Research

Red Rust of *Neoregelia* Bromeliads Caused by a Parasitic Alga *Cephaleuros parasiticus* in Florida

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Abstract

A newly emerging disease affecting proprietary hybrids of *Neoregelia* bromeliads was found in nursery production throughout south Florida. Initial symptoms appeared as small, reddish leaf spots with signs of fungal-like growth on the abaxial leaf surface closely resembling a rust. Closer examination of diseased tissue revealed the growth to be an alga with morphological and molecular characteristics of *Cephaleuros parasiticus*. This genus of plant parasitic thalloid green algae occurs worldwide in tropical and subtropical regions. Isolates of *C. parasiticus* collected from *Neoregelia* hybrid bromeliads were compared with two common

Cephaleuros spp. (*C. parasiticus* and *C. virescens*) causing leaf spots on guava (*Psidium guajava*) in south Florida. Further, pathogenicity tests were conducted on healthy *Neoregelia* hybrids using *C. parasiticus* isolates collected from diseased leaves of bromeliads and guava. Isolates from both sources produced symptoms consistent with those originally described as “red rust” on *Neoregelia* hybrids. Florida has 165 plant species belonging to 53 families that have been reported as hosts of *Cephaleuros* spp., but this is the first report on *Neoregelia* hybrid bromeliads.

Cephaleuros species were first reported in Florida by Swingle (1894). A large diversity of hosts has been listed of *Cephaleuros* species in Florida (up to 165 species), including ornamental plants such as *Ligustrum* spp., *Aglaonema* spp., *Spathiphyllum* spp., *Ficus* spp., and a wide range of broadleaf plants (Marlatt and Alfieri 1981).

Cephaleuros species are filamentous algae that parasitize numerous species of vascular plants. Most *Cephaleuros* species are obligate parasites that grow subcuticularly or intercellularly and cause damage to their host plant. Symptoms are often found on leaves but can be found on stems and fruits (Brooks et al. 2015). The severity of diseases caused by *Cephaleuros* spp. depends on the host plant, the environment, and the species. *Cephaleuros* species are usually found in tropical and subtropical climates, because warm weather and frequent rains are favorable conditions for their growth and reproduction (Brooks et al. 2015; Nelson 2008).

The macroscopic structure of *Cephaleuros* species consists of pigmented yellow-orange thalli that are produced on the surface tissue, with filaments bearing sporangiophores and zoosporangia (Thompson and Wujek 1997). The pigmentation of the thalli is owing to accumulation of carotenoids (López-Bautista et al. 2002). Species of *Cephaleuros* exhibit two different growth habits, and this is one characteristic that can be useful in identifying species. Some are subcuticular species, for which their growth is between the cuticle and epidermis of their hosts. The remainder are intercellular species, for which the alga penetrates the epidermis and grows

among the cells of their hosts (Brooks et al. 2015). Thompson and Wujek (1997) suggested that the features that are most valuable in determining the species of *Cephaleuros* are the thallus growth habit, the manner of bearing head cells or sporangiate-laterals, and the kind of lesion produced.

This paper presents a new host record for *Cephaleuros parasiticus* causing red rust on select hybrids of *Neoregelia* bromeliads. A severe outbreak of red rust appeared in production nurseries and landscapes with a loss up to 90% in one of the locations in December 2014 in south Florida. Morphological characteristics of the alga causing this new disease on hybrids of *Neoregelia* bromeliads were compared with descriptions in the monograph by Thompson and Wujek (1997), along with two *Cephaleuros* species (*C. virescens* and *C. parasiticus*) occurring on *Psidium guajava* in south Florida. To confirm its role as a plant pathogen, pathogenicity tests were conducted on *Neoregelia* hybrids with *C. parasiticus* isolates collected from diseased leaves of bromeliads and guava.

Host and Location of *Cephaleuros* Species

Specimens of *Cephaleuros* species were collected in Homestead, FL, from June 2015 to August 2016 and included two different plant hosts affected with “red rust” on leaves: *P. guajava* and *Neoregelia* hybrids. Algal specimens were collected from a variety of *P. guajava* cultivars including ‘Patillo’, ‘Blitch’, ‘Ruby × Supreme’, ‘Webber × Supreme’, ‘Asian White’, ‘Hong Kong Pink’, and ‘Barbie Pink’.

Samples were randomly collected from diseased bromeliads in a local nursery and from a guava orchard located at the University of Florida (UF) Tropical Research and Education Center (TREC) in Homestead, FL (latitude, 25°30′40.809″N; longitude, 80°30′3.983″W).

Specimens of *C. parasiticus* collected from *Neoregelia* bromeliad hybrids and *C. parasiticus* and *C. virescens* collected from *P. guajava* ‘Webber × Supreme’ were deposited in the Smithsonian

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Institution, Department of Botany, Washington DC (accession numbers not assigned by this institute).

Morphological Characteristics

Morphological characteristics of *Cephaleuros* thalli were observed using a Keyence microscope (model VHX-900F, Keyence Corporation of America, Itasca, IL) and transverse leaf sections were observed using a Laborlux microscope (Leitz, Wetzlar, Germany), and images were taken with a Pixelink camera (model PL-AA62, Pixelink, Ottawa, Canada). Several sections were made by hand using a sterile blade and were placed in a drop of Shear's fluid as described in Suto et al. (2014) on a glass slide with a cover slip sealed to observe under the microscope. The leaf cuticle was carefully removed from some of the sections as described in Preece (1962), modifying the antibacterial reagent. Plant preservative mixture (Caisson Laboratories, Smithfield, UT) was used to prevent bacterial contamination, and after 2 and 5 days, cuticles floated free in the solution. Sporangiophores and zoosporangia dimensions were measured ($n = 50$).

Pathogenicity Tests

Pathogenicity tests were performed on an asymptomatic healthy-appearing *Neoregelia* hybrid as well as a commercial variety, *Neoregelia carolinae* 'Tricolor perfecta'. The trial was run from March to September 2016 at UF TREC in Homestead, FL. Bromeliads were inoculated with zoosporangia from three *C. parasiticus* isolates (strains NEO 141075, NEO 4 N 41516, and NEO B1 111715) collected from a *Neoregelia* hybrid and strain GUA-AW 1.7, collected from *P. guajava*. *C. virescens* from *P. guajava* was not included in the pathogenicity test owing to the lack of an

inoculum source. Inocula were prepared from small pieces of symptomatic leaf spot tissue processed in a blender (Magic Bullet Blender, model MB 1001) prior to filtering through sterile cheesecloth (gauze sponges, McKesson, Richmond, VA). A zoosporangia suspension ranging from 1×10^2 to 1×10^4 zoosporangia/ml was sprayed over the foliage until runoff with a handheld air pump sprayer. Two plants were inoculated with each strain, and two noninoculated control plants were included. Noninoculated controls were sprayed with sterile deionized water. The pathogenicity experiment was repeated.

Plants were located in a humidity chamber under 73% shade (outdoor shadehouse) and arranged in a randomized complete block design. They were monitored for symptom development weekly. Temperature and relative humidity (RH) were recorded with a HOBO data logger (model U12-012, Onset, Bourne, MA) throughout the duration of the trials.

C. parasiticus on *Neoregelia* Hybrid Leaves

Small reddish elliptical lesions approximately 0.1 to 10 cm in length and 0.1 to 4 cm wide resembling "red rust" initially developed on the adaxial leaf surface and progressed through to the abaxial surface. The red spots appearing on the adaxial leaf surface eventually turned brown, forming characteristic red halos (Fig. 1A). The first red spot symptoms on the adaxial leaf surface developed 8 weeks after inoculation, and at 10 weeks spots turned brown with red halos (Fig. 1A). Symptoms caused by *C. parasiticus* (from the two isolates of different hosts) appeared at an average temperature of 26°C and 80% RH on both *Neoregelia* species. On the abaxial leaf surface, brown spots became covered with orange tufts of sporangiophores releasing zoosporangia (Fig. 1B). Over time, these

FIGURE 1

Leaf lesions caused by *Cephaleuros parasiticus* on *Neoregelia* hybrids. **A**, Brown lesions surrounded by red halos on the adaxial leaf surface. **B**, Brown lesions covered with tufts of sporangiophores on the abaxial leaf surface. **C**, Sporangiophores with zoosporangia on the abaxial leaf surface. **D**, Sporangiophore with zoosporangia. H = head cell; SC = sufflatory cell; SL = sporangiote-lateral; SP = sporangiophore; and Z = zoosporangia. **E**, Transverse section of leaf lesion showing *C. parasiticus* filaments growing among the cells of their *Neoregelia* hybrid host (intercellular). Red arrows show the *C. parasiticus* filaments. **F**, Transverse section of leaf lesion showing necrosis of epidermal (EP), palisade (PS), and spongy mesophyll cells (SP). Scale bars represent 100 μ m.

tufts were observed on the adaxial leaf surface. Evidence of sporangiophores releasing zoospores on the abaxial leaf surface appeared at 12 weeks.

Sporangiophores were cylindrical, erect, 150 to $375 \pm 21.74 \mu\text{m}$ long and 9.4 to $15 \pm 0.84 \mu\text{m}$ wide, with 3 to 7 cells ($n = 50$). Head cells were borne terminally on sporangiophores and produced 3 to 6

sporangiate laterals. Zoosporangia and their suffultory cells were flexed and bent (Fig. 1C and D). Zoosporangia were elliptical, 15 to $25 \pm 1.2 \mu\text{m}$ long and 12.5 to $20 \pm 1.03 \mu\text{m}$ wide, and appeared white to orange in color ($n = 50$) (Fig. 1D).

Thalli grew subepidermally, developed vertically beneath epidermal cells, and emerged intercellularly through the epidermis,

FIGURE 2

Red leaf spot symptoms caused by *Cephaleuros parasiticus* of *Neoregelia* species. **A**, Symptoms on *Neoregelia* hybrids inoculated with *C. parasiticus* isolated from *Neoregelia* hybrids. Arrows show the red leaf spot caused by *C. parasiticus*. **B**, Pathogenicity tests on *Neoregelia carolinae* cv. 'Tricolor perfecta'. Arrows show the symptoms of *C. parasiticus* isolated from *Psidium guajava* causing red leaf spot.

FIGURE 3

Lesions caused by *Cephaleuros* spp. on *Psidium guajava*. **A**, *Cephaleuros* spp. on *Psidium guajava* leaves. Black arrows show *Cephaleuros virescens* causing orange spots, and red arrows show *Cephaleuros parasiticus* causing dark-reddish brown spots. Photo taken from the adaxial leaf surface. **B**, Algae leaf spot on *P. guajava* caused by *C. parasiticus*. **C**, Algae spot on *P. guajava* caused by *C. parasiticus* on leaves and on fruits. **D**, Algae leaf spot on *P. guajava* caused by *C. parasiticus* on the abaxial surface. Arrows show the yellow-orange algae thalli on the abaxial leaf surface.

spongy mesophyll cells, and palisade cells (Fig. 1E and 1F). Necrosis was observed in epidermal, palisade, and spongy mesophyll cells, appearing brown to dark brown (Fig. 1F).

Koch's postulates and the host range test demonstrated the pathogenicity of the causal agent of the disease on *Neoregelia* hybrids. Additionally, *N. carolinae* cv. 'Tricolor perfecta' was also susceptible but to a lesser degree than *Neoregelia* hybrids (Fig. 2A and B). *C. parasiticus* from *P. guajava* also infected both *Neoregelia* species, and leaf spots symptoms closely resembled the original disease samples 8 weeks after the first inoculation (Fig. 2B). *C. parasiticus* was reisolated from symptomatic leaf tissue and was confirmed based on molecular and morphological characteristics. No symptoms developed on any noninoculated control plant.

C. parasiticus on *Neoregelia* hybrids was described from red spots turning brown with time, eventually producing orange tufts of sporangiophores producing zoosporangia with head cells borne terminally on the abaxial leaf surface. Thalli of *C. parasiticus* on *Neoregelia* hybrids grow subepidermally, develop vertically beneath epidermal cells, and grow into intercellular space of cells, showing necrosis of epidermal, palisade, and spongy mesophyll cells. The same characteristics were described for *C. parasiticus* on *P. guajava* (Sunpapao et al. 2016), *C. parasiticus* on *Syzygium aromaticum* (Suto et al. 2014), and *C. parasiticus* on *Magnolia grandiflora* (Chapman and Henk 1985).

Cephaleuros Species on *P. guajava* Leaves

Two species of the genus *Cephaleuros* have been observed causing leaf and fruit spots on *P. guajava* in south Florida. *C. virescens* causes orange leaf spots that are generally much less severe than the "red rust" caused by *C. parasiticus* (Fig. 3A). *C. parasiticus*

symptoms are attributed to dark, reddish brown spots occurring on the fruit and on both sides of the leaf surface of *P. guajava* (Fig. 3). Figure 3B and C shows the high incidence and severity of *C. parasiticus* on *P. guajava* leaves and fruit in south Florida. Pigmented yellow-orange thalli of *C. parasiticus* occur on the abaxial surface (Fig. 3D), whereas pigmented orange thalli occur on the adaxial surface for *C. virescens* (Fig. 3A).

Leaf and fruit spots caused by *C. parasiticus* on *P. guajava* were characteristically circular, dark red in early symptoms, and dark reddish-brown at maturity, approximately 0.1 to 6 cm in diameter (Fig. 3B and C). Yellow-orange tufts of sporangiophores with zoosporangia were produced in clusters and were observed solely on the abaxial leaf surface (Fig. 4A, B, and C). Sporangiophores were cylindrical, erect, 100 to 128 $\pm$ 3.53 μ m in length and 10 to 15 $\pm$ 0.69 μ m wide, with 1 to 4 cells (n = 50). Head cells were borne terminally on the sporangiophores and produced 1 to 6 sporangiate laterals, zoosporangia, and their suffultory cells. Zoosporangia were elliptical, 15 to 25 $\pm$ 0.67 μ m in length and 15 to 25 $\pm$ 0.96 μ m wide, white to orange (n = 50) (Fig. 4C). Sporangiophore initials of *C. parasiticus* were observed on the abaxial leaf surface of *P. guajava* (Fig. 4B). The thalli grew subepidermally and caused necrosis of epidermal, palisade, and spongy mesophyll cells (Fig. 4D and E).

Leaf spots caused by *C. virescens* on *P. guajava* were characteristically small orange circular leaf spots approximately 0.1 to 0.4 cm in diameter (Fig. 3A). Orange tufts of sporangiophores with zoosporangia developed solely on the adaxial leaf surface (Fig. 5A and B). Sporangiophores were cylindrical, erect, 225 to 450 $\pm$ 21.5 μ m long and 10 to 20 $\pm$ 0.9 μ m wide, with 1 to 6 cells (n = 50). Head cells were borne terminally on the sporangiophores and produced 2 to 7 sporangiate laterals, zoosporangia, and their suffultory cells,

FIGURE 4

Leaf lesions caused by *Cephaleuros parasiticus* on *Psidium guajava*. **A**, Sporangiophores with zoosporangia on the abaxial leaf surface. **B**, Sporangiophore initials of *C. parasiticus* on the abaxial leaf surface of *P. guajava* host. **C**, Sporangiophores with zoosporangia on the abaxial leaf surface. **D**, Transverse section of leaf lesion showing with red arrows *C. parasiticus* filaments growing among the cells of their *P. guajava* host (intercellular). Note necrosis of epidermal (EP), palisade (PS), and spongy mesophyll cells (SP). **E**, Transverse section of leaf lesion showing with red arrows *C. parasiticus* filaments growing among the cells of their *P. guajava* host (intercellular). Scale bars represent 100 μ m.

FIGURE 5

Leaf lesions caused by *Cephaleuros virescens* on *Psidium guajava*. **A**, Orange alga thalli of *C. virescens* on adaxial leaf surface. **B**, Sporangiate lateral of *C. virescens*. H = head cell; SC = suffultory cell; SL = sporangiate lateral; and Z = zoosporangia. **C**, Transverse section of leaf lesion showing *C. virescens* on the adaxial leaf surface and necrosis of epidermal (EP) and palisade cells (PS) of *P. guajava*. Scale bars represent 100 μm .

which were flexed (Fig. 5B). Zoosporangia were globular to elliptical, 16 to 30 ± 1.1 μm long and 15.6 to 25 ± 0.7 μm wide, appearing white to orange in color ($n = 50$). Thalli of *C. virescens* on *P. guajava* grew subcuticularly on the adaxial and not on the abaxial leaf surface and caused necrosis of epidermal and palisade cells (Fig. 5C).

Molecular Characterization of *Cephaleuros* Species

Three representative isolates of *C. parasiticus* were assessed at DNA level from different various locations from *Neoregelia* hybrids plants (strain NEO 141075, strain NEO 4 N 41516, and strain NEO B1 111715), in addition to one isolate of *C. parasiticus* (strain GUA-AW 1.7 cultivar ‘Asian White’) and one isolate of *C. virescens* (strain GUA-WS 4.14 cultivar ‘Webber $\times$ Supreme’).

Total DNA from isolates of all *Cephaleuros* species collected was extracted from symptomatic leaf spots. Leaves were rinsed with sterilized water to remove potential contamination from other organisms, and afterward *Cephaleuros* cells were carefully scraped by blade into a 1.5-ml tube with sterile distilled water. Cells were disrupted with a Tissuemiser (Fisher Scientific, Waltham, MA), and the DNA was extracted as described with the DNeasy Plant Mini kit (Qiagen, Valencia, CA). DNA was stored at 4°C prior to use. The integrity of DNA was checked by electrophoresis 1% agarose gels previously premixed with GelRed Nuclei Acid Stain (Phenix Research Products, Candler, NC).

PCR was performed for the 18S ribosomal RNA (rRNA) gene. Primers used for 18S rRNA were PCRA (5'-AACCTGGTTGAT CCTGCCAGT-3') (Medlin et al. 1988), 18H (5'-GCCCTTCCG TCAATTCCTTTAAGTTTCAGC-3') (Hamby and Zimmer 1988), and/or RP8 (5'-ACCTGGTTGATCCTGCCAGTAG-3') and RP9

(5'-ACCTTGTTACGACTTCTCCTTCCTC-3') (Zhang et al. 2008). RP8 and RP9 primers were used to detect and eliminate the contaminating presence of unicellular *Chlorella*-like green algae (Ma et al. 2013; Zhang et al. 2008).

Template DNA (2 μl , 50 ng/ μl) was amplified in a total reaction volume of 25 μl containing 2.5 μl of 10 $\times$ reaction buffer, 1.25 μl of MgCl_2 (50 mM), 0.5 μl of deoxynucleotide mixture (containing dATPs, dTTP, dCTP, and dGTP, 10 mM), 1.25 μl of each primer (10 μM), 0.1 μl of Taq (1U, New England Biolabs, Ipswich, MA), and sterile water. The PCR protocol for 18S rRNA for primers PCRA and 18H consisted of an initial denaturing phase of 3 min at 95°C, followed by 30 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min, with a final extension of 7 min at 72°C. The PCR protocol for RP8 and RP9 primers consisted of an initial denaturing phase of 5 min at 94°C, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 2 min, with a final extension of 10 min at 72°C (Ma et al. 2013). PCR fragments for 18S rRNA for *Neoregelia* hybrids were cloned into PCR 2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Prior to ligation into the cloning vector, PCR products were cleaned using QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's recommendations. A 3' A-overhang was added to the PCR product after purification to facilitate the ligation into the cloning vector by mixing, with a final volume of 20 μl , the purified PCR product with 2 μl of 10 $\times$ reaction buffer, 5 μl of dATP (10 mM), and 0.1 μl of Taq (1U, New England Biolabs). The reaction was placed in a thermocycler at 72°C for 20 min, and the product was immediately ligated into the cloning vector. *Escherichia coli* DH5 α competent cells (Invitrogen) were

used for transformation with the recombinant plasmid. *E. coli* colonies were grown overnight at 37°C on Luria–Bertani (Becton, Dickinson, and Co., Franklin Lakes, NJ) medium plates containing ampicillin (100 µg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (20 µg/ml, X-Gal, Thermo Fisher Scientific, Waltham, MA). Colonies were screened by PCR, and plasmid DNA from the positive colonies was purified using the PureYield Miniprep System (Promega, Madison, WI). Samples were sequenced using the standard cycle sequencing technology (dideoxy chain termination/cycle sequencing) on ABI 3730XL sequencing machines (Eurofins MWG Genomics, Louisville, KY). Primers used for sequencing were PCRA, 18H, and GRC (Hamby and Zimmer 1988; Rindi et al. 2009). Sequences were deposited into the National Center for Biotechnology Information (NCBI) GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). The accession numbers at GenBank for the three isolates of *C. parasiticus* of *Neoregelia* hybrids plants (strain NEO 141075, strain NEO 4 N 41516, and strain NEO B1 111715) were KY305499, KY305498, and KY305497, respectively. The accession number for *Cephaleuros* species from *P. guajava* were KY305496 for *C. parasiticus* strain GUA-AW 1.7 cultivar ‘Asian White’ and KY305495 for *C. virescens* strain GUA-WS 4.14 cultivar ‘Webber x Supreme’.

The sequences obtained had 99% identity (100% query; E-value 0.0) with either *C. parasiticus* (accession number KM020146) and *C. virescens* (accession number KM020145). Phylogenetic analysis based on 18S rRNA sequences was performed using the maximum likelihood method based on the Tamura–Nei model (Tamura and Nei 1993). Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). The strains of *C. parasiticus* from *Neoregelia* hybrids and from *P. guajava* formed a clade with 90% support between them (1,000 replicates) (Fig. 6).

Conclusion and Management Implications

C. virescens appears to be the most common widespread species of the genus (Brooks 2004; Brooks et al. 2015; Holcomb 1986; Thompson and Wujek 1997). In the Homestead area of south Florida, it has recently been observed causing leaf spot on adaxial

leaf surface on *Vitex negundo*, *Pachira aquatica*, *Litchi chinensis*, *Mangifera indica*, and *Dimocarpus longan* (UF/IFAS Florida Extension Plant Diagnostic Clinic, Homestead, FL, reports). This is the first report in south Florida of the appearance of both *Cephaleuros* species, *C. virescens* and *C. parasiticus*, in the same leaf host on *P. guajava*, *L. chinensis*, and *M. indica* (UF/IFAS Florida Extension Plant Diagnostic Clinic reports; in this study).

Based on morphology, *Cephaleuros* isolates found in this study were identified to genus and species levels. Sequences of the *Cephaleuros* isolates found in this study cluster in the genus of *Cephaleuros*, and the strains of *C. parasiticus* from *Neoregelia* hybrids cluster with the strain of *C. parasiticus* from *P. guajava*. Rindi et al. (2009) also showed that a high number of strains from *Cephaleuros* sequences found in different places worldwide cluster with *Cephaleuros* genera with high support sequences in a clade with either the 18S rRNA or the rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase, *rbcl*) genes. More DNA sequences need to be analyzed in that genus, but one of the challenges is that DNA sequences from that order have only recently become available (Rindi et al. 2009), and DNA extraction can be easily contaminated with unicellular algae from the Trebouxiophyceae class that are found in terrestrial environments where *Cephaleuros* spp. grow.

C. parasiticus infections on *P. guajava* (guava) have been misidentified as guava rust caused by *Puccinia psidii* (Nelson 2008). Similarly, red leaf spot on *Camellia sinensis* is caused by *C. parasiticus* but is often misdiagnosed as “red rust” (Brooks et al. 2015). Yellow-orange tufts of sporangiophores with zoosporangia observed solely on the abaxial leaf surface of *C. parasiticus* on *P. guajava* sometimes resemble symptoms of guava rust. *P. psidii* produces pale yellow uredia, with pustules on the foliage, and it was first described on *P. guajava* in Brazil (Burnett and Schubert 1985). Subsequently, it has been recorded on several genera of native Myrtaceae in Central and South America. In Florida, *P. psidii* was first found in 1977 (Rayachhetry et al. 1997), where it severely damaged native Myrtaceae, and also the introduced Australian *Melaleuca quinquenervia* (Huq et al. 2010). In 2006, three different host of *P. psidii* rust were identified in Homestead (south Florida),

FIGURE 6

Phylogenetic tree from maximum likelihood analysis using 18S rRNA sequences of *Cephaleuros* strains from this study and other *Cephaleuros* species. *Cephaleuros parasiticus* from *Neoregelia* hybrids (strain NEO 141075, strain NEO 4 N 41516, and strain NEO B1 111715) cluster with *C. parasiticus* from *Psidium guajava* (strain GUA-AW 1.7). The tree was rooted using an outgroup sequence of *Bryopsis plumosa*. The bootstrap values are shown on the branches, and the GenBank accession numbers are shown on parenthesis.

Myrcianthes fragrans, *Syzygium jambos*, and *Pimenta dioica* (allspice) (UF/IFAS Florida Extension Plant Diagnostic Clinic, Homestead, FL, reports). However, to date, rust caused by *P. psidii* on guava in Florida has never been observed. Although rust diseases on many plants are often managed through use of fungicides, control of algal leaf spot is not successful with algaecides (A. R. Chase, *personal communication*). Even use of copper-based fungicides is not effective in preventing algal leaf spot. The relatively close relationship of the host crop with the parasitic algal pathogen makes chemical control unlikely without damage to the host crop. Therefore, cultural controls are the sole means of addressing algal leaf spot on most plants.

Major predisposing factors to severe algae infection are considered to include plant stress caused by insufficient water or nutrients, poor soil conditions, mechanical harvesting or overharvesting, wounding, and disease (Brooks et al. 2015). Therefore, cultural practices on reducing plant stress and improving plant vigor are the best strategy to address the disease (Ramya et al. 2013; Suto and Ohtani 2013). These practices include proper irrigation and plant nutrition, thinning to improve aeration in the canopy, proper harvesting techniques, and control of pests and diseases (Brooks et al. 2015; Ramya et al. 2013; Suto and Ohtani 2013).

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