Table 6.1. Orders of Chytridiomycota following D. J. S. Barr (2001) and Kirk *et al*. (2001).

Order	Number of described taxa	Examples
Chytridiales (see Section 6.2)	80 genera 600 spp.	*Cladochytrium*, *Nowakowskiella*, *Rhizophydium*, *Synchytrium*
Spizellomycetales (see Section 6.3)	13 genera 86 spp.	*Olpidium*, *Rhizophlyctis*
Neocallimastigales (see Section 6.4)	5 genera 16 spp.	*Anaeromyces*, *Caecomyces*, *Neocallimastix*, *Orpinomyces*, *Piromyces*
Blastocladiales (see Section 6.5)	14 genera 179 spp.	*Allomyces*, *Blastocladiella*, *Coelomomyces*, *Physoderma*
Monoblepharidales (see Section 6.6)	4 genera 19 spp.	*Gonapodya*, *Monoblepharella*, *Monoblepharis*

6.2 Chytridiales

This is by far the largest order, comprising more than 50% of the total number of chytrids described to date. It is difficult to characterize members of the Chytridiales because they lack any specific features by which species have been assigned to the other four orders. The classification of the Chytridiales has traditionally been based on thallus morphology (Sparrow, 1973) but, as pointed out by D. J. S. Barr (2001), this is unsatisfactory because of the great variability in thallus organization shown by the same fungus growing on its natural substratum and in culture. Future systems of classification will be based on zoospore ultrastructure and the comparison of several different types of DNA sequences, but too few examples have yet been studied to provide a definitive framework. Because of this we shall study genera which illustrate the range of morphology, life cycles and ecology of the Chytridiales without attempting to place them into families.

6.2.1 *Synchytrium*

In this genus the thallus is endobiotic and holocarpic, and at reproduction it may become converted directly into a group (sorus) of sporangia, or to a **prosorus** which later gives rise to a sorus of sporangia. Alternatively the thallus may turn into a resting spore which can function either directly as a sporangium and give rise to zoospores, or as a prosorus. The zoospores are of the characteristic chytrid type (Lange & Olson, 1978). Sexual reproduction is by copulation of isogametes, resulting in the formation of thalli which develop into thick-walled resting spores. *Synchytrium* includes about 120 species which are biotrophic parasites of flowering plants. Some species parasitize only a narrow range of hosts, e.g. *S. endobioticum* on Solanaceae, but others, e.g. *S. macrosporum*, have a wide host range (Karling, 1964). Most species are not very destructive to the host plant but stimulate the formation of galls on leaves, stems and fruits.

Synchytrium endobioticum

This is the cause of wart disease affecting cultivated potatoes and some wild species of *Solanum*. It is a biotrophic pathogen which has not yet been successfully cultured outside living host cells. Wart disease is now distributed throughout the main potato-growing regions of the world, especially in mountainous areas and those with a cool, moist climate. Lange (1987) has given practical details of techniques for studying the fungus but in most European countries handling of living material by

unlicensed workers is illegal. Diseased tubers bear dark brown cauliflower-like excrescences. Galls may also be formed on the aerial shoots, and they are then green with convoluted leaf-like masses of tissue (the leafy gall stage; Plates 3a,b). Heavily infected tubers may have a considerable proportion of their tissues converted to warts. The yield of saleable potatoes from a heavily infected crop may be less than the actual weight of the seed potatoes planted. The disease is thus potentially a serious one, but fortunately varieties of potatoes are available which are immune from the disease, so that control is practicable. The possible life cycle of *S. endobioticum* is summarized in Fig. 6.6.

The dark warts on the tubers are galls in which the host cells have been stimulated to divide by the presence of the fungus. Many of the host cells contain resting spores which are more or less spherical cells with thick dark brown walls folded into plate-like extensions (see Fig. 6.7a). The resting spores are released by the decay of the warts and may remain alive in the soil for over 40 years (Laidlaw, 1985). The outer wall (exospore) bursts open by an irregular aperture and the endospore balloons out to form a vesicle within which a single sporangium differentiates (Kole, 1965; Sharma & Cammack, 1976; Hampson *et al*., 1994). Thus the resting spore functions as a **prosporangium** on germination. Germination of the resting spore may occur spontaneously but can be stimulated by passage through snails. It is presumed that abrasion and digestion of the spore wall

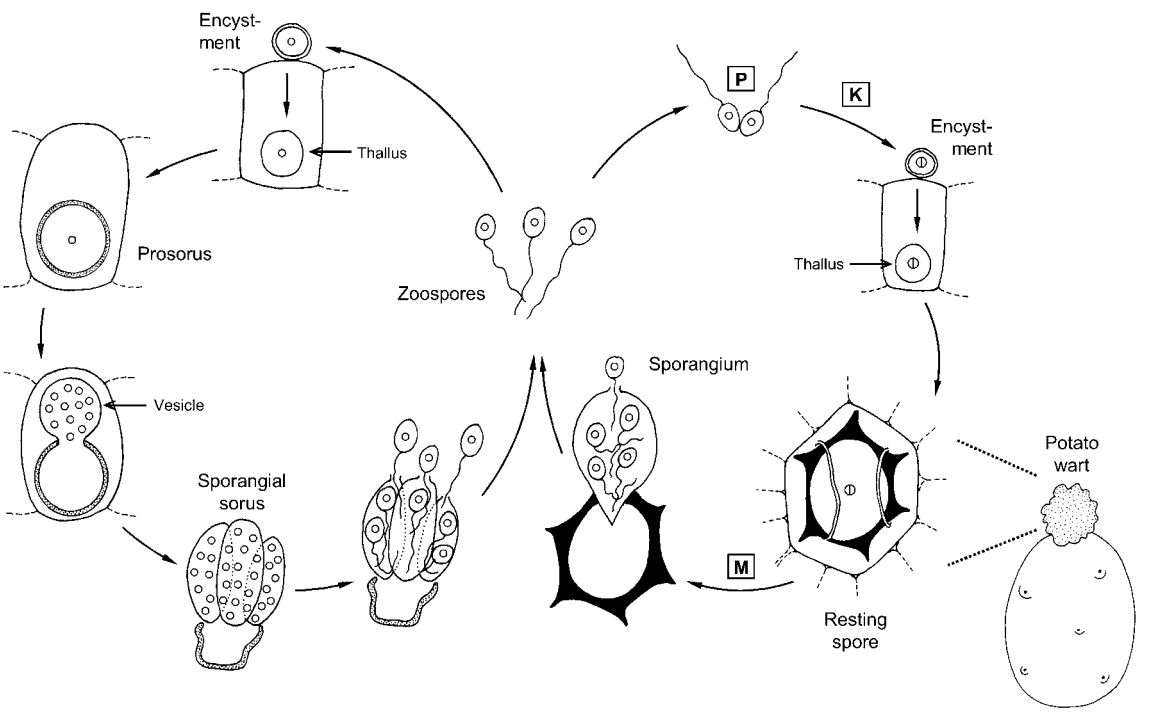

Fig 6.6 Schematic outline of the probable life cycle of *Synchytrium endobioticum*. Haploid and diploid nuclei are represented by small empty and larger split circles, respectively. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M). Resting spores within a warted potato contain a single nucleus which undergoes meiosis upon germination. Haploid zoospores are released from a single sporangium. If two zoospores pair up, a zygote is formed and penetration of a potato cell gives rise to a diploid thallus and, ultimately, a resting spore. Diploid infections cause host hyperplasia visible as the potato wart symptoms. If a zoospore infects in the haploid state, a haploid prosorus (summer spore) is formed, and hypertrophy of the infected and adjacent host cells ensues. A sorus of several sporangia is ultimately produced, with each sporangium releasing a fresh crop of haploid zoospores. *Synchytrium endobioticum* appears to be homothallic.

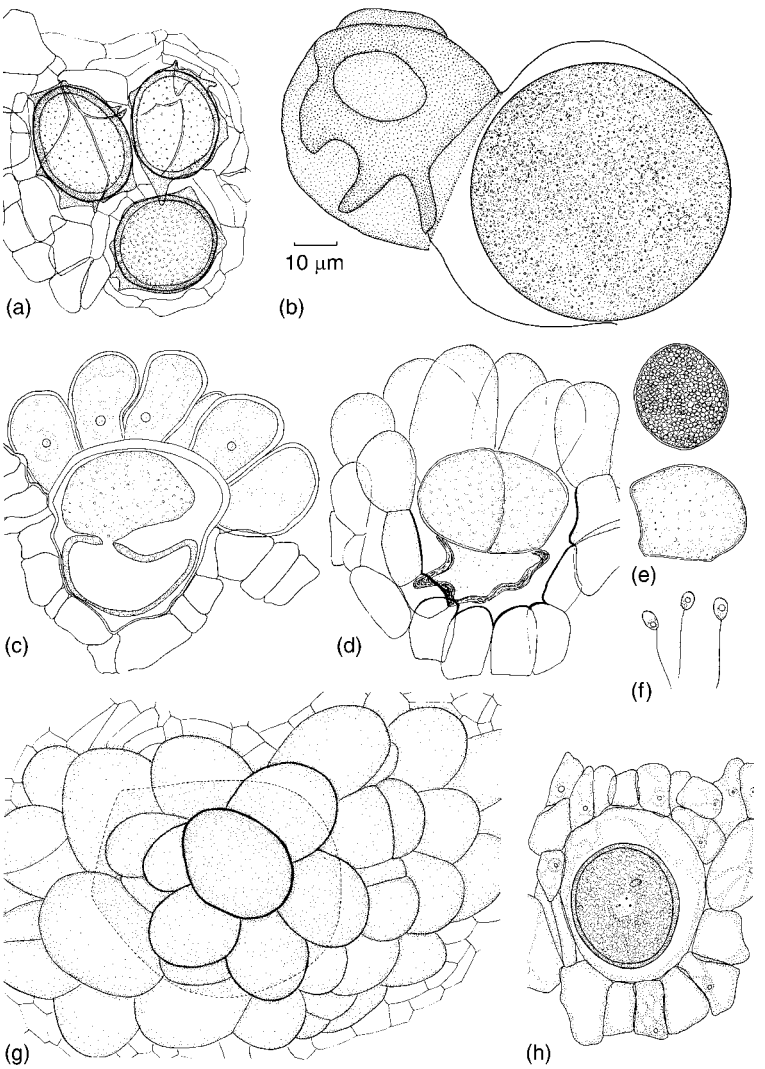

Fig 6.7 *Synchytrium endobioticum*. (a) Resting spores in section of wart. (b) Germinating resting spore showing the formation of a vesicle containing a single globose sporangium (after Kole, 1965). (c) Section of infected host cell containing a prosorus. The prosorus is extruding a vesicle. Note the hypertrophy of the infected cell and adjacent uninfected cells. (d) Cleavage of vesicle contents to form zoosporangia. (e) Two extruded zoosporangia. (f) Zoospores. (g) Rosette of hypertrophied potato cells as seen from the surface. The outline of the infected host cell is shown dotted. (h) Young resting sporangium resulting from infection by a zygote. Note that the infected cell lies beneath the epidermis due to division of the host cells.

in the snail gut causes breakdown of the thick wall which contains chitin and branched-chain wax esters, so overcoming dormancy related to the impermeability of the wall (Hampson *et al*., 1994).

The zoospores are capable of swimming for about two hours in the soil water. If they alight on the surface of a potato 'eye' or some other part of the potato shoot such as a stolon or a young tuber before its epidermis is suberized, they come to rest and withdraw their flagellum. During penetration, the contents of the zoospore cyst are transferred to the host cell whilst the cyst wall remains attached to the outside. When a dormant 'eye' is infected, dormancy may be broken and the tuber may begin to sprout. If the potato variety is susceptible to the disease, the small fungal thallus inside the host cell will enlarge. The infected host cell as well as surrounding cells also enlarge so that a rosette of hypertrophied cells surrounds a central infected cell (Fig. 6.7c). The walls of these cells adjacent to the infected cell are often thickened and assume a dark brown colour. The infected cell remains alive for some time but eventually it dies. The pathogen thallus passes to the bottom of the host cell, enlarges and becomes spherical. A double-layered chitinous wall which

is golden brown in colour is secreted around the thallus, now termed a prosorus or summer spore. Further development of the prosorus involves the protrusion of the inner wall through a pore in the outer wall, and its expansion as a vesicle which enlarges upwards and fills the upper half of the host cell (Fig. 6.7c). The cytoplasmic contents of the prosorus including the single nucleus are transferred to the vesicle. The process is quite rapid and can be completed in about 4 h. During its passage into the vesicle the nucleus may divide, and mitoses continue so that the vesicle contains about 32 nuclei. At this stage the cytoplasmic contents of the vesicle become cleaved into about 4–9 sporangia (Fig. 6.7d), forming a sorus. After the deposition of sporangial walls, further nuclear divisions occur in each sporangium, and finally each nucleus with its surrounding mass of cytoplasm becomes differentiated to form a zoospore. As the sporangia ripen, they absorb water and swell, causing the host cell containing them to burst open. Meanwhile, division of the host cells underlying the rosette has been taking place, and enlargement of these cells pushes the sporangia out onto the surface of the host tissue (Fig. 6.7e). The sporangia swell if water is available and burst open by means of a small slit through which the zoospores escape. There may be as many as 500–600 zoospores in a single large sporangium. The zoospores resemble those derived from resting sporangia and are capable of initiating further asexual cycles of reproduction throughout spring and early summer. Sometimes several zoospores succeed in penetrating a single cell so that it contains several fungal protoplasts.

Alternatively, zoospores may function as gametes, fusing in pairs (or occasionally in groups of three or four) to form zygotes which retain their flagella and swim actively for a time. Since zoospores acting as gametes do not differ in size and shape, copulation can be described as isogamous. However, the gametes may differ physiologically. Curtis (1921) has suggested that fusion may not occur between zoospores derived from a single sporangium, but only between zoospores from separate sporangia. Köhler (1956) has claimed that the zoospores are at first sexually neutral. Later they mature and become capable of copulation. Maturation may occur either outside the sporangia or within, so that in over-ripe sporangia the zoospores are capable of copulation on release. At first the zoospores are 'male', and swim actively. Later the swarmers become quiescent ('female') and probably secrete a substance which attracts 'male' gametes. After swimming by means of its two flagella, the zygote encysts on the surface of the host epidermis and penetration may then follow by a process essentially similar to zoospore penetration. Multiple infections by several zygotes penetrating a single host cell can also occur. Nuclear fusion occurs in the young zygote before penetration.

The results of zygote infections differ from infection by zoospores. The host cell reacts to zoospore infection by undergoing hypertrophy, i.e. increase in cell volume, and adjacent cells also enlarge to form the characteristic rosette which surrounds the resulting prosorus. In contrast, when a zygote infects, the host cell undergoes hyperplasia, i.e. repeated cell division. The pathogen lies towards the bottom of the host cell, adjacent to the host nucleus, and cell division occurs in such a way that the fungal protoplast is located in the innermost daughter cell. As a result of repeated divisions of the host cells, the typical gall-like potato warts are formed and fungal protoplasts may be buried several cell layers deep beneath the epidermis (see Fig. 6.7h). During these divisions of the host tissue the zygote enlarges and becomes surrounded by a two-layered wall, a thick outer layer which eventually becomes dark brown in colour and is thrown into folds or ridges which appear as spines in section, and a thin hyaline inner wall surrounding the granular cytoplasm (Lange & Olson, 1981). The host cell eventually dies and some of its contents are deposited on the outer wall of the resting sporangium, forming the characteristic brown ridges. During its development the resting spore remains uninucleate. Resting spores are released into the soil and are capable of germination within about 2 months. Before germination, the nucleus divides repeatedly to form the nuclei of the zoospores whose further development has

already been described. It has been claimed that the zygote and the young resting spore are diploid, and it has been assumed that meiosis occurs during germination of the resting sporangia prior to the formation of zoospores, so that these zoospores, the prosori and the soral zoospores are also believed to be haploid. These assumptions seem plausible in the light of knowledge of the life history and cytology of other species (e.g. Lingappa, 1958b), and an essentially similar life cycle has been described for *S. lagenariae* and *S. trichosanthidis*, parasitic on Cucurbitaceae, which differ from *S. endobioticum* in that their resting spores function as prosori instead of prosporangia (Raghavendra Rao & Pavgi, 1993).

Control of wart disease

Control is based largely on the breeding of resistant varieties of potato. It was discovered that certain varieties such as Snowdrop were immune from the disease and could be planted on land heavily infected with *Synchytrium* without developing warts. Following this discovery, plant breeders have developed a number of immune varieties such as Maris Piper. However, some potato varieties that are susceptible to the disease are still widely grown, including the popular King Edward. In most countries where wart disease occurs, legislation has been introduced requiring that only approved immune varieties be planted on land where wart disease has been known to occur, and prohibiting the movement and sale of diseased material. Within the British Isles, the growing of immune varieties on infested land has prevented the spread of the disease, and it is now confined to a small number of foci in the West Midlands, northwest England and mid and south Scotland. It has also persisted in Newfoundland. The majority of the outbreaks are found in allotments, gardens and smallholdings.

The reaction of immune varieties to infection varies (Noble & Glynne, 1970). In some cases when 'immune' varieties are exposed to a heavy inoculum load of *S. endobioticum* in the laboratory, they may become slightly infected, but infection is often confined to the superficial tissues which are soon sloughed off. In the field such slight infections would probably pass unnoticed. Occasionally infections of certain potato varieties may result in the formation of resting spores, but without the formation of noticeable galls. Penetration of the parasite seems to occur in all potato varieties, but when a cell of an immune variety is penetrated it may die within a few hours, and since the fungus is a biotrophic parasite, further development is checked. In other cases the parasite may persist in the host cell for up to 2–3 days, apparently showing normal development, but after this time the fungal thallus undergoes disorganization and disappears from the host cell.

Unfortunately, it has been discovered that new physiological races (or pathotypes) of the pathogen have arisen, capable of attacking potato varieties previously thought to be immune. About 20 pathotypes are now known, and the implications are obvious. Unless their spread can be prevented, much of the work of potato plant breeders over the past century will have to be started all over again.

Other methods of control are less satisfactory. Attempts to kill the resting spores of the fungus in the soil have been made, but this is a costly and difficult process, requiring large-scale fungicide applications to the soil. Copper sulphate or ammonium thiocyanate have been applied in the past at amounts of up to 1 ton $acre^{-1}$, and local treatment with mercuric chloride or with formaldehyde and steam has been used to eradicate foci of infection (Hampson, 1988). Control measures based on the use of resistant varieties seem more satisfactory. An interesting method of control developed in Newfoundland is the use of crabshell meal placed above seed tubers at the time of planting. This technique has resulted in significant and sometimes complete control (Hampson & Coombes, 1991) which may be due to selective enhancement of chitinolytic soil micro-organisms degrading the chitinous walls of the resting spores of *S. endobioticum*.

Other species of *Synchytrium*

Not all species of *Synchytrium* show the same kind of life cycle as *S. endobioticum*. *Synchytrium fulgens*, a parasite of *Oenothera*, resembles *S. endobioticum*

in that both summer spores and resting spores are formed (Lingappa, 1958a,b), but in this species the zoospores from resting sporangia can also function as gametes and give rise directly to zygote infections from which further resting spores arise (Lingappa, 1958b). It has been suggested that the same phenomenon may occasionally occur in *S. endobioticum*. In *S. taraxaci* parasitic on *Taraxacum* (Fig. 6.8; Plate 3c), as well as a number of other *Synchytrium* spp., the mature thallus does not function as a prosorus but cleaves directly to form a sorus of sporangia, and the resting spore also gives rise to zoospores directly. In some species, e.g. *S. aecidioides*, resting sporangia are unknown, whilst in others, e.g. *S. mercurialis*, a common parasite on leaves and stems of *Mercurialis perennis* (Fig. 6.9), only resting sporangia are known and summer sporangial sori do not occur. *Mercurialis* plants collected from March to June often show yellowish blisters on leaves and stems. The blisters are galls made up of one or two layers of hypertrophied cells mostly lacking chlorophyll, surrounding the *Synchytrium* thallus during its maturation to form a resting sporangium. In this species the resting sporangium functions as a prosorus during the following spring. The undivided contents are extruded into a spherical sac which becomes cleaved into a sorus containing as many as 120 sporangia from which zoospores arise. The variations in the life histories of the various species of *Synchytrium* form a useful basis for classifying the genus (Karling, 1964).

6.2.2 *Rhizophydium*

Rhizophydium is a large, cosmopolitan genus of about 100 species (Sparrow, 1960) which grow in soil, freshwater and the sea. The thallus is eucarpic, with a globose epibiotic zoosporangium which develops from the zoospore cyst, and endobiotic rhizoids which penetrate the host. Whilst some species are saprotrophic, others are biotrophic pathogens of algae and can cause severe epidemics of freshwater phytoplankton. Saprotrophic forms such as *R. pollinispini* and *R. sphaerocarpon* colonize pollen grains and are easily isolated by sprinkling pollen onto the surface of water overlying soil (Fig. 6.10). Within 3 days, sporangia with exit papillae are

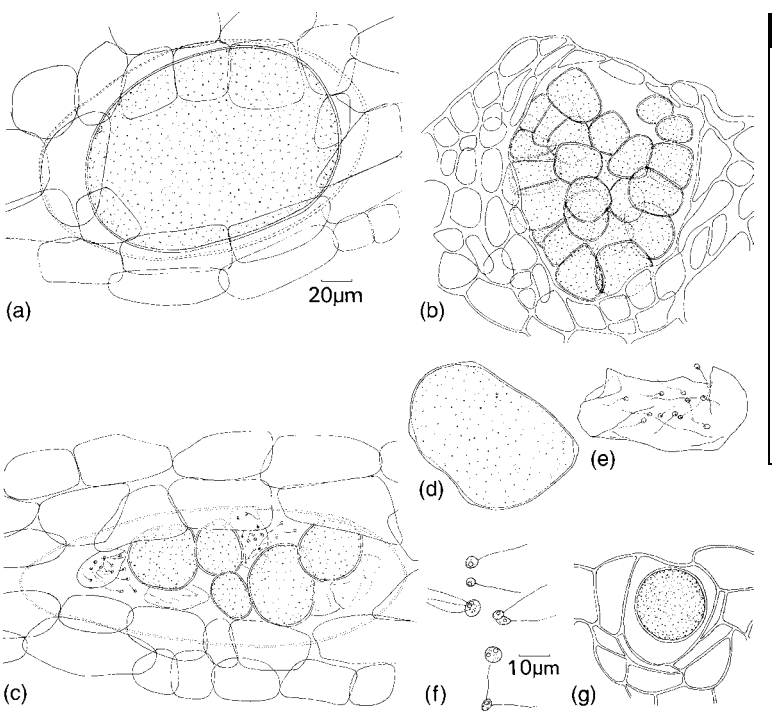

Fig 6.8 *Synchytrium taraxaci*.
(a) Undivided thallus in epidermal cell of scape of *Taraxacum*. Outline of host cell shown dotted. (b) Section of *Taraxacum* scape showing thallus divided into a sorus of sporangia. (c) A sorus of sporangia seen from above. Two sporangia are releasing zoospores. (d) A ripe sporangium. (e) Sporangium releasing zoospores. (f) Zoospores and zygotes. The triflagellate zoospore probably arose by incomplete separation of zoospore initials. (g) Section of host leaf showing a resting sporangium. (a–e) and (g) to same scale.