American Journal of Life Sciences
2022; 10(3): 45-52
http://www.sciencepublishinggroup.com/j/ajls
doi: 10.11648/j.ajls.20221003.13
ISSN: 2328-5702 (Print); ISSN: 2328-5737 (Online)

Potential Biocontrol Agents Diversity for Coffee Leaf Rust *Hemileiavastatrix* from Southwesten Ethiopia

Kifle Belachew Bekele

Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Phytopathology Research Section, Jimma, Ethiopia

Email address:
kiflekef@gmail.com

To cite this article:
Kifle Belachew Bekele. Potential Biocontrol Agents Diversity for Coffee Leaf Rust *Hemileiavastatrix* from Southwesten Ethiopia. *American Journal of Life Sciences*. Vol. 10, No. 3, 2022, pp. 45-52. doi: 10.11648/j.ajls.20221003.13

Received: May 10, 2022; **Accepted**: June 6, 2022; **Published**: June 29, 2022

Abstract: Coffee leaf rust (CLR), caused by Hemileiavastatrixis the main coffee disease, occurring in the main coffee producing countries worldwide. Effective management of CL Rintegrated approch including the development of resistant varieties, cultural management, biocontrol and chemicals. Considering both the potential source of potential bioagents by this ecological niche and the possibility of practical uses as a tool to be deployed against coffee leaf rust. White colourmycoparasitic growth is often observed on H. vastatrixpustules. The common report is a complex of "white colony forming-taxa" which has been promptly labeled as Verticilliumsp., Lecanicilliumsp. or, at a supposedly more precise level, as Verticilliumlecanii. Taxonomic information about such potential biocontrol fungi is lacking in Ethiopia. In order to investigate the identity of mycoparasites of H. vastatrixand to obtain potential biocontrol agents for the pathogen of coffee, a survey of fungicolous associated with coffee leaf rust was conducted at major coffee producing areas of Ethiopia. One hundred and ten different mycoparasite isolates from H. vastatrixpustules have been captured and characterized. The identified isolates belonging to twelve genera: Akanthomyces, Alternaria, Cladosporium, Fusarium, Gonatophragmium, Lecanicillium, Ochroconis, Paraphaeosphaeria, Phoma, Pleurodesmospora, Sarcopodiumand Simplicillium. Biological control of CLR with mycoparasites remains promising possibility for the future. Nevertheless, without firm taxonomic knowledge and the aid of modern tools, the rapid and precise identification of potential biocontrol agents remain a bottleneck. Therefore, morphological and cultural characterization of these mycoparasites should be supported with appropriate molecular tools and the phylogenic tree approach. Although very little is known about mycoparasites, this work provide piece of informationaboutpotential biocontrolagents deserving more attention by plant pathologists and related scientists for exploiting their service in fight against CLR.

Keywords: Biocontrol Agents, Coffee Leaf Rust, Diversity, *Hemileiavastatrix*

1. Introduction

Coffee is the second traded commodity next to oil and the most economically significant agricultural product in the world trade [1]. *Coffeaarabica*and *C. canephora*are the two main cultivated species in the world. These two species represent, respectively 66% and 34%, of commercially planted coffee [2]. Coffee production has been challenged worldwide by several biotic and abiotic factors, especially climate extremes and fungal diseases, both of which can drastically decrease yield and quality [3, 4].

Coffee leaf rust (CLR) is the main disease affecting coffee worldwide. It is caused by *Hemileiavastatrix* (*Pucciniaceae*) which is a host-specific obligate parasite [5, 6]. It attacks the coffee leaves reducing the photosynthetic area and leading to defoliation and occasionally causing branch dieback and even to plant death. High incidences of CLR can cause the loss of up to 50% of the foliage and up to 70% of berries – leading to yield reductions of 30% to 50% [6-10]. Recently economic losses reaching up to 90% have been reported in some Central American countries due to epidemic of CLR [10].

CLR control is based on the use of resistant varieties, fungicide applications [11] and on escaping the disease by establishing highland plantations [12]. However, there are limitations for each of these approaches. Breeding for resistance has contributed significantly to reduce losses provoked by *H. vastatrix* [13]. However, due to high variability of *H. vastatrix*, possible fuelled by cryptosexuality [8], the emergence of new races of the pathogen, as well as the occurrence of a complex of races challenges the quest for

obtaining durable resistant for coffee leaf rust [14 -16].

The use of fungicides, although effective for rust control in plantation situations may be too costly and unpractical for smallholders [8]. It may also exclude, particularly in the case of systemic fungicides, the product from the high value organic market. The continuous use of systemic fungicides can also lead to the selection of fungicide resistant populations whereas the use of broad-spectrum fungicides may harm the population of beneficial organisms (including the broad, diversity of fungal antagonists of *H. vastatrix*) as well as contribute to environmental pollution [17].

Biological control is an environmentally benign and potentially attractive alternative for CLR management, although relatively underexplored. As other rust pathogen *H. vastatrix* is attacked by several mycoparasites fungi [18, 19]. However, the most common and noticeable evidence of mycoparasitism of *H. vastatrix*is promptly recognized under the generic names *Verticillium*sp. or*Lecanicillium*sp. Therefore, the use of this name for mycoparasitic isolates on *H. vastatrix*should be regarded as questionable and never supported by recent advanced studies. This fungus is commonly found attacking green coffee scale insect at a number of coffee fields (*Coccusviridis*) [20].

Several studies demonstrated experimentally an existing effect of antagonistic microbes against *H. vastatrix* [21, 22]. Nevertheless, examples of systematic surveys of mycoparasitic fungi on *H. vastatrix*are limited to a few studies performed outside the center of origin of coffee and *H. vastatrix*in Mexico [18]. Recently, fungal communities associated with CLR were investigated in Mexico and Puerto Rico using molecular tools and unexpected presence of a hyperdiverse fungal community associated with *H. vastatrix*emerged from the study [19].

However, there is limited work and results of systematic surveys for fungal antagonists of *H. vastatrix*has ever been performed and published from the center of origin of *Coffeaarabica*Ethiopia. This study is of strategic importance, since without proper collection, isolation and characterization of the mycoparasites on *H. vastatrix,* it is unlikely that progress in biological control of CLR will be achieved with antagonistic fungi. Therefore, in order to obtain potential biocontrol of mycoparasites associated with *H. vastatrix*agents for coffee leaf rust, this study was initiated for collection, isolation and characterization of mycoparasites associated with CLR.

2. Material and Methods

2.1. Description of Sample Collection Areas

Collection of samples was conducted from the two major coffee growing regions (Oromia and SNNPR) of Ethiopia during 2017/2018 cropping season between September and January. From Oromia region three administrative zones namely, Jimma, Illuababor and Bale Zones were considered. Moreover, two to three districts within each zone were selected for collection, i.e. from Jimma zone (Seka, Gomma and Gera), from Illuababor (Mettu and Yayo) and twodistricts from Bale zone (Harena and Delomena) were considered. In SNNP Region, the districtsDecha, Chena and Gimbo (Kafa zone), Sheko and Gurafarda from Bench-Maji Zone and from Sheka zone (Yeki and Andracha) were considered.

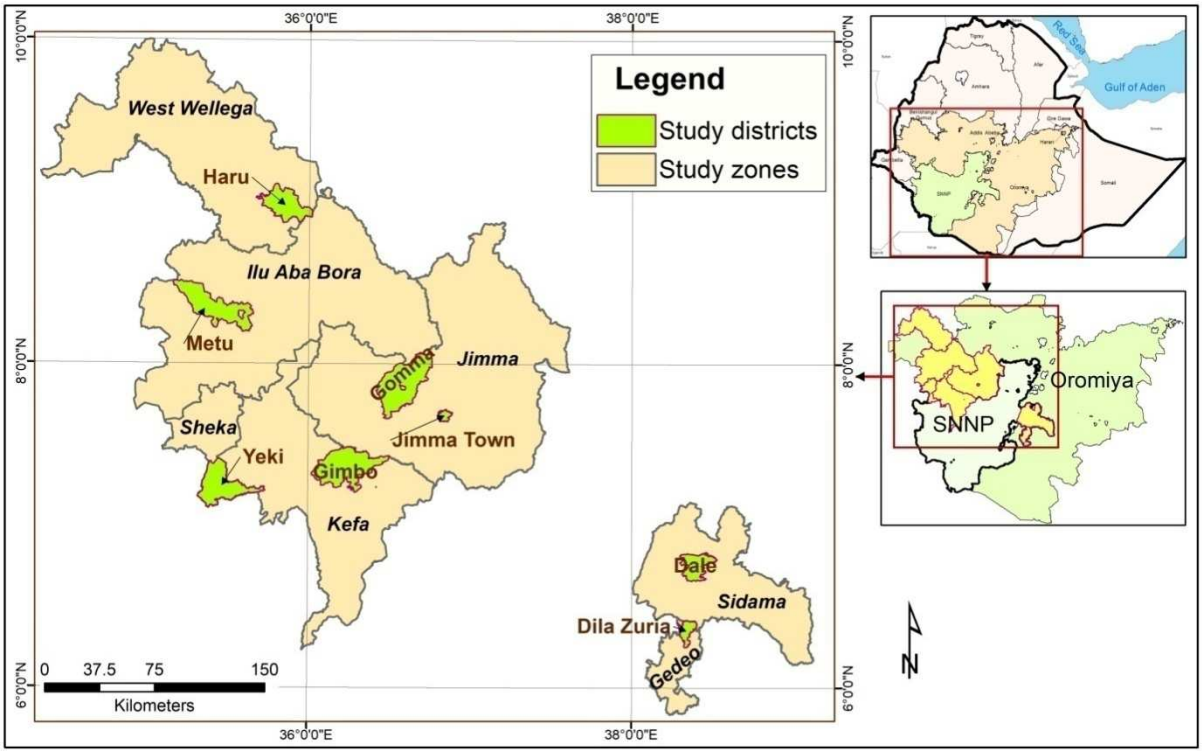

Figure 1. *Map of South Western part of Ethiopiaand from where CLR bio-agents collected. The Zonal map and district name was original and constructed using geographic coordinates and elevation data gathered from each collection sites using global positioning system (GPS).*

2.2. Sample Collection and Isolation

Coffee plant leaf samples with clear symptoms of coffee leaf rust or bearing rust pustules seemingly hyperparasitizedleaves were collected randomly at every locality, special attention being given to leaves showing overgrown by other fungi (with the naked eye or under a 40 × hand lens). Nevertheless, even when there was no obvious presence of mycoparasites, a representative samples were still collected of rust infected leaves for later observation under a higher magnification dissecting microscope in Jimma Agricultural Research Center pathology laboratory.

Coffee plant leaf samples were dried in a plant press and later placed in paper envelopes. The dried samples were stored at Jimma Agricultural Research Center pathology laboratory and representative samples were preserved at this laboratory. The samples were carefully screened for isolation of mycoparsites. Pustules of *H. vastatrix*bearing sporulatingmycoparasites were selected and spores or other fungal structures (mostly conidia) were transferred aseptically onto plates containing potato dextrose agar (PDA Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) supplemented with antibiotic streptomycin sulfate with the help of a sterile fine pointed needle. After incubation at 25°C for 4 to 6 days, single spore cultures were obtained. Pure cultures were preserved by following three methods. On a short term scale, by regular transfer of cultures in tubes containing potato-carrot-agar (PCA) and kept in the fridge. For long term storage, these were maintained in silica-gel, or in cryotubes containing glycerol 10% and maintained at -80°C in a deep-freezer, as described in [23].

2.3. Morphological and Cultural Characterization

Study of the fungus morphology, conidial development and branching patterns of conidial chains were made based on slides containing representative structures of the fungus and mounted in lactophenol or lactofuchsin. These were either scraped directly from the infected rust pustules, from fungal structures from colonies formed on Potato-Carot agar (PCA) or synthetic nutrient-poor agar (SNA) or Malt Extract Agar 2% (MEA) plates based on its preference, or slide cultures were prepared with SNA [24] for 14 d at 25°C with 12 h light regime as described in [25]. The conidial morphology was studied and their shape, colour and presence of septation, were recorded. Moreover, the conidia were mounted in lactophenol and 30 measurements at ×1000 magnification were made for all fungal morphology and illustrations were made under an Olympus BX 51 light microscope (Olympus, Tokyo, Japan) equipped with differential interference contrast (DIC) illumination. Photographs of the fungi were taken with OLYMPUS BX 53 compound microscope fitted with a digital camera (OLYMPUSQ-Color 5). Culture descriptions i.e colony characters and pigment production were based on observations of the colonies formed in plates containing PDA and PCA. These were incubated at 25°C under a 12 h daily light regime for 7 days. Growth rates measurement and colony characters were noted after 7 days. Colony colours (surface and reverse) of the plate were rated according to the color terminology chart of [26].

3. Results and Discussion

With this study 110 different mycoparasites which were identified at genera elevel were isolated from *H. vastatrix*pustules collected from major coffee growing areas of Ethiopia. Eleven genera were recognized, based on a combination of morphological and cultural characteristics. The identified isolates obtained from *H. vastatrix* pustules composed of myoparasites belonging to genera of; *Acremonium, Alternaria, Cercospora, Cladosporium, Digitopodium, Fusarium, Gonatophragmium, Lecanicillium/Akanthomyces, Phoma, Pleurodesmospora,* and *Simplicillium* (Table 1). The locations mycoparasites isolated and recorded were diverse. Based on districts 15 isolates were found from Gimbo districts of Kaffa zone, 12 isolates from Goma, 11 from Andracha and Yeki of Sheka zone, and 10 from Gera and Sheko each. The most diverse Genera of mycoparasites isolates were collected from Kaffa Zone followed by Jimma and Sheka Zones. However, the least diverse mycoparasits were recorded from *Mettu* and *Ale* districts with five genera each (Table 1).

Table 1. *Genera of H. vastatrixmycoparasites and locations they obtained, Ethiopia.*

No	Mycoparasites genera			Collection location			
	Genera	Frequency	%	Zone	Districts	Isolates no	%
1	*Acremonium*sp.	7	6.36	Illuababor	Ale	5	4.55
2	*Alternaria*sp.	3	2.73	Sheka	Andracha	11	10.00
3	*Cercospora*sp.	1	0.91	Kaffa	Chena	8	7.27
4	*Cladosporium*sp.	11	10.00	B/Maji	D. Bench	7	6.36
5	*Digitopodium*sp.	2	1.82	Jimma	Gera	10	9.09
6	*Fusarium*sp.	14	12.73	Kaffa	Gimbo	15	13.64
7	*Gonatophragmium*sp.	4	3.64	Jimma	Goma	12	10.91
8	*Lecanicillium*sp.	32	29.09	B/Maji	Gurafarda	8	7.27
9	*Phoma*spps	5	4.55	Illuababor	Mettu	5	4.55
10	*Pleurodesmospora*spps	15	13.64	B/Maji	Sheko	10	9.09
11	*Simplicillium*spps	16	14.55	Illuababor	Yayo	8	7.27
				Sheka	Yeki	11	10.00
	Total	110	100%			110	100

Note: Lecanicillium sp. is synonyem to Akanthomycessp.

Isolates from *Lecanicillium/Akanthomyces*sp, *Simplicilliums*p, *Pleurodesmospora*sp*, Acremonium sp.* And *Digitopodium*sp. were subjected for cultural, morphological and phylogenetic characterization and presented below.

3.1. Lecanicillium Species

3.1.1. Lecanicillium sp. Isolate I

Culture characteristics: Colonies on PDA 15 - 17mm diam. after 10 days at 25°C, convex, margin entire, aerial mycelium floccose, whitish, reverse brownish centrally, sporulation scarce. Colonies on PCA 16- 20mm diam. after 10 days at 25°C, umbonate, margin entire, aerial mycelium floccose white, reverse brown centrally, sporulation abundant (Table 2).

Morphological character: phialides produced singly or in whorls of up to 2–3 on prostrate hyphae, length 26–74 µm and width of 1–1.5 µm. Conidia of the isolate produced in globose mucilaginous heads, cylindrical, oblong, or ellipsoid, with length 3–7 µm and width of 1.5–2 µm, aseptate, hyaline, smooth. Conidiophores erect, verticillately branched, 15–110 × 1.5–2µm, with 1–4 verticils, each verticil with 2–4 conidiogenous cells. Conidiogenous cells holoblastic, monoblastic to polyblastic, with sympodial and sparingly denticulate fertile apex, swollen at base, gradually tapering towards the apex, 10–22 × 1–2µm, hyaline, smooth; denticles 1 µm long. Conidiao void to ellipsoid, 4–11 × 1–2µm, aseptate to rarely 1-septate, hyaline, thin and smooth-walled.

Morphologically, this species resembles fungi placed in *Calcarisporium*, a genus including several hyperparasites of other fungi such as *Cordyceps* and *Xyalria* [27]. Nevertheless, the molecular study placed it clearly outside *Calcarisporium*and within *Lecanicillium. Lecanicillium*sp. *isolate I*is close to *L. antillanum*, butdiffers morphologically from this species because of the presece of denticulate conidiogenous cells (absence in *L. antillanum*) and conidial size and shape (Table 2). Conidia of *L. antillanum* are longer (11–18µm) than those of *Lecanicillium*sp. isolate 1 (4.0–11µm). Another similar taxon recorded from *H. vastatrix* in Mexico [18] is *C. ovalisporum*. Nevertheless, [18] placed their specimen in *Calcarisporiumovalisporum*based on the cultural and morphological features available at this pre-molecular time. The absence of DNA sequence information renders it impossible to confirm that this fungus was correctly identified as *C. ovalisporum.* It is possible that Carrión & Rico-Gray's fungus was in fact the fungus described herein as a new species of *Lecanicillium*.

3.1.2. Lecanicilliumsp. IsolateII

Culture characteristics: Colonies on PDA 30 - 32 mm diam. after 10 days at 25°C, convex, margin entire, aerial mycelium cottony, whitish, humid centrally, reverse white to cream centrally, sporulation scarce. Colonies on PCA 24- 25 mm diam. after 10 days at 25°C, umbonate, margin entire, aerial mycelium floccose, white, reverse white to cream centrally, sporulation scarce (Table 2).

Morphological character: phialides produced singly or in whorls of up to 2–3 (–4) on prostrate hyphae, 20–45 × 1 –2 µm, hyaline, smooth. Conidia cylindrical to oblong, produced in globosemucilagionous heads, 3– 8 × 1–2 µm, with rounded ends, aseptate, hyaline, smooth.

3.1.3. Lecanicillium Dimorphum

The complete description is found at Zare R, Gams W, 2001. A revision of *Verticillium* section *Prostrata*. *Lecanicilliumdimorphum* is a species morphologically similar to *L. psalliotae* and *L. aphanocladi*, but it differs from these by possessing aphanophialides and macroconidia [28]. This species is commonly found on *Agaricus* spp. and was also recorded on *Pucciniacoronata* [29]. Recently, using the PacBiosequensing technology, a survey of fungi associated pustules in Mexico and Puerto Rico has indicated the frequent presence of*L. fusisporum*in association with this substrate [19]. Our isolates were only found on a single. This sample from Ethiopia appears to be the first complete report of *L. dimorphum*on *Hemileia* spp. pustules worldwide.

3.1.4. Lecanicillium Redinophilum

The detailed description is found at Park *et al.* (2015) [30]. *Lecanicilliumuredihophilum*is phylogenetically close to others species but differ in phialides length, conidial shape and size. However *L. uredinophilum*has been reported as mycoparasite of *Pucciniastriumagrimoniae* and *Coleosporium* sp. in Korea. This species was the most frequent mycoparasite of *H. vastatrix*in Ethiopia observed in this study.

3.2. Simplicillium-Species

3.2.1. Simplicilliumsp. Isolate 1

Culture characteristics: Colonies on PDA 30- 40 mm diam. after 10 days, convex, aerial mycelium lanose, whitish with floccose aerial mycelium, margin entire, reverse brownish centrally, sporulation scarce. Colonies on PCA 40-45 mm diam. after 10 days at 25°C, umbonate, margin entire, aerial mycelium floccose, white, reverse brown centrally, sporulation abundant (Table 3).

Phialides arising from prostrate hyphae, solitary, straight or slightly curved, aculeate, 26- 55 × 1- 1.5 µm, hyaline, smooth. Conidia in small mucilaginous globose heads at the apex of the phialides, subglobose to ellipsoidal, 2- 5 × 1- 2 µm, aseptate, hyaline, smooth, octahedral crystals present (Table 3).

The genus *Simplicillium* is characterized by predominantly solitary phialides arising from aerial hyphae and conidia which are arranged either in globose slimy heads, in short chains, or formed in sympodial succession such as in *S. sympodiophorum* [28, 31]. *S. subtropicum* and other four species have been originally isolated from soil samples in Japan [31]. Among the species in Nonaka et al. publication two (*S. cylindrosporum* and *S. minatense*) have been listed by [19], as associated to *H. vastatrix* pustules in Puerto Rico. *S. subtropicum*is morphologically similar to *S. lanosoniveum,* a species with well-known association with rust hosts. Nevertheless, the subglobose to ellipsoidal conida of *S.*

subtropicum provides easy distinction from the *S. lanosoniveum*.

Table 2. *Biometric data of Akanthomyces/Lecanicillium species associated to H. vastatrix.*

Species	*"A. lecanii"*	*A. fusisporum*	*A. dimorphum*
Phialides (µm)	11-20 (−30) × 1.3-1.8	10-22 × 1-1.5	14-30 × 1-2
Conidia (µm)	2.5-3.5(−4.2) × 1-1.5	3-5 × 1.5-2.0	Macro: 6-11 × 1.4-2.4
	elipsoidaltocylindrical	Ovoid toelipsoidal1-septate	Falcate with sharply pointed ends Micro: 2.5-4.2 × 1.1-1.6 oval toellipsoidalaphanophialides
Conidial mass	Globose heads	Subglobose to elipsoidal heads	Globose heads
Octahedral crystals	Present	Present	Present
Species	*Lecanicillium. isolate I*	*Lecanicillium isolate II*	*L. uredinophilum*
Phialides (µm)	25-70 × 1.0-2.5	20-45 × 1.0-2.0	16-30 × 1.0-1.5
Conidia (µm)	4.5-9.0 × 1.0-2.0	3.0-8.0 × 10-2.0	Macro: 4.0-10 × 1.5-3.0
	Cylindrical to oblong or ellipsoidaseptate	Cylindrical to oblong aseptate	Cylindrical with rounded ends, 1-septate Micro: 4.0-6.0 × 1.0-1.5
Conidial mass	Globoseheads	Globose heads	Goblose heads
Octahedral crystals	Present	Present	Present

3.2.2. Simplicillium Lanosoniveum

For a complete description see: [28]. A revision of *Verticillium* section *Prostrata*. *Simplicilliumlanosoniveum*has been repeatedly found associated with rusts, particularly with *H. vastatrix*on coffee [28] and *Phakopsorapachyrhizi* on soybean [32]. This species was frequently found in samples from Brazil now from Ethiopia. Immature ascomata were abundantly formed either on the natural substrate on CLR pustules or in culture. Our molecular analyses have demonstrated that cultures obtained from ascomata of VIC44419 are identical to those obtained from conidia reinforcing the hypothesis that *Torrubiella* (Cordycipitaceae) now *Gibellula* [33] is the teleomorph of *Simplicillium* as proposed by [28].

3.2.3. Simplicillium Subtropicum

For a complete description see: [31]. *S. subtropicum* and other four species have been originally isolated from soil samples in Japan [31]. Among the species in Nonaka et al. publication two (*S. cylindrosporum* and *S. minatense*) have been listed by [19] as associated to *H. vastatrix* pustules in Puerto Rico. *S. subtropicum*is morphologically similar to *S. lanosoniveum,* a species with well known association with rust hosts. Nevertheless, the subglobose to ellipsoidal conida of *S. subtropicum* provides easy distinction from the *S. lanosoniveum*. This is the first report of *S. subtropicum*asocciated with *H. vastatrix*from Africa.

Table 3. *Biometric data of Simplicillium species associated with H. vastatrix.*

Species	***S. lanosoniveum***	***S. lamellicola***	***S. subtropicum***
Phialides (µm)	15 - 35 × 0.7- 1.5	15 - 50 × 0.7- 1.0	20 - 42 × 1.0- 2.3
Conidia (µm)	1.5- 3.0 × 0.7- 1.3	Macro: 4.5 - 9.0 × 0.8- 1.2	2.3-4.0 × 1.5- 3.3
	Oval or elipsoidal to subcylindrical	Spindle-shapedMicro: 2.0 - 3.0 × 0.7- 1.2	Subglobosetoellipsoidal
Conidialmass	Globoseheads	Subglobosetoelipsoidal heads	Globoseheads
Octahedralcrystal	Present	Present	Present
Species	*S. minatense*	*S. cylindrosporum*	*Simplicillium*sp. nov.
Phialides (µm)	13 - 31 × 1.0 - 1.7	15 - 50 × 0.7- 1.0	26 - 55 × 1.0 - 1.5
Conidia (µm)	2.0 - 3.5 × 1.8- 2.5 (- 2.8)	Macro: 4.5 - 9.0 × 0.8- 1.2	2.0 -5.0 × 1.5- 2.0
	Globose to subglobose (sometimes ellipsoidal)	Cylindrical	Subglobose to ellipsoidal
Conidial mass	Globose heads	Goblose heads	Globoseheads
Octahedralcrystal	Present	Present	–

3.3. Pleurodesmospora Species

3.3.1. Pleurodesmospora sp. Isolate 1

Culture characteristics: Colonies on PDA 20 - 23 mm diam. after 10 days, flat, margin entire, aerial mycelium floccose, powdery at sporulating areas, white, humid centrally, reverse white to cream, abundant sporulation. Colonies on PCA 18 - 20 mm diam. after 10 days, umbonate, margin entire, aerial mycelium floccose, powdery at sporulating areas, white, diurnal zonation present, reverse white to cream, abundant sporulation.

Conidiophores cylindrical erect or procumbent in groups, hardly differentiated from the vegetative hyphae, 56 - 220 × 1.5 - 2µm, usually branched, smooth, hyaline. Conidiogenous cells holoblastic, terminal and intercalary, bearing numerous short-cylindrical, 1-2 µm long and 0.5µm wide conidiogenous pegs located mainly at the distal cells of the conidiophores or often in whorls below the septae. Conidia in short chains (7-8 conidia), dacryoid, 3 - 6 (-9) × 2 - 3 µm, base acuminate, apex, aseptate, hyaline, smooth.

The genus *Pleurodesmospora*was proposed by [34] to accommodate an entomogenous fungus *Pleurodesmosporacoccorum*. Morphologically, *P*. sp. isolate 1 resembles *P. coccorum* [34]. But conidia of *P.* sp. isolate 1 are longer than those of *P. coccorum* (3 - 4 × 2.0 - 2.8). Additionally, long conidial chains are absent in *P. coccorum.* It is phylogenetically close but distinct from the other species of *Pleurodesmospora*. It also bears morphological differences from such species as shown in.

3.3.2. Pleurodesmosporasp. Isolate 2

Culture characteristics: Colonies on PDA 20- 22 mm diam. after 10 days, convex, margin entire, aerial mycelium floccose, becoming powdery at sporulating areas, whitish, diurnal zonation present, reverse white to creamy, abundant sporulation. Colonies on PCA 20- 20 mm diam. after 10 days, umbonate, margin entire, aerial mycelium floccose, powdery at sporulating areas, white, reverse white to cream, abundant sporulation.

Conidiophores cylindrical erect or procumbent in groups, hardly differentiated from the vegetative hyphae, (55-) 120-445 (-643) × 1.5 - 2 µm, usually branched, smooth, hyaline. Conidiogenous cells holoblastic, terminal and intercalary bearing numerous short-cylindrical, 1 - 5 µm long and 1 - 1.5µm wide conidiogenous pegs located mainly at the distal cells of the conidiophores or often in whorls below the septae. Conidia dacryoid, in short branched chains (5-9 conidia), 2.5 - 4 × 1.5 - 2 µm, aseptate, base truncate and apex rounded, hyaline, smooth.

The morphology of *Pleurodesmospora*.sp.isolate 2 resembles that of *P. coccorum*but contrarily to the latter the new species does form conidial chains. *Pleurodesmospora* sp. isolate 2 is phyllogenetically close but distinct from the other novel species of *Pleurodesmospora*. For instance, it has longer conidiophores and smaller conidia than in *P*. sp. isolate 1 (3 - 6 (-9) × 2 - 3) µm.

3.3.3. Pleurodesmosporasp. Isolate 3

Culture characteristics: Colonies on PDA 20 - 23 mm diam. after 10 days, convex, margin entire, aerial mycelium floccose, becoming powdery at sporulating areas, whitish, white to cream reverse, sporulation abundant. Colonies on PCA 18 - 20 mm diam. after 10 days, umbonate, margin entire, aerial mycelium floccose, becoming powdery at sporulating areas, white, white to cream reverse, sporulation abundant.

Conidiophores cylindrical erect or procumbent, hardly differentiated from the vegetative hyphae, 40 - 180 (-300) × 1.5 - 3µm, unbranched, smooth, hyaline. Conidiogenous loci holoblastic, terminal and intercalary bearing numerous short-cylindrical, 1 - 5 µm long and 0.5µm wide conidiogenous pegs located mainly at the distal cells of the conidiophores or often in whorls below the septae. Conidia in short chains (5-6 conidia), one-celled, hyaline, smooth-walled, dacryoid with truncate base, 3 - 5 × 1.5 - 3µm.

Morphologically, *P.* sp. isolate 3 resembles *P. coccorum*. Nevertheless it is phylogenetically close but distinct from the other species of *Pleurodesmospora*. It also bears morphogical differences from such species. For instance it has smaller conidiophores and conidia than those *P.*sp. isolate 1 (3 - 6 (-9) × 2 - 3) µm.

This study make clear that potential biological control agent for CLR naming *Lecanicilliumlecanii*or*verticiliums*ppis mistaken andcurrently the name changed to *Akanthomyceslecanii*, according to a recently published phylogenetic study [33], used as label in many cases, is inappropriate. Although *Akanthomyces*former*Lecanicillium* species are commonly associated with insects and mushrooms [28], the present work indicates that *A. lecanii* is absent from CLR pustules collected from Ethiopia, strongly suggesting that the name has been incorrectly associated to *H. vastatrix*. This is also indirectly supported by [19] single molecule- DNA sequencing study. A consequence result, of such mistaken taxonomic treatment adopted in the past is most of the literature dealing with the evaluation of such fungi, as biocontrol of coffee rust with mycoparasites should now be regarded as being of little use. Particularly since reference cultures haven't been deposited, no matter how promising they were.

Interestingly, two main studies have been published involving surveys and attempted identification of *H. vastatrix*mycoparasites. One was conducted before the "molecular era of mycology" by [18] involving morphological information alone - and the other, more recent, based on modern single molecule-DNA sequencing [19] excluding any cultural and morphological information. The present work helps filling the gap between those two studies. Carrión & Rico-Gray's study was pioneer and correctly indicated that there was more diversity of maycoparasities on CLR infected leaves, but, lacked in precision because of the absence of molecular evidence for placement and separation of distinct taxa. Although it can be expected that the work by [19] will be followed by a culture based polyphasic approach to the study of the identity of the fungicolous fungi on CLR in Mexico and Puerto Rico.

In this study, it is shown that the diversmycoparasites on coffee leaf rust was identified and belong to a number of distinct genera. They include taxa *Acremonium, Alternaria, Cladosporium, Digitopodium, Fusarium, Gonatophragmium, Lecanicillium/Akanthomyces, Phoma, Pleurodesmospora, Sarcopodium*and*Simplicillium*. The *Lecanicillium*-like fungi obtained from coffee leaf rust from this study were found to belong to three different genera: *Akanthomyces*, *Simplicillium* and *Ovicillium*. Until recently *Akanthomyces* was a genus including only arthropod parasites. Finding *Akanthomyces* in such a distinct substrate, suggests that fungi in this genus have jumped between rather different niches. Morphology of *Akanthomyces* species isolates from *H. vastatrix* mostly overlap each other and also resemble that of *A. uredinophilum* isolated from *Coleosporium*sp. and*Pucciniastrumagrimoniae* in Korea [30]. However, there are some morphological features which may be used for distinguishing different species. The type of conidiogenesis, conidial and phialides size and conidial dimorphism are examples of discriminating characters.

Two of known species of *Simplicillium* obtained in this study (*S. lanosoniveum*and *S. lamellicola*) had been reported as mycoparasites of various fungi, including *H. vastatrix* [28, 19]. Moreover, *S. subtropicum* was isolated from soil in Japan (Nonake*et al.,* 2013). They may represent a polyphagous group of mycoparasites, possibly in contrast to the more specialized *Akanthomyces*. *Simplicillium* species can be distinguished using morphological characteristics and *ITS* sequences [31]. Morphological and phylogenetic analyses show that *Simplicillium*sp. *isolate I* differs from *S. coffeanum* a recently described endophyte species described from

Coffeaarabica in Brazil [35]. Differently from *Simplicillium*, phylogenetic analyses of *Akanthomyces*have previously been based on the *ITS* [28] and multilocus phylogeny using *SSU*rRNA, *LSU*rRNA, *TEF*, *RPB1* and *RPB2* [30, 36, 37]. Circumstantial evidence that *S. lanosoniveum* is a mycoparasite of *H. vastatrix* relied on repeated association between the two fungi alone. But this was confirmed by [38] who presented ultrastructural microscopic evidence of penetration of urediniospores of *Phakopsorapachyrhizi* by *S. lanosoniveum* through germ pores of urededinio spores and signs of organelle degradation 24h after inoculation.

The recognition of *Pleurodesmospora* as mycoparasitic on *H. vastatrix*was yet good finding resulting from this activity. *Pleurodesmospora*was a monotypic genus based on the species *P. coccorum* a species obtained from various hosts including insects and fungi, but mostly from arthropods [34]. [29], initially transferred a strain, obtained from the black mildew *Meliola* sp., to *Aphanocladiummeliolae*, but later corrected the earlier diagnosis with base on morphological characters based on denticles of conidiophores.

4. Conclusion

The discovery of number of fungal taxa from such a microniche as *H. vastatrix*sori is extraordinary and indicates that mycoparasites are ultra-diverse group of fungi. This study only involved a major coffee growing limited sampling area in Ethiopia. It is likely that expanding such surveys will also increase the number of taxa on CLR. Biological control of CLR with mycoparasites remains promising possibility for the future. It may represent a much-needed alternative to other management parctices. Therefore, taxonomic and precise identification of these mycoparasites should be supported with appropriate molecular tools and the phylogenic tree should be developed. Nevertheless, without firm taxonomic knowledge and precise identification of potential biocontrol agents, chances of consistent progress are likely to be low.

Acknowledgements

The author want to acknowledge and thank Ethiopian Institute of Agricultural Research, Plant Protection Process for financial and facility support. Moreover, the author was grateful to Jimma Agricultural Research Center, Plant Pathological Laboratory staffs, for providing cooperation during conducting field and laboratory activity.

References

[1] Batista KD, Araujo WL, Antunes WC, Cavatte PC, Moraes GA, Martins SC. 2012. Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors, Trees 26: 459–468.

[2] Somarriba, E., Harvey, C., Samper, M., Anthony, F., Gonzalez, J., Staver, C. and Rice, R. A. 2004. Biodiversity conservation in neotropical coffee (*Coffeaarabica*) plantations. edt: G. Schroth, G. A. B. da Fonseca, C. Harvey, C. Gascon, H. L. Vasconcelos, and A. M. N. Izac,. *Agroforestry and biodiversity conservation in tropical landscapes.* Island Press, Washington, D.C.

[3] Rodrigues-Junior, C. J. 1990. Coffee rust: history, taxonomy, morphology, distribution and host resistance. *Fitopatol Bras,* 15: 5–9.

[4] Menezes-Silva, P. E., LMPV, Sanglard, Ávila, R. T., Morais, L. E., Martins, S. C. V., Nobres, P., Patreze, C. M., Ferreira, M. A., Araújo, W. L., Fernie, A. L. and DaMatta, F. M. 2017. Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. *J. Exp. Bot,* 68: 4309–4322.

[5] Zambolim L. 2016. Current status and management of coffee leaf rust in Brazil. Trop Plant Pathol 41: 1–8.

[6] AvelinoJ, Cristancho M, Georgiou S, Imbach P, Aguilar L, Bornemann G, Läderach P, Anzueto F, Hruska AJ, Morales C. 2015. The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Security* 7: 303-321.

[7] Bhat SS, Naidu R, Daivasikamani S. Nirmala K. 2000. Integrated disease management in coffee. In: IPM System in Agriculture – Cash Crops. Volume 6.

[8] Capucho AS, Zambolim L, Cabral GC, Maciel ZE, Caixeta ET. 2013. Climate favorability to leaf rust in Conilon coffee. Austral Plant Pathol 24: 511–514.

[9] Honorato JJ, Zambolim L, Aucique Pérez CE, Resende RS, Rodrigues FA. 2015. Photosynthetic and antioxidative alterations in coffee leaves caused by epoxiconazole and pyraclostrobin sprays and *Hemileiavastatrix*infection. Pest. Biochem. Physiol. 123: 31–39.

[10] Avelino, J., Cristancho, M., Georgiou, S., Imbach, P., Aguilar, L., Bornemann, G., Läderach, P., Anzueto, F., Hruska, A. J. and Morales, C. 2015. The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Security*, 7: 303-321 https://doi.org/10.1007/s12571-015-0446-9.

[11] Zambolim, L. 2016. Current status and management of coffee leaf rust in Brazil. *Tropical Plant Pathology*, 41: 1-8.

[12] Dangour, A. D., Green, R., Sutherland, J., Watson, L. and Wheeler, T. R. 2015. Health impacts related to food and nutrition insecurity. In: Levy, B. S. and Patz, J. A. (Eds). *Climate change and public health. Oxford University Press*. 184–194.

[13] Carvalho, C. R, Fernandes, R. C., Carvalho, G. M. A., Barreto, R. W. and Evans, H. C. 2011. Cryptosexuality and the genetic diversity paradox in coffee rust, Hemileiavastatrix. *PLoS ONE 6* (11): 26387.

[14] Varzea VMP, Marques DV. 2005. Population variability of *Hemileivastatrix*vs coffee durable resistance. In: Zambolim, L., Maciel-Zambolim, E., Várzea, V. M. P. (Eds) Durable resistance to coffee leaf rust. UFV, Viçosa, pp 53–74.

[15] Zambolim, L., Maciel, Z. E., Vale, F. X. R., Pereira, A. A., Sakyiama, N. S. and Caixeta, E. T. 2005. Physiological races of *Hemileiavastatrix* in Brazil: physiological variability, current situation and future prospects. In: Zambolim L, Maciel-Zambolim E, Várzea V. M. P. (Eds) *Durable resistance to coffee leaf rust. UFV, Viçosa*, pp: 53–74.

[16] Cabral PGC, Maciel ZE, Zambolim L, Lelis TP, Capucho AS, Caixeta ET. 2009. Identification of a new race of *Hemileiavastatrix*in Brazil. Austral Plant Dis Notes 4: 129–130.

[17] Daivasikamani S, Rajanaika A. 2009. Biological control of coffee leaf rust pathogen, *Hemileiavastatrix*Berkeley and Broome using *Bacillus subtilis*and *Pseudomonas fluorescens*. J Biopest 2: 94–98.

[18] Carrión G, Rico-Gray V. 2002. Mycoparasites on the coffee rust in Mexico. Fungal Diversity 11: 49–60.

[19] James TY, Marino JA, Perfecto I, Vandermeer J. 2016. Identification of putative coffee rust mycoparasites via single-molecule DNA sequencing of infected pustules. Appl Environ Microbiol 82: 631–639.

[20] Vandermeer J, Perfecto I, Liere H. 2009. Evidence for hyperparasitism of coffee rust (*Hemileiavastatrix*) by the entomogenous fungus, *Lecanicilliumlecanii*, through a complex ecological web. Plant Pathol 58: 636–641.

[21] Shiomi HF, Silva HS, Melo IS, Nunes FV, Bettiol W. 2006. Bioprospectingendophytic bacteria for biological control of coffee leaf rust. Sci. agric. 63: 32–39.

[22] Haddad F, Maffia LA, Mizubuti ESG, Teixeira H. 2009. Biological control of coffee leaf rust by antagonistic bacteria under field conditions in Brazil. Biol Control 49: 114–119.

[23] Dhingra OD, Sinclair JB. 1996. Basic plant pathology methods. Boca Raton: CRC Press. 434p.

[24] Nirenburg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Canadian Journal of Botany 59: 1599–1609.

[25] Waller, J. M., Bigger, M. and Hillocks, R. J. 2007. Coffee Pests, Diseases and their Management. Wallingford, UK: *CAB International*.

[26] Rayner RW. 1970. A mycological colour chart. Common wealth Mycological Institute, Kew, Surrey. British Mycological Society.

[27] Sun, J. Z., Liu, X. Z., Hyde, K. D., Zhao, Q., Maharachchikumbura, S. S. N., Camporesi, E., Bhat, J., Nilthong, S. and Lumyong, S. 2017. *Calcarisporiumxylariicola* sp. nov.and introduction of *Calcarisporiaceae* fam. nov.inHypocreales. *Mycological Progress*. 16: 433–445.

[28] Zare, R. and Gams, W. 2001. A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 71: 1–50.

[29] Gams, W. 1971. *Cephalosporium*-artigeSchimmelpilze (Hyphomycetes). *Gustav Fischer Verlag, Stuttgart,* 262 pp.

[30] Park, M. J., Hong, S. B. and Shin, H. D. 2015. *Lecanicilliumuredinophilum* sp. nov.associated with rust fungi from Korea. *Mycotaxon*, 130: 997–1005.

[31] Nonaka, K., Kaifuchib, S., Ômuraa, S. and Masuma, R. 2013. Five new *Simplicillium* species (Cordycipitaceae) from soils in Tokyo, Japan. *Mycoscience*, 54: 42–53.

[32] Ward, N. A., Schneider, R. W. ameAime, M. C. 2011. Colonization of soybean rust sori by *Simplicilliumlanosoniveum*. *Fungal Ecology*, 4: 303–308.

[33] Kepler, R. M., Luangsa, A. J., Hywel, N. L., Quandt, C. A., Sung, G. H., Rehner, S. A., and Chen, M. 2017. A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus*, 8: 335-353.

[34] Samson, R. D., Gams, W. and Evans, H. C. 1980. *Pleurodesmospora*, a new genus for the entomogenoushyphomycete *Gonatorrhodiellacoccorum. Persoonia*, 11: 65-69.

[35] Gomes, A. A. M., Pinho, D. B., Cardeal, Z. L., Menezes, H. C., Queiroz, M. V. and Pereira, O. L. 2018. *Simplicilliumcoffeanum*, a new endophytic species from Brazilian coffee plants, emitting antimicrobial volatiles. *Phytotaxa* 333 (2): 188–198.

[36] Chen, W. H., Han, Y. F., Liang, Z. Q. and Jin, D. C. 2017. *Lecanicilliumaraneogenum* sp. nov., a new araneogenous fungus. *Phytotaxa,* 305 (1): 029–034.

[37] Chiriví-Salomón, J. S., Danies, G., Restrepo, S. and Sanjuan, T. 2015. *Lecanicilliumsabanense* sp. nov. (Cordycipitaceae) a new fungal entomopathogen of coccids. *Phytotaxa,* 234: 63–74.

[38] Gauthier, N. W., Maruthachalam, K., Subbarao, K. V., Brown, M., Xiao, Y., Robertson, C. L. and Schneider, R. W. 2014. Mycoparasitism of *Phakopsorapachyrhizi* the soybean rust pathogen, by *Simplicilliumlanosoniveum*. *Biological Control,* 76: 87-94.