growth representing the primary root which develops side roots at very short distances creating the globose structure (Imhof 1997).

In conclusion, although the two root systems of *V. truncata* and *V. tenella* seem to be very different, the root system of *V. tenella* is easily interpreted as an extremely abbreviated root system of *V. truncata*. This notion is supported by intermediate root systems linking *V. truncata* and *V. tenella*, e.g., *V. aphylla* (Imhof 1999c, Fig. 4.19h–j), *V. rosea* (Maas and Ruyters 1986), *V. chionea* (Progel 1865), and *V. obconica* (Imhof and Weber 2000, Fig. 4.18d, e). The only African representative, *V. primuloides*, which is considered to be sister to *V. chionea* (Albert and Struwe 1997), is the only species in the genus with prominent root hairs (Raynal 1967b).

The root anatomy and mycorrhizal colonization patterns in *Voyria* also support the progression proposed above. *Voyria truncata* has an almost identical root anatomy to a young *Gentiana lutea* (Perrot 1898), including a distinct dimorphic exodermis with short and long cells (Fig. 4.19f, g) and a substantial central cylinder with lignified tracheary elements, but lacking secondary thickening. Instead, a prolonged primary thickening is established (Fig. 4.19f), which constantly enhances the essential tissue for fungal colonization and is interpreted as an important adaptation to its mycoheterotrophy (Imhof and Weber 1997). The type of arbuscular mycorrhiza is also very similar to other gentians (Neumann 1934; Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983), except for the lack of lateral arbuscules formed from the coiled intracellular hyphae, typical for a *Paris*-type AM. The degradation of hyphal coils in the cells, best explained as a digesting process of the plant to absorb carbon and nutrients from the hyphae, happens after about 15 cell passages (Imhof and Weber 1997, Fig. 4.19f).

The roots of *V. aphylla* (Fig. 4.19h, i) have all anatomical elements of *V. truncata* but are reduced in their size and there is a tendency for a radiating formation of side roots at the shoot bases (Fig. 4.19i, j). The mycorrhizal associations are also similar. However, some new features have been acquired: (1) a longitudinal spread of straight hyphae within the epidermis as well as the innermost cortex layers (Fig. 4.19k, l) and (2) the development of root hairs only where roots of neighboring plants are attached (Imhof 1999c, Fig. 4.19m). Whereas the hyphae in roots of *V. truncata* are largely restricted regarding the cortical spread, the nondegenerating hyphae in the epidermis of *V. aphylla* are able to reach more distant segments of the root as well. Direct hyphal bridges from attached roots of neighboring plants are frequent sources of fungal root penetrations in several *Voyria* species. The locally developed root hairs of *V. aphylla* increase this contact zone and, in fact, receive most of the external fungal penetrations (Imhof 1999c; Fig. 4.19k + m). Hyphal passage through the exodermis still is exclusively via the short cells, although those are not as anatomically distinct as they are in *V. truncata* (Imhof and Weber 1997). Digestion of fungal material takes place in the cortex parenchyma except for its innermost layers, where a still imperfect internal spread along the central cylinder can be seen. The latter feature foreshadows the highly efficient colonization pattern of the further derived *Voyria tenella*, *V. obconica* and *V. flavescens* (Fig. 4.20i).

The root anatomy of the most advanced *V. tenella* had been investigated by Johow (1885) and Vigodsky-de Philippis (1938, under the synonym *Leiphaimos brachyloba*). Both stress the reduced character of the vascular system, the lack of suberization of the endodermis in *V. tenella*, and the voluminous root cortices (more details in Imhof 1997, Fig. 4.20a–e). Johow (1885) also recognized the coiled fungal mycelium within the cortex cells, but called it "parasitic" and, in accepting Drude's hypothesis (1873), assigned its

presence to the attraction through a "particularly rich flow of organic nutrients" (translated from German) he assumed to be a compulsory attribute of the roots of this "saprophytic" plant. He was aware of Kamienski's (1882) new notion of a symbiotic association between fungus and plant, yet with cautious criticism. Vigodsky-de Philippis (1938) called the hyphae a "micelio micorrhizico." However, these classical papers did not recognize the specialized colonization pattern. Root penetrating hyphae initially grow straight towards the innermost cortex layers and proceed in longitudinal direction along the reduced central cylinder. From there, hyphal branches grow back into the outer cortex parenchyma where they begin to coil, quickly inflate, and finally collapse into amorphous clumps (see details in Imhof 1997; Imhof and Weber 2000; Franke 2002, Fig. 4.20f–h). By this means, a sustained use of only few external penetrations of the fungus is attained, maintaining the hyphae alive in the inner cortex and only digesting branches of them in the outer cortex: an intraradical fungus garden (Imhof 1997, Fig. 4.19i). In summary, within *Voyria*, we can retrace not only morphological and anatomical reductions but also the evolutionary progression of a mycorrhiza (compare Figs. 4.19g, n and 4.20i), resulting in a highly efficient system to benefit from a fungus.

The mycorrhizal fungi of several *Voyria* species have been determined by molecular identification methods. Almost all of the endophytes belong to *Glomus*-group A of the Glomeromycota. However, *Voyria* spp. seem to not be as specific regarding their mycorrhizal associates as other mycoheterotrophic species (Bidartondo et al. 2002; Merckx et al. 2010).

Since mycoheterotrophic plants are very difficult to cultivate, the unexpected emergence of a *Voyria* species in the Botanical Garden in Hamburg (Germany) shall be briefly reported here. As an epiphytic stowaway on the trunk of a tree fern (*Alsophila salvinii*) imported in the mid seventies, a yellow *Voyria* (perhaps *V. aphylla*, which is known to grow also epiphytically, Groenendijk et al. 1997) was discovered around 1980. Unfortunately, it died in 1983, when its host fern tree was placed outside during summer (Poppendieck 1997).

4.7 Selected Species of Questionable Trophic Status

4.7.1 *Buxbaumia* spp. (Bryophyta, Fig. 4.1k)

The genus *Buxbaumia* is comprised of 12 species in the northern hemisphere (Crosby et al. 2000; Goffinet et al. 2008). In contrast to the majority of mosses, the up to 3 cm high sporophyte is the prominent phase of this genus and consists of a bulky, oblique-oval capsule on top of the seta (Eastwood 1939). This appearance has led to its enchanting vernacular names, e.g., Elfcap Moss, Humpbacked Elves, Bug-on-a-stick (Fig. 4.1k). While most species have greenish sporophytes (Udar et al. 1971; Ligrone et al. 1982; Stone 1983; Düll and Düll-Wunder 2008), the sporophytes of *Buxbaumia aphylla* and *B. minakatae* (Okamura 1911; Iwatsuki and Sharp 1967) seem to be largely devoid of chlorophyll. The gametophyte of *Buxbaumia aphylla* is minute and achlorophyllous (Goebel 1892; Dening 1928; Mueller 1972; Hancock and Brassard 1974). The possibly perennial protonema (Steven and Long 1989), consisting of single-lined threads of thick walled cells (Mueller 1972), which may form velvety mats (McClymont 1950), is green but of questionable trophic relevance (Haberlandt 1886; Eastwood 1939). Early stages of the sporophyte may show some green color (Goebel 1892; Dening 1928; Eastwood 1936; Hancock and Brassard 1974; Schoepe and Philippi 2000; van Rompu and Stieperaere 2002), but many recent as well as older publications consider it to have a heterotrophic mode of life (e.g., Haberlandt 1886; Eastwood 1936; Mueller 1972; Watson and Dallwitz 2005 onwards; Düll and Düll-Wunder 2008). It is clear therefore that the relation of auto- vs. heterotrophy in the whole genus is completely unknown and should be studied.

In contrast to Eastwood (1939), who believed in direct absorption by *Buxbaumia* of organic substances from humus or neighboring green mosses, we know today that plants are unable to do that directly, but are either parasitic (Kuijt 1969; Weber 1993) or mycoheterotrophic when lacking chlorophyll (Leake 1994, 2005). However,

no information is yet available on mycorrhizal, endophytic, or parasitic associations, which could possibly explain the strange habit of these mosses. The ultrastructural investigation on the green *B. piperi* did not find any association with fungi (Ligrone et al. 1982). Neither did the detailed description (Stone 1983) on the foot and vaginula of *B. novae-zelandiae* mention fungal hyphae, but explained the dense indumentum with anastomosing elements as "rhizoidal outgrowth from the epidermal cells of the fertile axis." Udar et al. (1971) also show drawings of longitudinal and transverse sections of the tomentose and slightly tuberous basis of the sporophyte of *B. himalayensis*, which superficially resembles an ectomycorrhiza. Unfortunately, the authors did not comment on this feature at all. However, Haberlandt (1886) has shown drawings of what he interpreted to be rhizoids. These "rhizoids" could well be fungal hyphae, and Haberlandt (1886) even explicitly remarked on their striking similarity to hyphae, particularly because of their frequent anastomoses, which, as far as we know, is not characteristic for moss rhizoids. Similarly, Goebel (1892) reports of anastomosing as well as achlorophyllous protonemata, which even show clamp-like connections (line drawing in Goebel 1892). In any case, contemporary studies providing photographic micrographs instead of drawings on the micromorphology and anatomy of *Buxbaumia aphylla* as well as stable isotope investigations (see Chap. 8) in order to elucidate the trophic mode, are urgently needed.

4.7.2 *Pyrola picta* (*Pyrola aphylla*, Ericaceae)

Pyrola aphylla was first described by James Edward Smith 1814 in Abraham Rees' Cyclopaedia (Vol. 29, No. 7), calling it "leafless." Other authors as well, such as de Candolle (1838) and Hooker (1840), described this species as having no leaves. However, Nuttall (1843, cited by Holm 1898) detected leaves of this species, and Holm (1898) ascertained subterranean shoots connecting apparently leafless specimens with rosettes of green leaves, proving that they belong to the same individual. Also Andres (1914) noted that *P. picta* can have few to no leaves. The same observation was made by Camp (1940), who revisited herbarium sheets and argued for a close relationship, if not identity, of *Pyrola aphylla*, *P. picta* and *P. dentata*. More recently, Haber (1987) eventually merged the three species within the highly variable *Pyrola picta* Sm.

In addition to the subterranean shoots (stolons) already mentioned, *P. picta* also has horizontally growing, runner-like, branching roots. Root-borne shoots as well as adventitious roots from the stolons can develop (Holm 1898). The only specific investigations on the mycorrhiza of *P. picta* is by Largent et al. (1980), calling it arbutoid and ericoid (both seen in specimens of *P. picta* var. *picta*) and ericoid (in *P. picta* var. *aphylla*). Other authors have called the mycorrhizas of *Pyrola* spp. arbutoid (Robertson and Robertson 1985; Massicotte et al. 2008; Vincenot et al. 2008), ectendomycorrhizal (Wang and Qiu 2006), or pyroloid (Cullings 1996). It also has been considered as a linking mycorrhizal type between arbutoid and ericoid mycorrhiza in a new classification of mycorrhizas (Imhof 2009).

Because *P. picta* has green leaves, and the extreme, leafless variant of *P. picta*, *P. aphylla*, still has chlorophyll in the shoot bark (Holm 1898), it actually should not be fully mycoheterotrophic. However, Hynson et al. (2009) found characteristic stable isotope signatures typical for mycoheterotrophic plants in *Pyrola aphylla* specimen. Interestingly, *Pyrola picta* with leaves, although being the same species taxonomically, did not show signs of mycoheterotrophy according to carbon stable isotope signatures (Hynson et al. 2009). Hence, the trophic status of this species is ambiguous. Possibly, dependency on the fungal carbon is not determined by the species in the taxonomical sense but on the actual ability for assimilation in a particular specimen.

4.8 Trends, Conclusions, and Future Directions

MH plants have distinctive structural necessities in contrast to autotrophic species due to their mycorrhizal dependence for carbon supply. Secondary growth of roots, for example, is deleterious since

it sheds the primary tissue, which alone can host the indispensable mycobiont. Moreover, the primary tissue, less important in autotrophic plants, must be present in sufficient quantity. Most importantly, intracellular (in contrast to intercellular) mycorrhizal colonization is a major prerequisite. In fact, there is no mycoheterotroph having an *Arum*-type AM or an ectomycorrhiza, both of which are characterized by predominantly intercellular hyphal growth. Obviously, the transfer of nutrients and carbohydrates provided by those mycorrhizal types is not sufficient to support achlorophylly. There also must be a high probability to become colonized by an appropriate fungus, keeping in mind that MH plants are often quite specific with respect to their endophyte (e.g., Kretzer et al. 2000; Taylor et al. 2002; Bidartondo et al. 2002; Franke et al. 2004; Ogura-Tsujita and Yukawa 2008). A widely branched allorhizic root system seems to be suitable for this, but in turn, is susceptible to functional failure of large parts by only a single blocking, collapsing or disconnection event in a proximal segment. This is particularly critical when secondary growth for securing the connection is impossible. In any case, the transfer of carbon to the reproductive parts must be either short or reliably assured. These challenges are reasons for the following convergent evolutionary trends concerning subterranean organs of MH plants in unrelated plant families:

1. Star-like root systems consisting of many roots radiating from the base of the shoot, either created by root-borne shoots or shoot-borne roots, reduce the risk of becoming disconnected to a major part of the root system (e.g., Figs. 4.2g, 4.4b, 4.6c + g + k, 4.7b, 4.8e + j, 4.10c + f + g + i, 4.12f + g, 4.13a + d, 4.14b, 4.15b, 4.18b + e + g + j, 4.19j, 4.20a).
2. Short and thick roots shorten the transport distance of carbon to the shoot while retaining the tissue volume of long and thin roots (e.g., Figs. 4.3a, 4.4b, 4.7b, 4.9d, 4.10f, 4.12f + g, 4.14b, 4.15b, 4.17e + g, 4.19c).
3. Specialized colonization pattern that enables a sustained use of a few fungal penetrations counterbalance the reduced probability to become colonized in short and thick roots compared to filiform roots (e.g., Figs. 4.3i, 4.4i, 4.5j, 4.8h, 4.9h, 4.13h, 4.14k, 4.16d, 4.19n, 4.20i).
4. Strong reinforcement of thin roots, either by tertiary endodermae (in monocots) or the development of multicellular fibrous tissue, protect the carbon supply of the shoot (e.g., Figs. 4.5d + f, 4.6c + d, 4.13h).

The contradicting needs for a large root surface for high infection probability and short distances for carbon transport, has been discussed as the "mycoheterotroph's dilemma" (Imhof 2010) and supposedly has shaped much of the subterranean organs in MH plants during evolution. As an effect, advanced MH plants within a family have stout, clumped roots and (in orchids) rhizomes mostly with a specialized fungal colonization pattern. This trend is best exemplified in *Voyria* (Gentianaceae, Imhof 1999c) and in Ericaceae (Furman and Trappe 1971). Gentianales and Ericaceae especially, having two fundamentally distinct groups of mycorrhiza (AM group vs. ECM group, Imhof 2009) but both show evolutionary reductions from trees to achlorophyllous herbs (Henderson 1919; Imhof 1999c) including changes in mycorrhizal pattern, turn out to be a textbook example for convergent evolution. In Triuridaceae (Imhof 2003), Burmanniaceae (Imhof 2001), *Thismia* (this chapter) and *Afrothismia* (both Thismiaceae, Imhof 2006), and Orchidaceae (Furman and Trappe 1971), this trend is partly detectable, but further investigations are necessary for more support. Research on taxa-like *Geosiris* (Iridaceae), *Corsia* (Corsiaceae), *Kupea* (Triuridaceae), *Haplothismia* (Thismiaceae) and others for which nothing is known concerning the fungal structures, will also help to understand the evolution of mycoheterotrophy. Moreover, given that 15 investigated vascular MH plants associated with AM fungi (i.e., Monotropoideae and orchids excluded) revealed 13 different mycorrhizal colonization patterns, there is a considerable chance for more fascinating novelties. In orchid mycorrhizas, although belonging to the oldest fields of mycorrhizal research (e.g., Schleiden 1845), comparatively little is known on the two existing types: tolypophagy and ptyophagy (Burgeff 1932; Wang et al. 1997; Rasmussen 2002; Imhof 2009). Since the latter type was found

exclusively in achlorophyllous orchids so far (e.g., Janse 1896; Campbell 1963, 1964; Wang et al. 1997), an examination of the mycorrhizal structures of other MH orchids is highly desired.

In conclusion, mycoheterotrophy is based on a number of specializations with respect to morphology and anatomy of the underground parts, and, most importantly, on the evolution of sophisticated mycorrhizal pattern.

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