Patellariopsidaceae Fam. Nov. With Sexual-Asexual Connection and a New Host Record for *Cheirospora botryospora* (Vibrisseaceae, Ascomycota)

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Helotiales is a polyphyletic order of Ascomycetes. The paucity of relevant molecular data and unclear connections of sexual and asexual morphs present challenges in resolving taxa within this order. In the present study, Patellariopsidaceae fam. nov., the asexual morph of *Patellariopsis atrovinosa*, and a new record of *Cheirospora botryospora* (Vibrisseaceae) on *Fagus sylvatica* (Fagaceae) from Italy are discussed based on morphology and molecular phylogeny. Phylogenetic analyses based on a combined sequence dataset of LSU and ITS were used to infer the phylogenetic relationships within the Helotiales. The results of this research provide a solid base to the taxonomy and phylogeny of Helotiales.

Keywords: Ascomycetes, Cheirospora botryospora, Leotiomycetes, Pezizomycotina, sporodochium

INTRODUCTION

The Leotiomycetes (Pezizomycotina) is a very diverse class and was erected when the super-class Leotiomyceta was split into seven classes by Eriksson & Winka (Eriksson and Winka, 1997). Leotiomycetes currently comprises 13 orders, out of which eight are monotypic, while over 200 genera are represented by one species only (Baral, 2016; Wijayawardene et al., 2018; Ekanayaka et al., 2019; Johnston et al., 2019). Among the orders in Leotiomycetes, Helotiales consists of the highest number of genera, *incertae sedis* within the familial rank (ca. 90–151) (Baral, 2016; Quijada et al., 2018; Wijayawardene et al., 2018). Hawksworth (2001) estimated that Helotiales consists of 70,000 species. Only 2,334 species belonging to 423 genera in 25 families have been recorded in Helotiales. This constitutes half of all known species in Leotiomycetes (Ekanayaka et al., 2019).
Recent phylogenetic studies based on ribosomal DNA analyses have reported the polyphyletic nature of Helotiales (Ekanayaka et al., 2019; Johnston et al., 2019). The lack of knowledge between asexual and sexual morph connections complicates the systematics of this order (Wang et al., 2006b). Many helotialean fungi are known based on a sexual morph, with their asexual morphs being either undiscovered or assumed to have been lost in evolution (Wang et al., 2006b). On the other hand, it is suggested that asexual morphs from various environmental samples are members of Helotiales, without mention of their sexual morphs (Sutton and Hennenber, 1994; Marvanova et al., 1997).

Helotiales is the largest group of non-lichen forming ascomycetes and occur in a wide range of niches (Ekanayaka et al., 2017; Wijayawardene et al., 2017). The members of Helotiales are recorded as plant pathogens, endophytes, nematode-trapping fungi, mycorrhizae, fungal parasites, terrestrial and aquatic saprobes, root symbionts and wood rot fungi (Wang et al., 2006a).

The objectives of this study are to introduce a new family with their sexual-axexual inter-connection and to provide a new host record for Cheirospora in Vibriisaceae.

### MATERIALS AND METHODS

#### Plant Sample Collection, Morphological Studies and Isolation of Pure Culture

Dead aerial branches of *Fagus sylvatica* L. (Fagaceae) and *Corylus avellana* L. (Betulaceae) were collected from Passo la Calla, Stia (province of Arezzo [AR]) Italy and Fiumicello di Premilcuore (province of Forlì-Cesena [FC]) Italy, respectively. Specimens were preserved and observed following the method of Karunarathna et al. (2017). Hand-cut sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. Specimens were examined with a Nikon ECLIPSE 80i compound microscope and photographed with a Canon EOS 600D digital camera fitted to the microscope. Measurements of morphological characteristics were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 (Adobe Systems, United States).

Single spore isolation was carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and grown at 10–16°C. Colony color and other characteristics were observed and measured after 1 week and 3 weeks. The specimens were deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. Living cultures were deposited in Mae Fah Luang Culture Collection (MFLUCC). Facesoffungi (FoF) and Index Fungorum numbers (IF) were acquired as in Jayasiri et al. (2015) and Index Fungorum (2019).

#### DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from fresh fungal mycelium grown on PDA media at 16°C for 4 weeks using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, China) following the instructions of the manufacturer.

The DNA amplification was performed by polymerase chain reaction (PCR). A partial sequence of the LSU rRNA gene region was amplified using the primer pair LR0R and LRS (Vilgalys and Hester, 1990). The internal transcribed spacer regions (ITS1, 5.8S, ITS2) were amplified using the primer pair ITS5 and ITS4 (White et al., 1990). PCR was carried out following the protocol of Phookamsak et al. (2014). The quality of PCR products was checked by gel electrophoresis on 1% agarose gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (Shanghai Sangon Biological Engineering Technology & Services Co., Shanghai, China). The sequence data acquired were deposited in GenBank (Table 1).

Sequencing of the ITS region of strain MFLUCC 17-1411 was failed due to an intron, of about 1.4 kb in length, positioned between the binding site of primer ITS5 and the start of the ITS region. To obtain a double-stranded ITS sequence, a piece of sporodochium <0.5 mm³ was removed from the specimen and added to a reaction tube with 5 µl of sterile distilled water (dH²O). The soaked specimen was frozen (−20°C) and thawed (+20°C) for five times and 0.5 µl of the solution was used for amplification. Based on initially obtained sequence information, a forward primer (Karu_F01: 5′-CAATGATCAAAGCAGTTGCG-3′) was designed, which has similar properties as the ITS4 primer and binds to the intron sequences close its 3′-end. The PCR reaction included 0.5 µl of the DNA-containing solution, 0.25 µl of each primer (Karu_F01 and ITS4; 10 µM, each), 5.25 µl of sterile dH₂O and 6.25 µl of the GoTaq® G2 Hot Start Colorless Master Mix (PRoMEGA; GoTaq® Hot Start Polymerase in 2 × Colorless GoTaq® Reaction Buffer (pH 8.5), 400 µM dNTPs, 4 mM MgCl₂). The PCR commenced with 3 min denaturation at 95°C, followed by 33 amplification cycles (27 s at 94°C, 60 s at 56°C, and 90 s at 72°C) and a final elongation at 72°C for 7 min. The PCR products were cleaned by successive incubation at 37°C for 30 min and 80°C for 15 min after adding 0.2 µl exonuclease I (20,000 U/ml), 0.2 µl Shrimp-Alkaline-Phosphatase (1,000 U/ml; both New England Biolabs) and 1.6 µl sterile dH₂O to 5 µl of PCR product. Purified PCR products were sequenced by the sequencing service of the Ruhr-Universität Bochum using a Genetic Analyzer 3130xl (Applied Biosystems).

#### Phylogenetic Analyses

Phylogenetic analyses were conducted separately based on LSU and ITS gene sequence data. Reference sequences (Table 1) of representative families in Leotiomyces were retrieved from GenBank. The related sequences were obtained from a BLAST search and from recently published data (Ekanayaka et al., 2019). Individual datasets for LSU and ITS genes were aligned using the default settings of MAFFT V.7.036 (Katoh et al., 2018) and improved manually where necessary using Bioedit. Aligned gene regions were concatenated using Bioedit v7.2 (Hall, 1999) and analyzed.

1http://mafft.cbrc.jp/alignment/server/
TABLE 1 | Taxa used in the phylogenetic analyses and their corresponding GenBank numbers (Newly generated sequences are indicated in black bold).

| Species                          | Strain/ Voucher No. | GenBank Accession No. |
|----------------------------------|---------------------|-----------------------|
|                                  | ITS                 | LSU                   |
| Acidornelania paniculica        | 61R8                | KF874619              |
| Amilocosica sp.                 | KUS_F51377          | JN006992              |
| Aquaporuticum pinicola          | ATCC_MYA-4213       | NR_111345             |
| Arcanophora aurata              | KUS-F52038          | JN033933              |
| Arcanophora aurelia             | KUS-F51520          | JN033409              |
| Ascorycnidula cylichnium        | KUS_F52351          | JN086709              |
| Ascorycnidula sarcoides         | HKAS 90651          | MK591999              |
| Bryocladuscus campyloto         | POD-101074          | JX93084               |
| Bryoglossum gracile             | DAO178087           | AY78926               |
| Bugliaria pulla                 | DHP 15-215          | KUB45540              |
| Cadophora fastigiata            | CBS 869.69          | MH895469              |
| Cadophora malorum               | A163                | AY249057              |
| Cheiropsis botryospora          | MFUCC 17-1399       | MN535816              |
| Chlorosplenium chloro           | BHI_F7394           | MG553994              |
| Chlorosplenium chloro           | BHI_F7394a          | MG553993              |
| Crucellorpus umtumvariae        | CBS 125742          | MH863669              |
| Dicenthaosa rubra                | MFU 18-1828         | MK591979              |
| Disculina boudieri              | HB3426              | KC412001              |
| Drepanopeziza ribis             | CBS 200.36          | MH855774              |
| Drepanopeziza salicis           | CBS 405.64          | MH858467              |
| Enceliopsis rhododendri         | CBS 905.69          | MH854799              |
| Geniculospora grandis           | CBS 261.84          | MH873440              |
| Godronia rubra                   | CBS 162.66          | MH85762               |
| Gradinella coracina             | ILS60491            | JN012009              |
| Haplographum delicatum          | CBS 196.73          | MH872362              |
| Heterosphaeria linearne         | MFU 15-2764         | MK591955              |
| Heterosphaeria patella          | G.M. 2014-08-04-1   | MF196187              |
| Hylacosphaena bicolor           | CBS 144009          | MH819392              |
| Hylacosphaena vitreola          | CBS 126276          | MH863954              |
| Hydrocora chactoleadia          | CCM F-10890         | KC834062              |
| Hymenotremellina madsonii       | ICMP 15648          | KJ606676              |
| Lachnum abnorme                 | KUS-F52080          | JN033935              |
| Lambertella seddsosa            | WU 3244             | KY493962              |
| Loramyceus junicola             | CBS 293.52          | MH85704               |
| Loramyceus macrosporus          | CBS 235.53          | MH857170              |
| Mitrulina ushualae              | POD-10964           | K2C73438              |
| Motisia cinerea                 | CBS 128349          | JF514865              |
| Neopyrenopeziza nigripigmentata | MFU 16-0599         | NR_163783             |
| Patellariosis atrovirens        | G.M. 2014-06-15-1   | KY462814              |
| Patellariosis atrovirens        | G.M. 2016-05-04-1   | KU70066               |
| Patellariosis atrovirens        | MFUCC 17-1411       | MN535817              |
| Patellariosis dennisi           | CBS 174.66          | MH858765              |
| Patellariosis dennisi           | G.M. 2017-09-04-3   | MK120898              |
| Peltigeromyces sp.              | HB 6432             | KO090803              |
| Phialocephala scopiformis       | CBS 468.94          | NR_161186             |
| Phialocephala urceolata         | UAM1 10827          | NR_111285             |
| Pulvinata tormentosa            | MFU 18-1819         | MK591965              |
| Rhexcercosporidium carotae      | CBS 418.65          | MH858647              |
|                                  |                     | MK584938              |

[Continued]

Initial alignment of LSU region included 7163 base pairs and ITS region included 6619 base pairs. In the phylogenetic analysis, LSU and ITS regions consisted of ambiguously aligned regions. Hence, manual alignment was performed where necessary and some unambiguous regions were removed from the analysis. The removed regions of LSU data set are 0–2658, 2738–2888, 2897–2955, 3037–3220, 3285–3338, 3421–3464, 3723–3730, 4029–4103, 4129–4562, 4581–7163. The excluded regions of the ITS data set are 0–146, 149–173, 181–2164, 2199–2234, 2253–2307, 2714–2923, 2936–2971, 2986–3052, 3065–3113, 3132–3270, 3331–6619. In the final alignment, LSU and ITS regions consist of 988 and 587 bp, respectively.

Phylogenetic constructions of combined gene trees were performed using maximum likelihood (ML), maximum parsimony (MP) and bayesian inference (BI) criteria. Maximum likelihood trees were generated using the RaxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al., 2008; Stamatakis, 2014) in likelihood trees were generated using the RAxML-HPC2 on parsimony (MP) and bayesian inference (BI) criteria. Maximum likelihood trees were performed to determine whether the trees inferred under different optimality criteria were different.

Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Nylander, 2004) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and MrBayes v. 3. Bayesian inference analysis was conducted with MrBayes v. 3.1.2 (Nylander, 2004; Swoford, 2002) using the heuristic search option, random stepwise addition, and 1000 replicates, with maxtrees set at 1000. Descriptive tree statistics for parsimony such as Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria.

The Kishino Hasegawa tests (Kishino and Hasegawa, 1989) were performed to determine whether the trees inferred under different optimality criteria were different.
gamma distribution with a proportion of invariant site (TrN+I+G) was applied for LSU gene region and symmetrical model with discrete gamma distribution coupled with a proportion of invariant sites (TIM2ef+I+G) was applied for ITS gene region. Two parallel runs were conducted, using the default settings, but with the following adjustments: Four simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation. The distribution of log-likelihood scores indicated the stationary phase for each search and were used to decide if extra runs were required to achieve convergence, using Tracer v. 1.6 (Rambaut et al., 2014). The first 20% of the generated trees represented the burn-in phase and were discarded. The remaining trees were used to calculate posterior probabilities of the majority rule consensus tree.

Phylogenetic Analyses
Phylogenetic trees obtained from LSU and ITS single gene analyses as well as the combined gene analyses share similar overall topologies at the generic level and are in agreement with previous studies (Johnston et al., 2014; Crous et al., 2015; Ekanayaka et al., 2019). The concatenated LSU and ITS dataset consisted of 58 taxa.

The RAxML analysis of the LSU dataset yielded a best scoring tree (Figure 1) with a final ML optimization likelihood value of $-16138.064322$. The matrix had 758 distinct alignment patterns, with 19.46% of undetermined characters or gaps. Parameters for the GAMMA+P-Invar model of the LSU and ITS were as follows: Estimated base frequencies; $A = 0.247456$, $C = 0.223115$, $G = 0.280101$, $T = 0.249328$; substitution rates indicated the stationary phase for each search and were used to decide if extra runs were required to achieve convergence, using Tracer v. 1.6 (Rambaut et al., 2014). The first 20% of the generated trees represented the burn-in phase and were discarded. The remaining trees were used to calculate posterior probabilities of the majority rule consensus tree.

Sexual Morph
Patellariopsis forms a well-supported (ML 74/BYPP 0.98) clade sister to Chlorospleniaeaceae, Loramycetaceae, Mollisiaceae and Vibrisseaceae. In Index Fungorum, Patellariopsis is included in Dermateaceae, but Wijayawardene et al. (2017) placed Patellariopsis in Helotiales genera incertae sedis based on morphology. Furthermore, in Ekanayaka et al. (2019), this clade was denoted as separate taxa based on phylogenetic analyses. Hence, we introduce this clade as a new family based on morphology and phylogeny.

Type Genus
Patellariopsis Dennis, Kew Bull. 19(1): 114 (1964)

Notes
Patellariopsis forms a well-supported (ML 74/BYPP 0.98) clade sister to Chlorospleniaeaceae, Loramycetaceae, Mollisiaceae and Vibrisseaceae. In Index Fungorum, Patellariopsis is included in Dermateaceae, but Wijayawardene et al. (2017) placed Patellariopsis in Helotiales genera incertae sedis based on morphology. Furthermore, in Ekanayaka et al. (2019), this clade was denoted as separate taxa based on phylogenetic analyses. Hence, we introduce this clade as a new family based on morphology and phylogeny.

Patellariopsis Dennis, Kew Bull. 19(1): 114 (1964)

Index Fungorum number: IF556217, Faces of Fungi number: FoF06575

The genus classified under Helotiales genera incertae sedis, Leotiomyctes (Wijayawardene et al., 2018). The type species is Patellariopsis clavispora (Berk. & Broome) Dennis. Five species are recorded in Index Fungorum (2019), $P$. atrovinosa (A. Bloxam ex Curr.) Dennis, $P$. carnea G.W. Beaton, $P$. clavispora (Berk. & Broome) Dennis, $P$. dennisii (E. Müll. & Hütter) Schläpf.-Bernh., and $P$. indica A. Pande. We were unable to find any reported described asexual morphs of Patellariopsis in the literature.

Taxonomy
In this section, Patellariopsidaceae Karun., Camporesi & K.D. Hyde, fam. nov. and the new record of Cheir ospora botryospora are described and illustrated. Helotiales includes several families with sporodochial asexual morphs viz. Gelatinoidiscaceae, Helotiaeaceae and Mollisiaceae. A morphological comparison among members of the Helotiales is given in Tables 2, 3.

Notes
Patellariopsis forms a well-supported (ML 74/BYPP 0.98) clade sister to Chlorospleniaeaceae, Loramycetaceae, Mollisiaceae and Vibrisseaceae. In Index Fungorum, Patellariopsis is included in Dermateaceae, but Wijayawardene et al. (2017) placed Patellariopsis in Helotiales genera incertae sedis based on morphology. Furthermore, in Ekanayaka et al. (2019), this clade was denoted as separate taxa based on phylogenetic analyses. Hence, we introduce this clade as a new family based on morphology and phylogeny.

Patellariopsis atrovinosa (A. Bloxam ex Curr.) Dennis, Kew Bull. 29(1): 167 (1974)

Index Fungorum number: IF 319233, Facesoffungi number: FoF06574

Sexual morph: Refer to Dennis (1974). Asexual morph:
**FIGURE 1** | RAxML tree based on a combined dataset of LSU and ITS partial sequence data. Bootstrap support values for maximum likelihood equal to or higher than 70%, maximum parsimony equal to or higher than 70%, and Bayesian posterior probabilities equal to or greater than 0.90 are displayed on the nodes, respectively. Newly generated sequences are indicated in white. The tree is rooted to Lambertella seditiosa and Rutstroemia longipes.

**Sporodochium** 33–37 µm high, 278–355 µm diam. ($\bar{x} = 35 \times 324 \mu m$, $n = 5$), sub-epidermal or sub-peridermal, solitary. **Conidiophores** 41–78 $\times 1–1.5 \mu m$ ($\bar{x} = 58 \times 1.3 \mu m$, $n = 20$) cylindrical, straight or slightly curved, branched over the conidiophore, sepetate, hyaline, expanding toward the apices, smooth. **Conidiogenous cells** 1.5–2 $\times 1–1.6 \mu m$.
| Family                                      | Hyphomycetous conidiomata | Conidiophore | Conidiogenous cell | Conidia                                      |
|--------------------------------------------|---------------------------|--------------|--------------------|----------------------------------------------|
| Amicodiscaceae (Ekanayaka et al., 2019)    | Hyphomycetous/stromatic   | Hyaline to cinnamon-colored glistening slimy heads, straight or flexuous, dark brown and thick-walled except at the apex | Terminal, cylindrical, sympodially proliferate | Cylindrical to cylindric-ellipsoidal, hyaline, aseptate, thin-smooth walled. |
| Discinellaceae (Ekanayaka et al., 2019)    | Hyphomycetous conidiomata | Holoblastic  |                    | Mostly hyaline, sometimes branched, filiform, globose, or fusoid some form dimorphic conidia |
| Drepanopezizaceae (Yoshikawa and Yokoyama, 1992; König et al., 2017) | Hyphomycetous/acervulus   | Holoblastic  |                    | Sometimes two types. Macroconidia-ellipsoid to fusoid, slight curved. Microconidia-ellipsoid to bacilliform Aseptate, hyaline and subglobose |
| Gelatinodiscaceae (Seaver, 1938; Johnston et al., 2010) | Sporodochial           |              |                    |                                              |
| Helotiales (Peláez et al., 2011; Jaklitsch et al., 2018) | Hyphomycetous, sporodochial or synnematal | Macroconidia – holoblastic/Microconidia – phialidic | Macroconidia – hyaline, filiform or stauroporous, dark brown, in chains, bulbs or solitary on conidiophores and 3–5-septate. Microconidia rarely pigmented, multicellular and appended |
| Heterosphaeriaceae (Leuchtmann, 1987)      | Synanamorphic, hyphomycetous acervulus and ceolomycteous |                     |                    |                                              |
| Hyaloscyphaceae (Jaklitsch et al., 2016)   | hyphomycetous sporochoical | Phialidic    |                    | Aseptate, hyaline or brown, branched and muriform or in chains |
| Hydrocinaceae (Ekanayaka et al., 2019)     | Hyphomycetous             | Long, hyaline, simple or branched, filiform | Proliferate, sympodial. | Filiform, branched, sometimes septate and fragment into microconidia. |
| Loramyctaceae (Digby and Goos, 1967; Walsh et al., 2014) | anguillospora-like | Conidiophores are simple or occasionally branch. Conidiogenous cells are hyaline and straight. Conidia are globose, sub-ellipsoid or sigmoid and hyaline | Conidiogenous cells are hyaline and straight. Conidia are globose, sub-ellipsoid or sigmoid and hyaline | Conidia are globose, sub-ellipsoid or sigmoid and hyaline |
| Mollisiaceae (Sutton and Ganapathi, 1978; Butin et al., 1996; Grünig et al., 2002) | Sporodochial | Hyaline to brown |                    | Unicellular, ellipsoid or phragmosporous, hyaline or brown and also in chains |
| Patellariopsidaceae                         | Sporodochium              | Cylindrical, straight or slightly curved, branched over the conidiophore, septate, hyaline, expanding toward the apices, smooth | Holoblastic, polyblastic, cylindrical, integrated, hyaline, smooth. | Sphaerical, acropetal, branched chains, globose to cylindrical mass of small, thick-walled, dark brown, septate, eguttulate, smooth, cheirid, conidium-complex |
| Phialcephala urceolata clade (Wang, 2009)  | Hyphomycetous              | Hyaline to darkly pigmented, septate and mononematos | Phialidic and conidiogenous cells are flask to urn-shaped and each with a prominent cylindrical and hyaline collarette | Globose, pedicellate and single or adhering in small clusters at the phialide apex |
| Plamtnetrulaeaceae (Marvanová and Bárlocher, 2001; Goodwin, 2002; Gönczöl and Révay, 2003; Grünig et al., 2011; Gonçalves et al., 2012; King et al., 2013; Travadon et al., 2015; Duarte et al., 2016; Walsh et al., 2018) | hyphomycetous or ceolomycteous | Hyaline to brown |                    | Ellipsoid to rod-shaped or filiform with pointed apices and 0–1-septate |
| Solenopeziaceae (Ekanayaka et al., 2019)   | Conidiomata hyphomycetous | Simple, sparsely branched or absent | Cylindrical to subclavate, sometimes apically slightly swollen | Hyaline or black, septate, branched, lunate, sometimes formed in a chain and becoming tortuous and appearing as terminal dictyospores, rarely appended |
| Vitrissaeaeeae (flurraiga and Israel, 1985; Goh and Hyde, 1998; Goh et al., 1998; Kirschner and Oberwinkler, 2001; Shinoy et al., 2010; Hernández-Pestrepo et al., 2012, 2017; Legon, 2012; Crous et al., 2015) | hyphomycetous, phialidic or acervulus | Straight, cylindrical, hyaline and sometimes branched | Holoblastic or polytretic | Ellipsoid or irregular in shape and unicellular or up to 7–septate |
TABLE 3 | Comparison of major sexual morph characteristics of families in order Helotiales based on Ekanayaka et al. (2019).

| Family                | Ascomata                                                                 | Excipulum Peridium                                                                 | Paraphyses                                  | Asci                                      | Ascospores                                                                 |
|-----------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------|-------------------------------------------|---------------------------------------------------------------------------|
| Amicodiscaceae        | Apothecial, cupulate, sessile or sub-stipitate, margins covered by hairs   | Ectal excipulum textura angularis or textura prismatica cells,                      | Filiform, cylindrical, separte, simple     | 8-spored, amyloid, sometimes arising     | Ellipsoid to fusoid, aseptate, guttulate, lemon-yellow pigmented         |
|                       |                                                                           | medullary excipulum loosely arranged hyphae                                        |                                             | from croziers                            |                                                                           |
| Aquapoterium          | Apothecial, cupulate, receptacle, sessile or tipitate,                      | Ectal excipulum textura prismatica cells or a single layer of parallel hyphae with enlarged, globose apices, medullary excipulum reduced or composed of loosely arranged hyphae | Filiform, hyaline, obtuse to lavate at apex, separte, smooth-walled, simple or branched | 8-spored, amyloid or non-amyloid, cylindric-clavate | Ellipsoid to clavate cylindric, hyaline, smooth-walled, 0–1-septate, surrounded by a gelatinous sheath |
| Unguicularia clade    | sessile or tipitate, sometimes margins covered with short cylindrical hairs |                                                                                   |                                             |                                           |                                                                           |
| Arachnopezizaceae     | Apothecial, covered by hairs                                              | Ectal excipulum textura angularis to prismatica cells, medullary excipulum textura prismatica to textura obita cells | Cylindrical, hyaline                        | 8-spored, cylindric clavate, amyloid, arising from croziers | Ellipsoid to fusoid, 0–7-septate                                         |
| Bryoglossaceae        | Apothecial, clavate to apitate or cupulate to turbinate, long stipitate, gelatinous | Ectal excipulum textura porrecta cells, medullary excipulum textura intricata cells | Filiform, swollen at the apex               | 8-spored, amyloid or non-amyloid, arising from croziers | Ellipsoid to fusoid, aseptate, gelatinate                                 |
| Bulgariella clade     | Apothecial or rarely cleistothelial, cupulate, discoid, turbinate or capitate, sessile or stipitate, margins and flanks are covered with hairs | Ectal excipulum textura prismatica cells, textura obita cells, medullary excipulum is composed of cells of textura intricata or textura obita cells | Filiform, lanceolate or cylindrical      | 8-spored, cylindric clavate, amyloid or non-amyloid, arising from croziers | Globose, elipsoid to filiform, separte or aseptate, hyaline or brownish, guttulate |
| Chlorosplenaceae      | Apothecial, cupulate or discoid, sessile or substipitate                   | Ectal excipulum textura angularis cells, medullary excipulum textura intricata cells | Filiform, separte                          | 8-spored, cylindric clavate, amyloid     | Ellipsoid to fusoid, hyaline and smooth walled                           |
| Colipila clade        | Apothecial cupulate, covered by long cylindrical hairs                     | Ectal excipulum and medullary excipulum textura prismatica cells                   | Dimorphic, sub cylindrical and not exceed the length of asci, or broadly lanceolate and exceed the length of asci | 8-spored, cylindric clavate, amyloid, arising from croziers | Ellipsoid to fusoid                                                       |
|Discinellaceae         | Apothecial, discoid to cupulate, circular, gelatinous,                      | Ectal excipulum textura prismatica or textura porrecta cells, medullary excipulum textura intricata to prismatica cells | Filiform, branched at the apex             | 8-spored, cylindrical, amyloid or non amyloid, sometimes arising from croziers | Ellipsoid, aseptate, hyaline, without sheath                              |
|                       | 0–1-septate, smooth-walled                                                |                                                                                   |                                             |                                           |                                                                           |
| Drepanopezizaceae     | Apothecial, cupulate, sessile, mostly immersed                             | A thin layer of textura angularis cells,                                           | Apically slightly swollen, straight         | 4–8– sporated, non amylloid             | Ellipsoid to fusoid, 0–2-septate                                         |
| Gelatinodiscaceae     | Apothecial, cupulate or discoid, some are tremellloid, form cerebiform masses which each lobule contains a tubinate apothecium | Ectal excipulum textura prismatica to textura angularis to globulousa cells, medullary excipulum textura obita to textura porrecta or textura intricata cells | Filiform, cylindrical, apically swollen, guttulate | 8-spored, amyloid, arising from croziers | Ellipsoid to fusoid, hyaline, yellowish or brownish, smooth, with a gelatinous sheath, guttulate, 0–5-septate |
| Godroniaceae          | Apothecial, urceolate, discoid or cupulate, mostly stromatic, erumpent,    | Ectal excipulum textura prismatica to angularis cells, medullary excipulum textura epidermoidea, prismatica to porrecta cells | Filiform or lanceolate, simple or branched, sometimes slightly swollen at the apex          | 8-spored, cylindric clavate, amyloid or non-amyloid                       | Fusoid, hyaline, separte, guttulate                                       |
|                       | sometimes covered with hairs                                              |                                                                                   |                                             |                                           |                                                                           |
| Helotiaceae           | Apothecial, cupulate, discoid, capitate to clavate, turbinate or globose, sessile or tipitate, margins and flanks smooth or covered with hairs | Ectal excipulum textura prismatica, intricata, globulousa-angularis, or tobita cells, medullary excipulum textura intricata or porrecta cells | Cylindrical, separte or aseptate, hyaline to yellowish, guttulate           | 4–8–spored, cylindric-clavate, amyloid or non amyloid, sometimes arising from croziers | Ellipsoid, fusoid or filiform, 1–3-septate, rarely ornamented            |
| Heterosphaeriaceae    | Apothecial, discoid, black, sessile, erumpent, gelatinous                  | Ectal excipulum textura angularis cells, medullary excipulum textura porrecta cells | Clavate contains many guttules             | 8-spored, amyloid, arising from croziers | Aseptate, ellipsoid to fusoid, without gel sheath                        |
| Hyaloscyphaceae       | Apothecial, cupulate or discoid, sessile or substipitate, sometimes         | Ectal excipulum textura globulousa cells, medullary excipulum textura porrecta, intricata to obita cells | Filiform, separte, branched, slightly swollen at the apices                      | 8-spored, cylindric clavate, amyloid, arising from croziers | Ellipsoid to fusoid, aseptate or separte, hyaline                        |

(Continued)
TABLE 3 | Continued

| Family                | Ascomata                                                                 | Excipulum Peridium                                                                 | Paraphyses                                                                 | Asci                                           | Ascospores                                      |
|-----------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Hydrocinaceae         | Apothecial, cupulate, sessile or substipitate                             | Ectal excipulum textura globulosa cells, medullary excipulum textura                 | Filiform, septate, branched, slightly swollen at the apices               | 8-spored, cylindric clavate, amyloid, arising from croziers | Ellipsoid to fusoid, aseptate or septate, hyaline |
| Lachnaceae            | Apothecial or discoid, sessile or stipitate, margins and flanks are covered with hair | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, septate, unbranched, sometimes apically swollen and pigmented   | 8-spored, cylindric clavate, amyloid or non-amyloid, sometimes arising from croziers | Fusiform, septate, sometimes with terminal appendages and gel sheath |
| Loramycetaceae        | Apothecial or perithelial, apothecia cupulate or pulvinate, perithecia    | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, cylindrical, with yellow carotenoid droplets                    | 8-spored, amyloid, cylindrical clavate, mostly arising from croziers | Ellipsoid to long filiform, 0–7-septate, guttulate |
| Mitrulaceae           | Apothecial, clavate, stipitate                                           | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, cylindrical, apically swollen, guttulate                         | 8-spored, cylindrical clavate, amyloid           | Ellipsoid to fusoid, hyaline, 3–7-septate       |
| Mollisiaceae          | Apothecial, discoid covered by hairs,                                     | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, and pigmented at the apices                                     | 8-spored, cylindrical clavate, amyloid           | Ellipsoid to fusoid, hyaline, 3–7-septate       |
| Patellariopsidaceae   | Apothecial, discoid, sessile                                              | Ectal excipulum textura globulosa cells to angularis cells, medullary excipulum     | Filiform, cylindrical, apically swollen, guttulate                         | 8-spored, cylindrical clavate, amyloid           | Ellipsoid to fusoid, hyaline, 3–7-septate       |
| Petigeromyces clade   | Apothecial, cartilaginous, thin, with a large variety of lobes             | Records are not available for micro morphological characters                         |                                                                           |                                                |                                                |
| Phialocephala urceola | clade                                                                      |                                                                                      |                                                                           |                                                |                                                |
| Phialocephala clade   | Apothecial, cupulate, discoid or urn-shaped, sessile or sub stipitate, sometimes covered with pigmented hairs | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, cylindrical or lanceolate, guttulate                             | 8-spored, conical apex, amyloid                 | Ellipsoid to long filiform, 0–3-septate, guttulate |
| Ploetnerulaceae        | Apothecial, cupulate, discoid or urn-shaped, sessile or sub stipitate, sometimes covered with pigmented hairs | Ectal excipulum textura globulosa cells to angularis cells, medullary excipulum textura prismatic cells | Filiform, lanceolate or cylindrical                                       | 8-spored, cylindrical clavate, amyloid or non-amyloid, sometimes arising from croziers | Globose, ellipsoid to fusiform, septate or aseptate, guttulate |
| Solenopziaceae        | Apothecial, cupulate, discoid or pulvinate, sessile or stipitate, sometimes covered with hyaline, whitish, yellow or brown, non-bristle like hairs | Ectal excipulum textura prismatic cells, medullary excipulum textura prismatic cells | Filiform, lanceolate or cylindrical                                       | 8-spored, cylindrical clavate, long stipitate, sometimes amyloid, arising from croziers | Globose, ellipsoid to fusiform, septate or aseptate, guttulate |
| Vibrisseaceae         | Apothecial, cupulate or clavate, sessile to stipitate                     | Ectal excipulum textura globulosa cells to angularis cells, medullary excipulum textura prismatic cells | Filiform, apically slightly swollen, sometimes branched                    | 8-spored, cylindrical clavate, long stipitate, sometimes amyloid, arising from croziers | Globose, ellipsoid to fusiform, septate or aseptate, guttulate |

(\(\bar{x} = 1.8 \times 1.4 \, \mu m, n = 20\)) holoblastic, polyblastic, cylindrical, integrated, hyaline, smooth. *Conidia* 1.5–2.7 \(\times\) 1.5–2.5 \(\mu m\) (\(\bar{x} = 2 \times 2 \, \mu m, n = 40\)), spherical, proliferating with several, short, lateral, acropetal, branched chains. Primary branches in turn develop secondary branches, which eventually form a globose to cylindrical mass of small, thick-walled, dark brown, septate, euguttulate, smooth, cheiriod, conidium-complex.

Colonies growing on PDA becoming 2 cm within 10 days at 16°C, circular, flat, cottony, irregular margin, with less aerial mycelium, olivaceous green to gray from above and dark brown from below.

**Material Examined**

ITALY, Forlì-Cesena [FC], Fiumicello di Premilcuore, dead aerial branch of *Corylus avellana* L. (Betulaceae), 5 September 2015, E. Camporesi, IT 3178 (MFLU 16-2950), living cultures, MFLUCC 17-1411.

**Cheirospora Moug. & Fr., in Fries, Syst. Orb. Veg. (Lundae) 1: 365 (1825)**

Index Fungorum number: IF 7614, Faces of Fungi number: FoF06593

The genus is in Helotiales genera incertae sedis, Leotiomycetes (Wijayawardene et al., 2018). Ekanayaka et al. (2019) placed this genus under Vibrisseaceae. The type species is *C. botryospora* (Mont.) Berk. & Broome. There are four species in Index Fungorum (2019), *C. alni* Shabunin., *C. betulina* (P. Karst.) Kuntze., *C. botryospora* (Mont.) Berk. & Broome and *C. oblonga* (Fuckel) Kuntze.
Cheirospora botryospora (Mont.) Berk. & Broome, Ann. Mag. nat. Hist., Ser. 2 5: 455 (1850)

Index Fungorum number: IF 294800, Facesoffungi number: FoF06594 (Figure 3)

Saprobic on dead branches of Fagus sylvatica L. Sexual morph: unidentified. Asexual morph: Sporodochium 1850–1854 µm high, 3728–3732 µm diam. ($\bar{x}$ = 1852 × 1730 µm, n = 5), sub-epidermal or sub-peridermal, solitary. Conidiophores 171–225 × 3–4 µm ($\bar{x}$ = 198 × 3.5 µm, n = 20) cylindrical, straight or slightly curved, branched only at the base, septate, hyaline, expanding toward the apices, smooth. Conidiogenous cells 8–7 × 10–11 µm ($\bar{x}$ = 7.5 × 10.5 µm, n = 20) holoblastic, polyblastic, cylindrical, integrated, hyaline, smooth. Conidia 10–11 × 9–11 µm ($\bar{x}$ = 10.5 × 10.5 µm, n = 40), sphaerical, proliferating with several, short, lateral, acropetal, branched chains. Primary branches in turn develop secondary branches which eventually form a globose to cylindrical mass of small, thick-walled, dark brown, septate, eguttulate, smooth, cheiroid, conidium-complex, enclosed in a gelatinous sheath.

Colonies growing on PDA to 2 cm diam. within 10 days at 16°C, circular, flat, cottony, irregular margin, with less aerial mycelium, olivaceous green to gray from above and dark brown from below.

Material Examined
ITALY, Province of Arezzo [AR], Passo la Calla - Stia, dead aerial branch of Fagus sylvatica (Fagaceae), 5 September 2015, E. Camporesi, IT 2609 (MFLU 15-2612), ex-type living culture, MFLUCC 17-1399.

DISCUSSION
The highly divergent morphological, ecological and biological characteristics of Helotiales makes it a focus for taxonomic studies in the Leotiomycetes, as it is one of the most problematic groups for traditional classification and molecular phylogeny (Wang et al., 2006a). It is a poorly studied order, within which...
about 19–27% of the genera have an uncertain position at the family level (Baral, 2016). Hence, the taxa in Helotiales have already been subjected to several nomenclatural reinterpretations (Wang et al., 2006a). Lantz et al. (2011) revealed that some genera related to members in Helotiales were traditionally placed in Rhytismatales.

*Patellariopsidaceae* is established herein based on morphological and phylogenetic support. Comparisons of
major sexual and asexual morph characteristics of families in Helotiales are provided in Tables 2 and 3. The asexual morph characteristics of this family are unique in having sporodichium with cheiroid conidium complex. The cheiroid conidium complexes are also present in C. botryospora in Vibriaseae. Patellariopsidaceae differs from Vibriaseae, in having highly branched conidiohores and thicker conidia complexes. Further, the sexual morph of the Patellariopsidaceae shows unique characteristics by having a sessile discoid apothecium, paraphyses with filiform, branched and pigmented apices, cylindric-clavate, amyloid asci and ellipsoid to hyaline septate ascospores. Patellariopsidaceae was further supported by phylogeny. Hence, herein we establish the Patellariopsidaceae under Helotiales.

Most of the Patellariops species were recorded from the United Kingdom with few exceptions (Beaton and Weste, 1978; Farr and Rossman, 2020). Patellariopsis atrovina on Prunus laurocerasus was also reported from the United Kingdom. In our study, we report the asexual morph of P. atrovina on Corylus avellana from Italy. Patellariopsis carnea on dead grass twigs was reported from Australia (Beaton and Weste, 1978). P. clavispora shows a wide host range, which includes Acer sp., Corylus sp., Crataegus sp., Fagus sp., Fraxinus sp., Ligustrum sp., Prunus sp., Quercus sp. and Symphoricarpos sp. from the United Kingdom (Dennis, 1978, 1986) and Mangifera indica from Pakistan (Ahmad, 1978).

Apart from ribosomal RNA sequence data, the use of protein-coding gene phylogenies involving helotialean fungi are slowly emerging (Wang et al., 2006b; Johnston et al., 2014). Most contemporary results suggest that the Helotiales and currently delimited families are not monophyletic and that the highly conserved small subunit (SSU) rRNA gene is not informative enough to resolve these lineages with confidence (Gernandt et al., 2001).

In our phylogenetic analyses, all the Patellariopsis strains available in the GenBank were included. Among them, the phylogenetic placement of P. dennisii (G.M. 2017 09 04.3) is ambiguous. No morphological descriptions are available in the literature for comparison (Ekanayaka et al., 2019; Vu et al., 2019) and the topology obtained in this study is similar to the topology obtained by Ekanayaka et al. (2019). Hence, we suggest the need for having more data to clarify the position of P. dennisii (G.M. 2017-09-04.3). The blast results for the Patellariopsis dennisii (CBS 174.66) strain include several other Ascomycetous fungi. Therefore, Patellariopsis dennisii (CBS 174.66) was excluded in our dataset after the preliminary phylogenetic analyses.

Genealogical Concordance Phylogenetic Species Recognition (GCP SR) analysis using multi-gene concatenated sequences is used to determine the recombination level within phylogenetically closely related species. Under this study three P. atrovina strains MFLUCC 17-1411, G.M. 2016-05-04.1, G.M. 2014-06-15.1, and P. dennisii G.M. 2017 09 04.3 were subjected to the GCP SR analysis. The analysis failed due to the lack of the informative characters in the highly similar sequences of P. atrovina strains MFLUCC 17-1411, G.M. 2016-05-04.1 and G.M. 2014-06-15.1.

Based on phylogenetic analyses, our strain MFLUCC 17-1411 forms a well-supported (ML 100/MP 100/BYPP 1.00) clade with specimens G.M. 2016-05-04.1 and G.M. 2014-06-15.1 (both P. atrovina). The phylogenetic relatedness is supported by the 100% similarity between sequences. The asexual stage of Patellariopsis is not recorded in literature. Hence, no morphological comparison can be done between the strains MFLUCC 17-1411 and P. atrovina. Nevertheless, no scientific evidence was provided to confirm this association. Hence, we justify MFLUCC 17-1411 belongs to the P. atrovina.

Cheirospora botryospora MFLUCC 17-1399 forms a well-supported clade with (ML 100/MP 100/BYPP 1.00) C. botryospora CPC 24607 and this was further supported by morphology. C. botryospora CPC 24607 was isolated from Fagus sylvatica in Germany. Danti et al. (2002) identified an endophytic Cheirospora sp. on Fagus sylvatica from Italy. However, they were unable to identify it to the species level. Therefore, in this study, based on morphology and phylogeny the first report of C. botryospora on F. sylvatica from Italy is provided (Farr and Rossman, 2020).

In this study, the Patellariopsidaceae fam. nov. is introduced with an asexual morph. Furthermore, a new host record for the C. botryospora (Vibriaseae) and updated phylogenetic tree for Helotiales are provided.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this manuscript are not publicly available. Requests to access the datasets should be directed to anumandrack@yahoo.com.

AUTHOR CONTRIBUTIONS

AK and KH designed the study. AK performed the morphological study and phylogenetic the data analyses with the help of DP, AE, KC, and RJ. DP did the primer design. SL and SK provided the grant. AK wrote the manuscript. RJ, DP, IG, KC, AE, SK, SL, RC, and KH reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

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