The Trentepohliales (Ulvophyceae, Chlorophyta): An Unusual Algal Order and its Novel Plant Pathogen, *Cephaleuros*

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Algae as Plant Pathogens

Traditional plant pathogens. Fungi, fungus-like stramenopiles, viruses, bacteria, and nematodes cause most diseases of vascular plants. They are responsible for stem rust of wheat, late blight of potato, cucumber mosaic, fire blight, and crown gall. These diseases are well known to plant pathologists and familiar to many others. Organisms that cause pathological effects such as algal spot, however, are less common, usually do not cause severe damage, and are not as well known. The aim of this article is to introduce readers to the fascinating order Trentepohliales and in particular to *Cephaleuros* Kunze in Fries 1829, a trentepohlialean genus well known as a unique algal plant pathogen.

The algae. The term “algae” includes a broad, diverse assemblage encompassing all organisms that perform oxygenic photosynthesis other than embryophyte land plants (Cavalier-Smith 2007). It is now well established that algae are an unnatural (i.e., nonmonophyletic) group, formed by many evolutionary lineages with separate origins. This heterogeneity is reflected in the huge diversity observed in their size, shape, color, life history, cell wall and chloroplast structure, and secondary metabolites. Nevertheless, there is a tendency to treat algae as a single group because they

occur mostly in aquatic habitats, have a simple body structure called a “thallus,” and in most cases an autotrophic lifestyle (see glossary).

Reading the term “algae,” most people will think about forests of giant kelp (*Macrocystis pyrifera*) that grow along the shores of California and Oregon, or the dense mats of filamentous green algae (e.g., *Cladophora* and *Spirogyra*) found in many streams and lakes. In the course of their evolution, however, several lineages of algae have been able to colonize subaerial (nonaquatic, not usually in or on soil) habitats and survive in conditions of complete desiccation (Lewis and Lewis 2005). Because of their small dimensions and habit, subaerial algae easily can be confused with mosses, lichens, or fungi (Box 1, Fig. 1). Nevertheless, algae are present in most ecosystems and represent an important component of the microbial flora in many nonaquatic sites.

The Order Trentepohliales

Similarities and differences. The order Trentepohliales belongs to the lineage of green algae identified as phylum Chlorophyta. This phylum is sister to the Streptophyta, the lineage that includes some green algae and all land plants (Leliaert et al. 2012). However, placement of this order at class level was a major taxonomic controversy until recently. This uncertainty arose because of a unique combination of morphological and ultrastructural features that make the Trentepohliales one of the most mysterious groups in the plant kingdom. These features include a characteristic type of sporangium (Thompson and Wujek 1997), absence of pyrenoids in the chloroplasts (Van den Hoek et al. 1995), a unique flagellar apparatus (Chapman and Henk 1985), cytokinesis by production of a phragmoplast similar to that of the vascular plants (Chapman and Henk 1986), and plasmodesmata

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in the cell walls (Chapman and Good 1978). It was only after an analysis of molecular data that placement of the Trentepohliales in the class Ulvophyceae was largely accepted (López-Bautista and Chapman 2003). However, the presence of a phragmoplast-mediated cell division in this group remains a mystery because this mode of cell division is otherwise found only in the Streptophyta.

At first approach, this placement is surprising because the Ulvophyceae are green seaweeds and therefore a strictly marine group: some examples are the sea lettuce *Ulva* and the Atlantic green sponge fingers *Codium fragile*. Even the color is strikingly different. Instead of green, the Trentepohliales often appear orange, yellow, or red because of the accumulation of carotenoid pigments in their cells (López-Bautista et al. 2002). The amount of carotenoids produced depends on environmental conditions and is highest when these algae are exposed to strong light and subjected to a shortage of nutrients (Abe et al. 1999; Hametner et al. 2014a; Ho et al. 1983). Conversely, in shaded habitats the green color of their chloroplasts may not be obscured by carotenoids like β -carotene or astaxanthin (Thompson and Wujek 1997), and some of the genera look nearly identical to filamentous green seaweeds, such as species of *Cladophora*.

Subaerial algae. The Trentepohliales is one of the most widespread and distinctive groups of subaerial algae. Their center of diversity and abundance is in tropical and subtropical regions, but they

can be found in cooler regions where the climate, or local microclimate, is sufficiently humid (Suto and Ohtani 2009). From an ecological point of view the Trentepohliales occupy a wide range of substrata: rocks, tree bark, leaves, stems, fruits, decaying wood, and occasionally bare soil. They also grow on and damage artificial surfaces such as concrete walls, tombstones, marble statues, and plastic (Fig. 2). In certain humid climates, such as western Ireland and Singapore, species of *Trentepohlia* Martius 1817 produce unsightly red streaks on the walls of many houses, requiring periodic cleaning or repainting (Ong et al. 1992; Rindi and Guiry 2002). One of the most peculiar characteristics is that this order includes two genera, *Cephaleuros* and *Stomatochroon* Palm 1934, which live respectively as pathogens and space parasites of vascular plants (Chapman and Good 1983).

General morphology. Morphologically, the Trentepohliales consist of uniseriate filaments (i.e., filaments formed by a single row of cells), with a variable branching pattern. The way these filaments are arranged is the main character used for distinction at genus level. In some genera the filaments remain separated and form small bushes or prostrate systems with an open branching pattern. In other genera they are united to produce compact disks or pseudoparenchymatous masses (Guiry and Guiry 2014; Printz 1939; Thompson and Wujek 1997). A morphological feature diagnostic of this order is the sporangiate-lateral (Fig. 3), consisting of a zoosporangium and

Glossary

Biflagellate swarmer—a cell bearing two flagella and capable of active movement even in small volumes of water. In the Trentepohliales, biflagellate swarmers are putative gametes released from a gametangium that do not fuse to form a zygote, but germinate directly and form a new thallus.

Filament—a single strand of cells that forms an algal thallus and its structures; similar to a fungal hypha.

Gametangium—a structure in which gametes are produced.

Gametophyte—one of the two generations of the life cycle typical of most algae and plants. It consists of a haploid thallus that produces gametes and originates from a meiozoospore released from a meiosporangium.

Meiosporangiate-lateral—a branch formed by the suffultory cell and attached meiosporangium; produced on the diploid sporophyte.

Meiosporangium—a sporangium formed on the sporophyte plant in which meiosis occurs.

Meiozoospore (meiospore)—a haploid spore with four flagella produced in a meiosporangium following meiosis.

Mycobiont—the fungal component of a lichen.

Phragmoplast—a structure produced during cell division, consisting of microtubules and small vesicles that fuse to form a cell plate; site of primary cell wall growth typical of plants and some groups of green algae.

Phycobiont—the algal component of a lichen.

Plasmodesmata—microscopic channels that transverse cell walls and link the symplasts (cytoplasm) of adjacent cells.

Pseudoparenchyma—tissue-like structure formed by laterally compacted, short-celled filaments resembling the parenchyma of higher plants.

Pyrenoid—a small body localized in the chloroplasts of many algae, in which intense fixation and concentration of carbon dioxide and accumulation of starch takes place.

Ramulus (pl. ramuli)—a lobe or branch of a thallus formed by laterally joined filaments

Rhizoid—a small appendage formed by one of more cells with the primary function of attachment to a substrate. In *Cephaleuros* it is a reduced, irregular cell growth from the underside of a thallus that runs beneath the host cuticle or between host cells and absorbs water and nutrients.

Space parasite—an endophyte; an alga that may only use its host plant for shelter.

Sporangiate-lateral—a short terminal or lateral branch consisting of a suffultory cell and a zoosporangium.

Sporangium—a structure in which spores are produced.

Sporophyte—one of the two generations of the life cycle typical of most algae and plants. It consists of a diploid thallus that develops from a zygote and produces spores (usually meiozoospores) in meiosporangia.

Subaerial—growing above, or occasionally on, the soil; not subaqueous.

Suffultory cell—a crooked cell to which a sporangium is attached.

Synaptonemal complex—a ladder-like protein complex that binds each homologous pair of chromosomes together and facilitates crossover during meiosis (prophase).

Thallus—the undifferentiated vegetative body of an alga, fungus, or lichen.

Trentepohlialean (adj.)—belonging to or relating to the order Trentepohliales.

Ulvophycean (adj.)—belonging to or relating to the class Ulvophyceae.

a highly modified branch, the “suffultory” cell, which supports it (Thompson and Wujek 1997).

Life history. Evidence suggests that the normal life history of three genera in the Trentepohliales, *Trentepohlia*, *Phycopeltis* Millardet 1870, and *Printzina* Thompson and Wujek 1992, is an isomorphic alternation of two generations: a diploid sporophyte and a haploid gametophyte. The other two genera, *Cephaleuros* and *Stomatochroon*, have a heteromorphic alternation of generations. However, it is well documented that the gametophyte thallus can produce both gametangia and zoosporangia, in which case the zoospores reproduce the same generation. There are some reports that biflagellate “swarmers,” or presumptive gametes, may germinate and reproduce new thalli without mating, therefore behaving as zoospores instead of gametes (Rindi and Guiry 2002; Suto and Ohtani 2013).

Taxonomy

Genera and species. Some species of Trentepohliales are among the earliest described organisms in the history of systematics. Linnaeus described *Byssus iolithus* (currently *Trentepohlia iolithus*) in 1753 and *B. aureus* (currently *T. aurea*) in 1759. This order has therefore been studied for a long time and its classification has undergone numerous rearrangements. In the current scheme (Guiry and Guiry 2014), it includes the single family Trentepohliaceae and five genera: *Cephaleuros* (17 species), *Phycopeltis* (25 species), *Printzina* (8 species), *Stomatochroon* (4 species), and *Trentepohlia* (48 species). The taxonomic validity of a sixth genus, *Physolinum* Printz 1920, is controversial and in recent literature this genus has not been separated from *Trentepohlia*.

Here we briefly present the main characteristics of each genus except *Cephaleuros*, which is discussed in detail in a following section.

- i. *Trentepohlia*. This genus consists of erect branched filaments, variously developed and arranged, arising from a prostrate system. Sometimes these filaments are compacted to form a crust-like structure. *Trentepohlia* occurs on rocks, tree bark, leaves, soil, and artificial substrata, mainly in exposed habitats (Fig. 2A and B). Bright red patches formed by *Trentepohlia flava* are a common sight on rocks facing the sea in many coastal areas of northern California and Oregon (Fig. 2C). This genus of the Trentepohliales has the widest geographical distribution and though most abundant in the tropics, species of *Trentepohlia* extend into temperate areas (Hametner et al. 2014a) and regions with cold climates such as Alaska, Norway, and Tierra del Fuego.
- ii. *Phycopeltis*. The thallus of *Phycopeltis* is formed by numerous prostrate, branched filaments that are usually joined to form a closed disk. In some species the thallus has a regular circular outline; in others it is irregular and formed by several lobes, called “ramuli.” Initially the alga forms a single layer of cells, and then

becomes multilayered in some parts of the thallus, especially where gametangia or zoosporangia are produced. *Phycopeltis* is largely an epiphyllous alga, occurring above the cuticle on the upper leaf surface of numerous vascular plants (Fig. 2D). Its thalli are occasionally found on other parts of plants—stems, branches, twigs, and fruits—and on inert surfaces. Partially shaded sites with constantly moist conditions, especially at the margins of tropical and temperate rainforests, are the most common habitat of *Phycopeltis*.

- iii. *Printzina*. This genus described by Thompson and Wujek in 1992 includes nine taxa previously belonging to *Trentepohlia*. The reclassification was based mainly on differences in shape of zoosporangia, arrangement of the sporangiate-laterals, and development of the prostrate parts of the thallus. *Printzina* is usually associated with shaded habitats, but species such as *P. bosseae* and *P. lagenifera* (Fig. 2E) are often found in exposed areas.
- iv. *Stomatochroon*. Hard to see because it is mostly in the leaf of its host, *Stomatochroon* is the least known and most rarely recorded genus of Trentepohliales. It is an endophytic alga found in the substomatal chambers of vascular plants. For this reason it has been considered a space parasite and may not produce symptoms on its host (Liu and Hu 2013; Zhu et al. 2014). However,

Fig. 1. Algal spot on avocado (*Persea americana*) caused by *Cephaleuros virescens*. Commonly called “red rust,” identifying *Cephaleuros* species has confused observers for decades. Even microscopic examination of the lesions reveals hyphae-like filaments, setae, sporangiophores, and swimming spores similar to fungi and the oomycetes.

Box 1. Algal parasites misidentified.

Species of the plant-parasitic alga *Cephaleuros* are often misidentified. At a glance, the fuzzy, reddish orange growths look like a rust disease (Fig. 1). As a result, leaf and stem infections on tea plantations over a century ago were misleadingly named “red rust” (Mann and Hutchinson 1907). In 1965, a fossilized organism from about 40 million years ago was reported as a fungus, *Pelicothallus villosus*, but later recognized as a species of *Cephaleuros* (Reynolds and Dunn 1984). These algae have been mistaken for coffee rust, powdery mildew, various leaf spots, and bacterial canker (Thompson and Wujek 1997; Wellman 1965).

A closer look at *Cephaleuros* species through a microscope reveals fruiting structures that resemble an unusual downy mildew or other sporangium-forming organism. In 1982, *Veralucia brasiliensis* was described as a fungus using mycological terms, but was later recognized as *Cephaleuros parasiticus* by the same authors (Reynolds and Dunn 1982). Embarrassingly, five mycologist colleagues and two taxonomists reviewed the earlier paper before publication and did not recognize the authors’ error. In the southeastern United States, the causal agent of a common leaf spot of *Magnolia grandiflora* previously published as *Glomerella cingulata* in 1947 was identified as *C. virescens* in 1984 (Holcomb and Henk 1984).

Hennen and Figueiredo (1981) tell the story of *Dasyscypha gregaria*, a tropical rust first collected in Surinam in 1827. Teliospores and spermatogonia were reported on the original material, as was the presence of a powdery mildew, *Oidium parasiticum*. Various collectors have since mistakenly described this powdery mildew as the aecia or uredinia of the *Dasyscypha* rust. Hennen and Figueiredo collected fresh material from Brazil in 1979 and had these asexual structures examined. They were not aecia, uredinia, or even powdery mildew, but a new species of the trentepohlialean alga *Stomatochroon*, which lives in the substomatal chambers of its host.

Cephaleuros species are especially difficult to recognize if they are lichenized by one of several fungi in the Ascomycota (Mann and Hutchinson 1907). Their appearance is typical of lichens but they are not usually associated with plant damage. Evidence suggests, however, that the algae cause damage to the plant before they are lichenized, not after (Brooks 2004; Schubert 1981). The presence of fruiting bodies and spores of the fungal component of the lichen may further hinder recognition of the algae.

severe infections have been associated with water soaking, chlorosis, and necrosis, possibly due to the inhibition of gas exchange from blocked stomata (Brooks 2004; Chapman and Good 1983; Thompson and Wujek 1997; Timpano and Pearlmutter 1983). The thallus of *Stomatochroom* is either a single large cell anchored in a substomatal chamber or a system of branched filaments spreading through the spongy mesophyll. In both cases, only gametangia or single-celled sporangiophores with sporangiate-laterals emerge from the stomata. This genus is probably widespread in tropical regions, but its exact distribution is not easy to assess because it is so difficult to detect.

Phylogeny

A puzzling order. The phylogeny of the Trentepohliales has long been one of the least understood aspects of this group. How were the Trentepohliales related to other groups of the previously mentioned green algae and what were the relationships at genus and species level within this order? The unusual combination of features listed in an earlier section as typical of the Trentepohliales suggested possible affinities with four different classes of green algae. These classes were the Charophyceae, Ulvophyceae, Chlorophyceae, and Trebouxiophyceae, with the first two classes generally considered as the main candidates (López-Bautista et al. 2002). As for many other algal groups, substantial advancements in our understanding became possible only with the availability of molecular data.

An answer. The first molecular data produced for the Trentepohliales were partial SSU rDNA sequences published by Zechman et al. (1990) for two cultured strains, *Trentepohlia* sp. UTEX 1227 and *Cephaleuros virescens* BR-50. López-Bautista and Chapman (2003) presented more complete SSU sequences for eight members of this order: strains of *Cephaleuros*, *Trentepohlia*, *Phycopeltis*, and *Physolinum*. The results of this study were the first solid piece of molecular evidence in support of the ulvophycean nature of the order. The analyses of

López-Bautista and Chapman (2003) placed the Trentepohliales with moderate to high support in the Ulvophyceae, in an intermediate position between the basal orders Ulotrichales and Ulvales and the derived orders Cladophorales, Bryopsidales, and the Dasycladales. Cocquyt et al. (2010) confirmed this placement in a more recent study based on limited taxon sampling, but on a higher number of molecular markers. Their study included sequences SSU, *rbcL*, and OEE1 mRNA of *T. aurea* in a concatenated dataset of seven markers, recovering this species with moderate support as a sister taxon to the clade formed by Cladophorales, Bryopsidales, and Dasycladales.

Genera and species. Phylogenetic relationships at genus and species level in the Trentepohliales have been examined in several studies based on SSU and *rbcL* sequence data (López-Bautista et al. 2006; Nelsen et al. 2011; Rindi et al. 2009; Zhu et al. 2014). Unfortunately the assessment is incomplete at present because of limitations in taxon sampling. High-quality sequences are usually obtained from axenic cultures and most trentepohlialean algae grow slowly and are difficult to isolate without contaminants. Consequently for some genera, *Phycopeltis* in particular, the number of sequences available is still limited. Even for *Trentepohlia* and *Printzina*, the genera from which the best datasets are available, the number of sequences reliably identified at species level is a minority. Nevertheless, data acquired in recent years have greatly advanced our knowledge of the phylogeny of this group, revealing a scenario in contrast with the traditional morphological classification (López-Bautista et al. 2006; Nelsen et al. 2011; Rindi et al. 2009; Zhu et al. 2014).

Five lineages. Studies based on the largest taxon sampling (Nelsen et al. 2011; Rindi et al. 2009) defined five main lineages as well-supported clades, but none correspond to a trentepohlialean genus as defined on morphological grounds (Fig. 4). A large group, clade 1 (Nelsen et al. 2011; Rindi et al. 2009), appears to be the most basal lineage and is sister to all other Trentepohliales. This lineage is formed by species of *Trentepohlia* and *Printzina* with different morphologies and widespread geographical distributions (*Printzina bosseae*, *T. flava*, *T. iolithus*, *T. umbrina*), as well as a number of

Fig. 2. Genera of the Trentepohliales are widely distributed and grow on living and nonliving substrates. **A**, Sinnamary, French Guiana: Species of *Trentepohlia* stain numerous porous surfaces reddish orange. **B**, American Samoa: *Trentepohlia* even lives on nonporous plastics. **C**, Devil's Punchbowl, Oregon: *Trentepohlia flava* growing on the inner rim of a rocky bowl carved by the sea. **D**, Moycullen, Ireland: *Phycopeltis* is a common epiphyte, often with beautifully symmetrical thalli. **E**, Heavy yellowish epiphytic growth of *Printzina lagenifera*. **F**, American Samoa: *Cephaleuros virescens* is one of several species that cause plant damage, like this algal spot of guava (*Psidium guajava*) fruit.

unidentified strains from lichens sequenced by Nelsen et al. (2011). Among the remaining taxa, a small, highly supported lineage, clade 4 (Nelsen et al. 2011), is formed by unidentified algae isolated from tropical lichens in the Old and New Worlds.

Sister to this lineage is a large, well-supported clade in which all other Trentepohliales are grouped. Several lineages are recovered within this clade. A large group, clade 2 (Nelsen et al. 2011; Rindi et al. 2009), contains many strains of *Trentepohlia* and *Printzina* from both tropical and temperate regions clustered with numerous lichen phycobionts (i.e., the algal component of a lichen). In this clade are two of the most common trentepohlialean species in tropical regions: *T. arborum* and *Printzina lagenifera*, the type species of *Printzina*. Notably, however, neither of these species represents a monophyletic entity.

Three taxa represent the most derived lineages: *T. aurea* (the type species of *Trentepohlia*), *Cephaleuros*, and *Stomatochroom*. In the studies of Rindi et al. (2009) and Nelsen et al. (2011), strains of *T. aurea* formed a well-supported clade closely related to the *Cephaleuros* lineage and the *T. arborum*–*P. lagenifera* lineages, but its exact placement remained unsupported. Strains of *Cephaleuros* formed a monophyletic group, although with limited support and with a species of *Trentepohlia*, *T. dialepta*, included in it (Nelsen et al. 2011; Rindi et al. 2009). Recently, Zhu et al. (2014) provided the first sequence data for *Stomatochroom*, recovering it as a sister

taxon to *Cephaleuros*. This is in agreement with the idea that the endophytic habits of *Cephaleuros* and *Stomatochroom* are derived characters. At present, the phylogenetic position of *Phycopeltis* is still waiting for a detailed assessment, as the relatively few SSU sequences available for this genus have only recently been released.

Lichenized Trentepohliales

Symbiosis. Trentepohlialean algae often exist in symbiosis with lichen-forming fungi and are one of the most common algal components of tropical and subtropical lichens (Brodo et al. 2001). Lichens are often grouped informally with the cryptogams, plants that reproduce by spores rather than seeds, but this is only partially true. Lichens are not plants; they are composite organisms consisting of a fungus, the *mycobiont*, and at least one alga, the *phycobiont* (Brodo et al. 2001; Chapman and Waters 2002; Grube et al. 2009). Lichens commonly reproduce when fragments of fungal and algal tissue are dispersed and grow to form a new lichen thallus (Brodo et al. 2001).

The mycobiont. Lichens are classified according to the mycobiont, most of which are in the Ascomycota, or “sac” fungi, with only a few known Basidiomycota, or “club” fungi (Brodo et al. 2001). The fungal component of trentepohlialean lichens is grouped in four distinct classes of the Ascomycota: Arthoniomycetes, Dothideomycetes, Eurotiomycetes,

Fig. 3. The sporangiate-lateral is unique to the Trentepohliales. It consists of a zoosporangium (z) borne at the end of a crooked “suffultory” cell (sc). When moisture is present the cell straightens, holding the sporangium away from the sporangiophore (sp) and head cell (hc) and promoting windborne dispersal. **A**, *Trentepohlia* sp.; **B**, *Printzina* sp.; **C**, *Cephaleuros minimus*; **D**, *C. virescens*.

and Lecanoromycetes (Nelsen et al. 2011; Schoch et al. 2009). These fungi are not monophyletic or represented by a single ancestor (DePriest 2004; Gargas et al. 1995). Most can reproduce sexually by ascospores and asexually by conidia. To be successful, however, the spores must come into contact with and colonize a compatible phycobiont and form a new lichen thallus.

The phycobiont. Ahmadjian (1993) estimated that worldwide 31% of lichen-forming fungi associate with trentepohlialean algae, though Nelsen et al. (2011) suggest this number is closer to 23%. These estimates, however, do not reflect the geographical distribution of trentepohlialean–fungal associations. Research indicates that the Trentepohliales are more prevalent in tropical regions. Therefore a relatively greater number of tropical lichens have trentepohlialean phycobionts, about 45% (Grube et al. 2009; Nelsen et al. 2011; Rindi et al. 2009), and they are especially frequent among lichens that grow on leaves (Ahmadjian 1993; Brodo et al. 2001; Nelsen et al. 2011). Unlike their mycobiont partners, once trentepohlialean algae are colonized by fungi they can no longer form reproductive structures (Hametner et al. 2014a). Recently Hametner et al. (2014a, 2014b) studied the identity of several lichen phycobionts using molecular data. Their results showed a considerable taxonomic diversity, indicating that *Trentepohlia abienta*, *T. aurea*, *T. arborum*, and *P. lagenifera* were the morphospecies to which these phycobionts were most closely related.

Cephaleuros: A victim? Lichens with *Cephaleuros* as the phycobiont are interesting because the algae are at the very least spatial parasites of their host. Most *Cephaleuros* species are obligate parasites that grow subcuticularly or intercellularly and cause damage to their host plants (Chapman 1976; Green 2012). The symbiotic relationship between the phycobiont and the mycobiont is often unclear, but it displays varying degrees of parasitism (Brooks 2004; Chapman 1976; Mann and Hutchinson 1907; Schubert 1981; Suto and Ohtani 2009, 2011a). For example, *Strigula smaragdula* (Lücking 2008) is an obligate leaf-inhabiting lichen (Fig. 5) and its mycobiont aggressively acquires several trentepohlialean phycobionts (Lücking 1999). The fungus first entangles itself among the algal filaments and assumes the same basic form as the phycobiont. It then uses its haustoria to extract water and nutrients from the algal cells. This process differs from plant-parasitic fungi, however, whose haustoria penetrate plant cell walls. An ultrastructural study of *S. complanata* (Matthews et al. 1989) showed that the cell walls of the phycobiont are able to expand and invaginate as haustoria of the mycobiont invade. Eventually, however, the algal cell wall appears to disintegrate and the cell dies (Matthews et al. 1989). In the *Strigula* species studied, therefore, the mycobiont can be described as a hyperparasite (Chapman 1976; Chapman and Good 1983; Thompson and Wujek 1997).

Ecosystem change and survival. Lichens undoubtedly play a role in establishing various trentepohlialean genera within natural ecosystems (Green 2012). Relatively little is known, however, about algae of the tropical regions. According to Rindi et al. (2006), “This is particularly worrying both because these are among the most endangered ecosystems on the planet and because recent investigations have shown that many new taxa, including some major evolutionary surprises, can be expected to be discovered in these environments.” The microbial communities of tropical and subtropical forests represent a potentially rich biological resource for our global community and a greater knowledge of their diversity is urgently needed (Rindi et al. 2006).

Recently, climate change has been predicted to significantly affect the frequency and severity of disturbances that shape the world’s forests and other natural ecosystems (Bentz et al. 2010). Average mean atmospheric temperature is expected to increase between 1.8 and 4°C by the end of the 21st century (Bentz et al. 2010). It has recently been documented that global warming has influenced the migration of trentepohlialean algae and the tropical lichens that associate with them. A study conducted by Aptroot and van Herk (2007) noted that a majority of the epiphytic lichens rapidly colonizing forests in Western Europe are associated with the Trentepohliales.

Temperature and disease distribution. Changes in the earth’s climate can profoundly affect ecological niches at all levels. According to Erickson et al. (2012), “The widening of the tropical belt has

many implications for ecological systems, including the potential expansion of tropical and subtropical diseases, their infectious agents, and vectors that are affected by climatic conditions.” In the southern United States, the Asian tiger mosquito (*Aedes albopictus*) has steadily increased its northern range due to warming temperatures. With this vector comes the threat of various bloodborne diseases such as dengue fever, yellow fever, and chikungunya fever (Erickson et al. 2012). This same scenario emerges with plant diseases. Sudden oak death, caused by an oomycete, has rapidly become entrenched in California’s temperate forests and is predicted to increase in severity as spring months continue to become warmer (Pautasso et al. 2012). The incidence and severity of damage associated with *Cephaleuros* are likely to be influenced by changing climate patterns, especially in regions experiencing increased precipitation and warmer temperatures.

Cephaleuros Species

History of the genus. Kunze created the genus *Cephaleuros* for specimens of *C. virescens* from Surinam. However, taxonomic confusion about the type species arose because Kunze’s specimens had been lost and his description was not sufficiently detailed. Cunningham (1879) described a parasitic alga from India as *Mycoidea parasitica* Cunningham, a new genus and species, but Hariot (1889) pointed out that Cunningham’s genus *Mycoidea* was identical to Kunze’s genus *Cephaleuros*. Karsten (1891) accepted the older genus name, *Cephaleuros*, added five species, and named *M. parasitica* as *C. mycoidea* Karsten, a new species. Mann and Hutchinson (1907), however, concluded that the reasons given by Karsten were not sufficient to justify the abandonment of *C. virescens*. Printz (1939) reported 13 species in his monograph, recognized the priority of *C. virescens*, and reduced *M. parasitica* and *C. mycoidea* to synonymy. Thompson and Wujek (1997) published a monograph that included 13 species of

Fig. 4. Summary of phylogenetic relationships within the Trentepohliales based on molecular data from López-Bautista et al. 2006, Nelsen et al. 2011, Rindi et al. 2009, and Zhu et al. 2014. *Stomatochroon* photo courtesy of G.-X. Liu.

Cephaleuros and one variety and in 2009, Suto and Ohtani added three species from Japan. Detailed histories of the genus have been described by Thompson and Wujek (1997), López-Bautista et al. (2002), and Suto and Ohtani (2009). Guiry and Guiry (2014) currently list 17 species and one variety of the genus (Box 2).

Identifying *Cephaleuros* species requires a close examination of their morphological characteristics. A supportive diagnostic method is counting chromosomes (Box 3, Fig. 6). Suto and Ohtani (2011b) discovered the number of chromosomes among five species of *Cephaleuros* were different yet remained consistent for each species. Current morphological identifications at genus and species levels, however, are being revised by advanced molecular techniques (López-Bautista et al. 2006).

Symptoms and Signs

Two growth habits. Species of *Cephaleuros* can be divided into those that grow between the cuticle and epidermis of their host plants (subcuticular) and those that penetrate the epidermis and grow among the cells of their hosts (intercellular) (see Box 2). Most species of *Cephaleuros* grow beneath the cuticle on the upper surface of leaves and on stems and fruits. The few intercellular species, however, grow through the epidermis, palisade, and spongy mesophyll cells and are the most damaging to their hosts.

Algal spot. The original name for *Cephaleuros* diseases was “red rust,” an apt description of the reddish orange thalli that infect tea (*Camellia sinensis*) in India (Mann and Hutchinson 1907). As mentioned earlier, this name has led to some unfortunate misidentifications (see Box 1). A currently accepted common name for this disease is algal spot, but red rust is still used. If “spot” refers to necrosis, the reaction of the plant to the alga, it is a symptom. If it refers to the thallus of the alga and its projections, then the “spot” is a sign.

Symptoms. Most subcuticular species of *Cephaleuros* only cause necrosis beneath their thalli (Fig. 7A and B) (Brooks 2004; Ogle 1997; Suto and Ohtani 2009). This necrosis is usually limited to the epidermal cells but occasionally includes palisade parenchyma and can extend to the lower leaf surface (Fig. 7C) (Chapman 1976; Thompson and Wujek 1997). On hosts in Japan, the subcuticular *C. japonicus* produces reddish brown necrosis 3 to 18 mm in diameter beneath thalli on the upper leaf surface, and corresponding yellow discolorations on the lower leaf surface (Suto and Ohtani 2009). Intercellular species cause translaminar necrosis that appears as circular, purplish black spots on upper and lower leaf surfaces. *Cephaleuros biolophus*, for example, destroys the leaf mesophyll and causes cracking of the epidermis of both leaf surfaces (Suto and Ohtani 2009). Lesion diameters of two intercellular species measured 3 to 6 mm for *C. biolophus* and 2 to 25 mm for *C. parasiticus* (Thompson and Wujek 1997). Algal spots on fruits are superficial and usually smaller than on leaves (Joubert and Rijkenberg 1971; Ogle 1997). The most serious plant damage is caused by *Cephaleuros* species that attack leaves and twigs of crops such as tea, citrus, and blackberry (Dewdney 2012; Holcomb et al. 1998; Ramaya et al. 2013; Wolf 1930). Infected twigs and stems are often swollen and

cracked (Holcomb et al. 1998). Thalli can coalesce and girdle twigs causing chlorosis, cankers, premature defoliation, and dieback (Joubert and Rijkenberg 1971; Suto and Ohtani 2009; Wolf 1930).

Signs. The greenish yellow to reddish orange thalli of subcuticular *Cephaleuros* species are quite distinctive (Fig. 7A, B, and D). They are usually slightly raised and have a velvety appearance due to sporangiophores and setae (sterile hairs) growing up through the plant cuticle. Macroscopically, gametangia appear as aggregates or concentric rings of greenish black granules (Suto and Ohtani 2009). The intercellular species are less obvious as their thalli are sometimes scant or below the epidermis, and sporangiophores are usually produced through stomata and the lower epidermis (Chapman and Henk 1985; Suto and Ohtani 2009; Thompson and Wujek 1997).

Subcuticular thalli develop as cells produced by germinated zoospores divide and form branching filaments. As thalli age, the central portion decays, turns whitish, and fresh thallus cells develop around it. In Japan, this condition is called “whitish algal disease” (Suto and Ohtani 2013). The decayed portion of the algal thallus and necrotic leaf tissue below it sometimes drops from the leaves, producing a “shot hole” appearance. When decayed tissue finally spreads to the periphery, the thallus dies (Brooks 2004; Chowdary and Jose 1979).

Morphology

The thalli of all *Cephaleuros* species, both subcuticular and intercellular, are composed of a prostrate system and an erect system.

Box 2. *Cephaleuros* species.

List of currently accepted subcuticular and intercellular *Cephaleuros* species (Guiry and Guiry 2014).

Subcuticular species (thallus mainly between host cuticle and epidermis)

Cephaleuros aucubae Suto & Ohtani
Cephaleuros diffusus Thompson & Wujek
Cephaleuros drouetii Thompson & Wujek
Cephaleuros endophyticus (F.E.Fritsch) Printz
Cephaleuros expansa Thompson & Wujek
Cephaleuros henningsii Schmidle
Cephaleuros japonicus Suto & Ohtani
Cephaleuros karstenii Schmidle
Cephaleuros lagerheimii Schmidle
Cephaleuros microcellularis Suto & Ohtani
Cephaleuros solutus Karsten
Cephaleuros tumidae-setae Thompson & Wujek
Cephaleuros virescens Kunze ex E.M.Fries (type species)

Intercellular species (thallus subcuticular, subepidermal, intracellular)

Cephaleuros biolophus Thompson & Wujek
Cephaleuros minimus Karsten
Cephaleuros parasiticus Karsten
Cephaleuros parasiticus var. *nana* Thompson & Wujek
Cephaleuros pilosa Thompson & Wujek

Fig. 5. A, The lichen *Strigula smaragdula* in various stages of colonizing the alga (phycobiont) *Cephaleuros virescens*. Black dots (arrows) in the thalli are fruiting bodies of the fungus (mycobiont). Orange thalli are only partially colonized, green thalli fully colonized, and the whitish thalli are deteriorating. Photo courtesy of E. Barnard. **B,** If *Cephaleuros* kills all the plant cells beneath its thallus and the tissue drops from the leaf, it creates a symptom called “shot hole.” Here, *S. smaragdula* appears to have lichenized the remaining thallus surrounding the shot hole except for a portion of the alga at the far right.

The prostrate system. The filamentous cells of *Cephaleuros* species are cylindrical to irregular in shape and develop by dichotomous or irregular branching of the apical cells (Fig. 8A). If these filaments grow radially and coalesce, they produce an open-ramulate thallus between the cuticle and epidermis (Fig. 8B). If the radial growth of the filaments is compressed laterally, it forms a disk-like, pseudoparenchymatous thallus (Fig. 8C). The margins of this disk can be entire, or lobed with deep sinuses. Intercellular species form loosely branching thalli beneath the cuticle and either above or below the host epidermis (Fig. 8D). Gametangia are spherical to elliptical and formed from terminal cells of the filaments. If the released gametes do not fuse, they form a spherical shape and shed their flagella as they come to rest. Rhizoids form as irregular cellular growth from the underside of the thallus and are thought to anchor the alga in position (Joubert and Rijkenberg 1971). The rhizoids and other cells of the thallus also absorb water and nutrients from the host plant (Ong et al. 1992).

The erect system. This system consists of sporangiophores (Fig. 9A–D) and setae (Fig. 9 E–F) that project externally through the cuticle of leaves, twigs, and fruits. Intercellular species may produce sporangiophores through the upper leaf cuticle but most emerge from stomata or erupt through the lower epidermis. Sporangioophores and setae develop from terminal cells of thalli, usually when one lobe of a branching filament becomes obstructed. Sporangioophores generally consist of 5 to 7 cells and setae from 1 to 33 cells (Thompson and Wujek 1997). Head cells usually form at the apex of sporangiophores and produce sporangiate-laterals (see Fig. 3). There are two exceptions: *C. biolophus* produces head cells and sporangiate-laterals both along sporangiophores and at their apices, and *C. minimus* produces head cells along sporangiophores but with sporangiate-laterals only on one side of sporangiophores (see Fig. 3C and 9D). Instead of terminal head cells, the last few cells of *C. minimus* sporangiophores are tapered and sterile (Brooks 2004; Thompson and Wujek 1997).

Life Cycle

Asexual cycle. Zoospores germinate and develop into thalli. Sporangioophores are produced from terminal thallus cells and develop sporangiate-laterals. When rain or dew wet the zoosporangia they release quadriflagellate zoospores (Fig. 10) as primary inoculum. From 8 to 64 zoospores are produced in each zoosporangium (Chapman and Henk 1985; Guiry and Guiry 2014; Thompson and Wujek 1997). At first, zoospores appear to have two flagella, but each flagellum consists of two flagella twisted together. Zoospores have the same shape as gametes of the same species, but are one-third to one-half larger. Thompson and Wujek (1997) provide an excellent review of the asexual and sexual life cycles of *Cephaleuros* species.

Sexual cycle. Gametangia develop from terminal and lateral thallus cells. From 8 to 64 biflagellate isogametes (Fig. 10) are produced in the gametangia and are half to two-thirds the size of zoospores of their respective species (Thompson and Wujek 1997). They are spindle shaped when released, becoming ellipsoid to fusiform during their active swimming phase. The gametes fuse outside of

gametangia if sufficient moisture is present; otherwise, they fuse within gametangia (Thompson and Wujek 1997). Zygotes grow into dwarf plants (2n) bearing meiosporangia on meiosporangiate-laterals. Meiosporangia produce quadriflagellate meiozoospores that germinate and produce new thalli (n). The diploid phase of the life cycle is considered to occur only in the dwarf plants and the site of meiosis is purported to be in the meiosporangia. This cycle is a heteromorphic alternation of generations and is repeated as more gametangia mature. Using transmission electron microscopy (TEM), Chapman and Henk (1981) discovered sporangia with multiple nuclei containing synaptonemal complexes in specimens of *C. virescens*. This observation indicated that meiosis had occurred. Based on the proposed life cycle, they concluded that the TEM specimen had contained a dwarf sporophyte growing on the gametophyte thallus. López-Bautista and others (2006) reported that the DNA content in 2C nuclei of *C. parasiticus* gametophytes closely approximated 50% of the 4C values in the sporophyte and was consistent with the presumptive sexual life cycle and alternation of haploid and diploid phases.

Presumptive gametangia and biflagellate swimmers. Although the above observations support the proposed existence of haploid and diploid generations, Suto and Ohtani (2013) reported no evidence of sexual conjugation of gametes in five species of *Cephaleuros*. They only recorded direct germination and formation of a haploid thallus. Moreover, they did not observe diploid dwarf plants among natural populations of these species at their study sites in Japan, or on three hosts from India (Suto et al. 2014). Rindi and Guiry (2002) reported that in some Trentepohliales from western Ireland, including *Printzina* and *Trentepohlia*, “biflagellate swimmers,” which have been called gametes, were liberated from “presumptive gametangia.” These swimmers germinated without conjugation and produced new plants. Therefore, Suto and Ohtani (2013) also suggest that in populations of *Cephaleuros*, gametangia be called “presumptive gametangia” and gametes be called “biflagellate swimmers.” Since gametes usually fuse near or within their gametangia, it could also be argued that if they mate it would likely be with genetically identical gametes, reducing the evolutionary value of sexual reproduction.

Seasonal Disease Cycle

Seasons and development. Most scientists agree that *Cephaleuros* species are plant pathogens. Reports by several researchers on its disease cycle vary, however, and are probably related to differences in climate. Algal spot is a monocyclic disease. In general, dispersal of gametes and zoospores, host infection, and development of thalli and lesions occur during the rainy season (Chowdary and Jose 1979; Mann and Hutchinson 1907; Salleh and Kamsare 1994; Wolf 1930). Reproduction, dispersal, and infection take place the following year (Mann and Hutchinson 1907; Suto and Ohtani 2013; Wolf 1930), with gametangia forming earlier than zoosporangia (Chowdary and Jose 1979; Marche-Marchad 1977; Suto and Ohtani 2013). The seasonal development of *Cephaleuros* has been studied in more detail in parts of Florida and Japan.

Subtropical Florida. Wolf (1930) reported similar annual disease cycles on *Magnolia grandiflora* and *Citrus* species in Orlando, Florida. *Magnolia* formed new leaves in May and infections (thalli) were

Box 3. Chromosomes.

Identifying *Cephaleuros* species using morphological characteristics can be difficult as their form varies with the environment. One approach to this problem was to determine if each species had a unique number of chromosomes. Jose and Chowdary (1977) found different numbers of chromosomes among isolates of both *Cephaleuros solutus* and *C. virescens* and called these isolates cytological races. The presence of these races compromised the effectiveness of this approach. However, Suto and Ohtani (2011b) recently reported stable numbers of haploid chromosomes for each of five species of *Cephaleuros* cultured on agar media. The numbers of chromosomes were as follows: 12 in the filaments of *C. microcellularis*; 22 in the filaments and gametes of *C. aucubae* and 34 in *C. biolophus*; and 18 in the filaments, gametes, and zoospores of *C. japonicus* and 24 in *C. virescens* (Fig. 6). Note: not all cultures produced gametes and zoospores.

Fig. 6. Suto and Ohtani (2011b) consistently reported a correlation between the morphologies of five species of *Cephaleuros* in Japan and the number of chromosomes in gametes, zoospores, or filament cells. Chromosomes in filament cells of **A**, *Cephaleuros aucubae* ($n = 22$) and **B**, *C. virescens* ($n = 24$).

visible by the end of the rainy season in September and October. By January of the next year, setae were protruding above the leaf cuticle. At the beginning of the rainy season in May, sporangiophores were producing zoosporangia and zoospores.

Temperate Japan. Suto and Ohtani (2013) described the seasonal development of *Cephaleuros* species in a temperate region of Honshu, Japan. They determined that young thalli appeared mainly on 1- and 2-year-old leaves of various woody hosts at the end of the rainy season in June and July. Thalli continued to develop from April

through September of the following year, producing gametangia and zoosporangia on 2- and 3-year-old leaves. Gametes of *C. microcellularis* were released from late March to late May and gametes of four other species from late April to late July. Zoospores of *C. biolophus*, *C. japonicus*, and *C. virescens* were released from mid-May to early August. As in subtropical Florida, one infection cycle was completed each year in temperate Japan.

Inoculum production. Inoculum is produced the year following infection and disease can be severe (Brooks 2004; Cunningham

Fig. 7. **A**, Tissue necrosis caused by *Cephaleuros* species is usually limited to cells beneath the thallus. The thallus of *C. virescens* and most of the plant cuticle have been removed from the upper lesion to show necrosis. **B**, On some hosts, *Cephaleuros* causes hyperplasia of the epidermis and palisade parenchyma, raising the thallus above the leaf surface. **C**, An infection of *Camellia japonica* by the subcutaneous alga *Cephaleuros japonicus*: cuticle (c), thallus (t), epidermis (e), palisade parenchyma (p), and corky tissue (ct) formation among spongy mesophyll cells in response to infection. **D**, These yellowish orange, fuzzy thalli on guava leaves are a typical sign of *C. virescens*.

Fig. 8. **A**, Irregular branching of an intercellular filament of *Cephaleuros minimus*. **B**, When individual filaments expand they form branches called "ramuli." This "open-ramulate" thallus of *C. karstenii* has spaces between the ramuli. **C**, The ramuli of *C. virescens* usually coalesce and form an "entire" or disk-like thallus. The blurred projections are multicellular setae. **D**, The filamentous thallus of the intercellular pathogen *C. minimus* is partially visible beneath the upper leaf cuticle.

1879; Mann and Hutchinson 1907; Marlatt and Campbell 1980; Suto and Ohtani 2013; Wolf 1930). Thompson and Wujek (1997) estimated zoospore production on *Abutilon* species as follows. A 2-mm-diameter thallus of *C. virescens* produced an average of 250 sporangiophores, each with 4 to 11 sporangiate-laterals (zoosporangia). If each zoosporangium contained 8 zoospores, *C. virescens* would produce 8,000 to 22,000 zoospores per 2-mm thallus. With an average of 9 thalli per cm² of leaf surface, this alga would produce 72,000 to 198,000 zoospores per cm². This is a conservative estimate as the reported number of zoospores per zoosporangium varies. Chapman and Henk (1985) found 8 or 16 zoospores in zoosporangia of *C. parasiticus* and 32 or 64 in *C. virescens*. The number of viable spores is much lower, however, as gametes and zoospores frequently burst a few minutes after their release (Cunningham 1879; Sarma 1986; Suto and Ohtani 2013; Ward 1884).

Inoculum distribution. Sporangiate-laterals extend from head cells on sporangiophores (see Fig. 3). The supporting branch, the suffultory cell, is wider at the base and tapers apically to a short neck that ends in a spherical or oval zoosporangium. Under moist conditions, the suffultory cell swells and straightens, holding the zoosporangium away from the head cell (López-Bautista et al. 2002). At maturity, the zoosporangium abscises and is carried by the wind. This is the main means of spore dispersal (Chapman and

Good 1978) and another unique feature of the Trentepohliales. Rain and moving water, insects, and “jarring or brushing” can also dislodge and move zoosporangia on the plant surface or from plant to plant (Joubert and Rijkenberg 1971; Mann and Hutchinson 1907; Thompson and Wujek 1997). Suto and Ohtani (2013) recorded the release of gametes and zoospores 2 to 5 min after placing pieces of thallus in water. Though most reports indicate only water is needed to release the motile cells, they observed that some form of physical (mechanical) disturbance was required.

Host penetration. Researchers have yet to determine how *Cephaleuros* species enter their host plants (Thompson and Wujek 1997). There is no evidence of enzymatic or mechanical penetration of the cuticle. Mann and Hutchinson (1907) thought zoospores germinated on the plant surface, formed small primary thalli, and then breached the cuticle by “pushing their way through.” Wolf (1930), however, observed that most of these superficial thalli died. Only spores that found breaks in the cuticle and developed thalli beneath it survived. In support of this, Suto and Ohtani (2011c) demonstrated that leaves of certain plants wounded by cutting or rubbing were more susceptible to infection than nonwounded plants.

Inoculation Studies and Host Range

Early work. Inoculation experiments can be used to determine the pathogenicity and virulence of a suspected causal agent of disease

Fig. 9. A, Typical lesion on an upper leaf surface caused by *Cephaleuros virescens*. The erect portion of the alga consists of sporangiophores with head cells and sporangiate-laterals, and setae. B, Head cells and sporangiate-laterals of *C. japonicus*. C, Cluster of *C. parasiticus* sporangiophores emerging from the lower leaf surface. D, Sporangiate-laterals of *C. minimus* form on one side of sporangiophores; the pointed, three-celled apex is sterile. E, Multicellular setae (arrows) of *C. karstenii*. F, Short setae (arrows) of *C. henningsii*.

and its host range. However, few inoculation studies with *Cephaleuros* species have been published. In the 1920s, Wolf (1930) detected no host infections following inoculations with zoosporangia, fragments of thalli, or filaments from pure cultures of *C. virescens*. Fifty years later Chowdary and Jose (1979) were also unable to produce infections using zoospores as inoculum.

Newer work. Suto and Ohtani (2011b) successfully inoculated seedlings of various woody plants in Japan with five species of *Cephaleuros*. The algae were cultured on agar media and then applied directly to leaves, or applied as a suspension of fragmented thalli. Seasonal development of disease in the controlled studies was the same as in natural inoculations. Inoculation experiments with biflagellate swimmers and zoospores, which are natural inocula in the field, should be used in future bioassays.

Host specificity. The host range of *Cephaleuros* species in the tropics and subtropics is extensive. It includes both angiosperms and gymnosperms, with angiosperms most affected (Brooks 2004; Holcomb 1986; Marlatt and Alfieri 1981; Suto and Ohtani 2009). The most commonly reported species is *C. virescens* (Thompson and Wujek 1997). Batista and Lima (1949) listed *C. virescens* on 448 hosts in the state of Pernambuco, Brazil, some by their Latin binomial and others only by their genus or common names. Holcomb (1986) reported *C. virescens* on 218 host genera, species, and cultivars in Louisiana alone. No other *Cephaleuros* species were listed by Batista and Lima or by Holcomb. Of five *Cephaleuros* species collected from 152 plant taxa in American Samoa, 115 were *C. virescens* (Brooks 2004). In Japan, Suto and Ohtani (2009) collected 45 plant species of which 25 were infected with *C. virescens*.

Though *C. virescens* has a relatively broad host range, other species appear to have a limited number of hosts. Reports of host specificity are usually based on field observations, but controlled field inoculation studies by Suto and Ohtani (2011c) in Japan indicated definite host preferences. The following combinations of algae and host plants produced infections: *Cephaleuros aucubae* on *Aucuba japonica*; *C. biolophus* on *Quercus phillyraeoides*; *C. japonicus* on *Camellia japonica*, *Camellia sinensis*, *Persea thunbergii*, and *Symplocos lucida*; *C. microcellularis* on *P. thunbergii* and *S. lucida*; and *C. virescens* on *Camellia japonica*, *Camellia sinensis*, and *Persea thunbergii*. It should be noted that molecular evidence is already

revising morphological identifications of *Cephaleuros* at the species level. For example, a phylogenetic study of *C. virescens* strains from the United States, South Africa, and Taiwan determined they are not a monophyletic group, though similar morphologically (López-Bautista et al. 2006). Ongoing genetic analyses and other approaches will continue to redefine our morphological classification of the genus and the host range of its species.

Disease Impact

Economics. Leaf infections caused by *Cephaleuros* species usually are not economically important (Dewdney 2012; Holcomb 1986; Joubert and Rijkenberg 1971). Superficial lesions on fruits such as guava, oranges, and smooth-skinned avocados, however, can affect their marketability. Leaf infections may also decrease the aesthetics of natural and ornamental landscapes. Slow-growing lesions on leaves seldom affect plant growth unless severe, with the exception of tea. Since the tea plant is cultivated for its leaves, foliar infection is a special problem and can affect quality and yield (Huq et al. 2010; Ponmurugan et al. 2010; Ramaya et al. 2013).

India and Japan. *C. virescens* and *C. parasiticus* have been important pathogens of tea in India since the 1800s (Cunningham 1879; Mann and Hutchinson 1907), with plantations affected in the north and south of the country (Joubert and Rijkenberg 1971; Ponmurugan et al. 2010; Ramaya et al. 2013; Suto and Ohtani 2013). Foliar infection is a source of inoculum and can increase the incidence of the more serious stem infections (Mann and Hutchinson 1907). The intercellular pathogen *C. parasiticus* damages the vascular tissue of young stems, leading to defoliation, dieback, and occasionally plant death. *Cephaleuros* species are commonly observed throughout southwestern Japan. They mainly infect leaves of evergreen trees in gardens, parks, and natural forests, damaging their aesthetic value (Suto and Ogawa 1988). Though algal leaf spot caused by *C. virescens* is widely distributed in tea plantations in Japan, the damage is not serious (Ezuka and Ando 1994).

Disease Management

Plant stress. Several authors have reported an inverse relationship between the severity of infection caused by *Cephaleuros* species and host vigor (La Rue 1923; Schubert 1981). Joubert and Rijkenberg (1971) proposed that only healthy plants are able to form the protective suberized tissue that limits algal growth. Plant stress caused by insufficient water or nutrients, poor soil conditions, mechanical or over-harvesting, wounding, and disease are considered major predisposing factors to severe algal infection (Huq et al. 2010; Ramaya et al. 2013; Suto and Ohtani 2011b).

Cultural control. Reducing plant stress and improving plant vigor is best addressed through cultural practices. These practices include proper irrigation and plant nutrition, thinning to improve aeration in the canopy, proper harvesting techniques, and control of pests and diseases (Huq et al. 2010; Ramaya et al. 2013).

Managing *Cephaleuros* on tea in India. Most of the research on management of algal infections has been done in India, prompted by heavy losses among the country's tea plantations. In general, copper-based sprays are the most commonly recommended chemical control method (Joubert and Rijkenberg 1971; Mann and Hutchinson 1907; Ramaya et al. 2013; Wolf 1930). One study determined that fungicides provided better protection than biological control agents, which were more effective than the algicides tested (Ramaya et al. 2013). More specifically, the systemic fungicides carbendazim and hexaconazole provided better disease control than the contact fungicides copper oxychloride and mancozeb. Two species of *Streptomyces* reduced the incidence of infection by *Cephaleuros* better than the bacteria *Pseudomonas* and *Bacillus*, or the fungus *Trichoderma*. Disinfecting pruning tools in various algicidal solutions between plants reduced disease incidence up to 25% and hand-plucking leaves produced fewer infections than either shearing or over-harvesting.

Resistant cultivars. A recent survey of tea plantations in southern India evaluated the resistance of various clonal tea cultivars to *Cephaleuros parasiticus* (Ponmurugan et al. 2010). A majority of the clones had either a moderate or low susceptibility and four of the most

Fig. 10. When released from their gametangia and zoosporangia, gametes and zoospores both appear to have two flagella (arrows). Zoospores have four flagella, however, twisted together in two pairs. **A**, Gametes of *C. virescens* with two flagella (left) and zoospores with four flagella (right). **B**, Gametes of *C. japonicus* with two flagella (left) and zoospores with four flagella (right), both stained with Safranin O.

popular clones were described as tolerant. Climate was also a factor in clonal susceptibility. Though these results are promising, breeding for resistance to *Cephaleuros* species appears to be relatively unexplored.

Conclusions and Future Research

The peculiar biology of the Trentepohliales presents opportunities for exciting and creative research. Despite recent advancements, the diversity of this order is still poorly understood. There is an urgent need for a modern systematic and taxonomic assessment founded on solid molecular data with wide taxon sampling. Although this is common to many algal groups, it is a mandatory requirement for the Trentepohliales, in which morphology offers relatively few characters and is severely affected by phenotypic plasticity. Accordingly, most in need of assessment is the widely reported paraphyletic species, *C. virescens*. This work should be supplemented with detailed observations of morphological characteristics in different environments, inoculation studies to determine host selectivity, and clarification of the infection process. Furthermore, assessing disease development under various field conditions will improve management options, as will resistance breeding for high-value crops.

The development of next-generation sequencing techniques in recent years has greatly expanded the body of genomic and transcriptomic (all mRNA present in a cell or population of cells) data available for algae. The first full genome sequencing project for a trentepohlialean alga, *Trentepohlia flava*, is in progress http://bridge.botany.ubc.ca/herbarium/details.php?db=ubcalgae.fmp12&=ubcalgae_web_details&recid=210232&ass_num=A89248. Similarly, the first transcriptomic analysis, for *Trentepohlia iolithus*, was recently published (Li et al. 2014). Analysis of genomes and transcriptomes of the Trentepohliales can provide great insights into their physiology and ecology. Pathogenic members of the order are good subjects for these studies. It would be particularly interesting to compare the transcriptomic profiles of subcuticular *Cephaleuros* species with its more virulent intercellular species. This could lead to a better understanding of plant-microbe interactions and improved crop management strategies.

General information on the ecology, natural history, and distribution of the Trentepohliales is always a valuable contribution. We hope this article will encourage plant pathologists, phycologists, and others based in tropical regions to look for and appreciate these algae.

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Dr. Brooks received his B.S. degree in ornamental horticulture from California State Polytechnic University, Pomona, in 1989 and his Ph.D. in plant pathology from the University of California, Riverside, in 1994. Dr. Brooks was in the Maltese Islands for three years with the U.S. Peace Corps and then continued as plant pathologist for the Maltese government. He was then plant pathologist for the land grant college in American Samoa and did research on various tropical diseases, including taro leaf blight, black leaf streak, and burrowing nematode (toppling) diseases of banana, brown root rot, and algal spot. Dr. Brooks is currently at the University of Hawaii at Manoa where he has taught basic plant pathology and pesticide safety education.

Dr. Rindi is a senior researcher in the Department of Life and Environmental Sciences at the Università Politecnica delle Marche, Ancona, Italy, which he joined in 2010. Dr. Rindi received his Masters in Biological Sciences (1992) and his Ph.D. (1999) from the university in his hometown of Pisa, Italy. Subsequently, he was a postdoctoral research fellow at the National University of Ireland, Galway (advisor Prof. Michael Guiry), and then at the University of Alabama, Tuscaloosa (advisor Prof. Juan Lopez-Bautista). Dr. Rindi's research interests focus on the systematics, evolution, taxonomy, ecology, and conservation of algae. Although work carried out during the early stages of his career was entirely focused on marine algae, in the course of his postdoctoral experiences Dr. Rindi's interests expanded to terrestrial and freshwater algae. The order Trentepohliales is now one of the main targets of his research and he has produced numerous publications concerning this group.

Dr. Suto is a forest pathologist in Shimane Prefecture, Japan. He received his Ph.D. in plant pathology from Kyoto University in 1981.

He began his career at Shimane Prefecture Forest Research Center (SPFRC) in 1963 and investigated tree diseases, especially several pine needle diseases, the pine wilt disease, and the resinous stem canker in *Chamaecyparis*. He retired from SPFRC in 1998. Since his retirement, he has been studying foliicolous algae and lichens in cooperation with Dr. Ohtani. He has a particular interest in the diversity of microorganisms on leaves and needles of woody plants and their ecology.

Dr. Ohtani is a taxonomist of freshwater algae. He received his Ph.D. from Hiroshima University in the taxonomy of Desmids (Chlorophyta). He visited Antarctica two times when he was a member of National Institute of Polar Research, Tokyo. He also studied cultured strains of freshwater algae near Syowa Station, Antarctica. He moved to Shimane University in 1992. He has been investigating the flora and yearly change of microalgae in the brackish lakes of Shimane prefecture. He has been studying foliicolous algae and lichens in cooperation with Dr. Suto.

Dr. Green was awarded his Ph.D. in Environmental Science from Tennessee Technological University. He is a visiting scholar in Dr. Juan López-Bautista's Phycolab at the University of Alabama, Tuscaloosa, where he is continuing research from his doctoral studies. This research was conducted in consultation with Dr. Francois Lutzoni of Duke University. His research focuses on investigating the biodiversity of subaerial and lichenized Trentepohliales using genetic sequencing data as applied to molecular-based phylogenetic circumscriptions. At present, he is integrating his research into undergraduate programs throughout the country while on temporary faculty assignment.

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