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New species of *Capronia* (*Herpotrichiellaceae*, *Ascomycota*) from Patagonian forests, Argentina

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Abstract. Three new species belonging to *Capronia* are described from plants native to the Andean Patagonian forests, Argentina. The first record of *C. chlorospora* in South America is also reported. The identity of the three new species is based on detailed morpho-anatomical observations as well as analyses of ITS and LSU nuclear rDNA. A key to the *Capronia* species present in Argentina is provided.

Key words: ITS, LSU, phylogenetics, systematics, three new species

Introduction

Capronia is an ascomycete genus with medically important asexual morphs in the *Exophiala-Ramichloridium-Rhinocladiella* complex, known as "black yeast", which is considered polyphyletic (Untereiner et al. 2011; Teixeira et al. 2017). It is characterized by small, dark and usually setose ascomata, with periphysate ostioles, the absence of interascal filaments, bitunicate, 8- to polysporus asci, and septate, hyaline to dark-colored ascospores (Barr 1987; Réblová 1996). A majority of the species are saprobic or hypersaprobic (Untereiner 2000) but about twenty species are reported to grow obligatory on lichens (Etayo & García Sancho 2008; Etayo et al. 2013; Flakus & Kukwa 2012; Halici et al. 2010; Tsurykau & Etayo 2017; Zhurbenko 2012; Zhurbenko et al. 2016). About eighty species of *Capronia* are described and, in spite of them being common and ubiquitous, their diminutive ascomata, which are seldom abundant on the substrate, makes them difficult to recognize.

Through our work on the biodiversity of ascomycetes on trees native to the Andean Patagonian forests in Argentina, three new species of *Capronia* along with *C. chlorospora* were found growing on dead wood and bark. All three new species of *Capronia* are described, illustrated and compared morphologically and phylogenetically to other known species in the genus. This is the first report of *C. chlorospora* in the Southern Hemisphere. A key to the *Capronia* species present in Argentina is provided.

Materials and methods

The samples were collected in Andean Patagonian forests where native species of *Nothofagus* along with *Cupressaceae*, *Proteaceae*, ferns and mosses prevail. Four parks were included in this survey: Parque Provincial Lago Baggilt (Chubut), Parque Nacional Lanín (Neuquén), Parque Nacional Los Alerces (Chubut) and Parque Nacional Nahuel Huapi (Río Negro). Leaves, small branches and bark showing fungal growth when observed under a 10× loupe were placed in paper bags and transported to the laboratory. The samples were dried at room temperature and deposited in the Bahía Blanca Biology Herbarium (BBB). For microscopy, sections were cut freehand under a Leica EZ4 stereomicroscope and mounted in tap water and 5% KOH with phloxine or Melzer's reagent. A Leica DM2000 microscope fitted with a Samsung NV10 digital camera was used to capture images of micromorphology. At least 10 measurements were taken for each structure mounted in tap water. Averages for ascospores are given in parentheses. Material was mounted in calcofluor 0.5% for examination of the hymenium under a Nikon Eclipse 80i fluorescence microscope with a Nikon DXM 1200F camera system and a Leica DM2000 with a Leica EC3 camera system. Single- and multiple-ascospore isolates were attempted after 1–5 months on three different agar media, including 2% malt extract agar (MEA), oatmeal agar (OA) and potato dextrose agar (PDA) at three different temperatures (10°C, 15°C, room temperature) and light conditions (white light, fluorescent light, white

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Figure 1. Phylogenetic tree (ln = 4183.8) of *Herpotrichiellaceae* taxa, based on the D1–D2 region of LSU nrDNA sequences constructed using the PhyML maximum likelihood method implemented in Seaview 4.7 with 1000 bootstrap replications. Bootstrap values ≥ 70% from the PhyML and RAxML analyses are shown above or below branches. Thickened branches indicate Bayesian posterior probabilities ≥ 95%. The three new species of *Capronia* are bolded. Source and GenBank accession numbers are given after taxon names.

fluorescent light alternating with periods of UV light). All attempts to obtain axenic cultures failed due to lack of growth, most likely due to the length of time between collection and isolation, so we extracted fungal DNA directly from the ascomata.

Extraction, amplification and sequencing of DNA followed Promputtha and Miller (2010). Briefly, DNA was extracted directly from ~30 ascomata using an E.Z.N.A.® Microelute Genomic DNA kit (Omega Bio-tek). The complete internal transcribed spacer (ITS) region and the first 600 bp of the 5' end of 28S nuclear ribosomal large subunit (LSU) including the D1 and D2 domains were amplified separately using puReTaq™ Ready-To-Go PCR Beads (Amersham Biosciences Corp.) according to the manufacturer's instructions, with primer sets ITS1F-ITS4 and LROR-LR3, respectively (Gardes & Bruns 1993; Rehner & Samuels 1995; Vilgalys & Hester 1990; White et al. 1990). PCR products were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega), and template DNA was used in 10 µL sequencing reactions with BigDye® Terminator v3.1 (Applied Biosystems), using the same primers as in PCR. Sequences were generated on an Applied Biosystems 3730XL high throughput capillary sequencer at the W.M. Keck Center at the University of Illinois Urbana-Champaign. Consensus ITS and LSU sequences were assembled with Sequencher 5.4 (Gene Codes Corp.).

The LSU dataset comprising 103 taxa was assembled with sequences from the three new species along with sequences from previous studies (Abliz et al. 2004; Crous et al. 2007; De Hoog et al. 2011; Feng et al. 2014; Gueidan et al. 2007; Hamada & Abe 2010; James et al. 2006; Marincowitz et al. 2008; Rakeman et al. 2005; Teixeira et al. 2017; Untereiner & Naveau 1999; Untereiner et al. 2008; Vitale et al. 2002; Vu et al. 2019) using the MUSCLE® multiple alignment program as implemented in Sequencher 5.4. The alignment was rooted with *Placocarpus schaereri* and *Verrucula inconnexaria*. Ambiguously aligned regions were removed from the final alignment using Gblocks (Castresana 2000; Talavera & Castresana 2007), employing the less stringent parameters. The general time reversible (GTR) model (Rodríguez et al. 1990) was determined as the best-fit model of evolution by jModeltest (Darriba et al. 2012; Guindon & Gascuel 2003) after evaluating 1624 possible evolutionary models and implementing the Akaike information criterion (AIC) (Posada & Buckley 2004). A maximum likelihood (ML) analysis with 1000 bootstrap replicates was performed using PhyML as implemented in Seaview 4.7 (Gouy et al. 2010), with all parameters optimized and the GTR model. Clades with bootstrap values (BV) ≥ 70% were considered significant and strongly supported (Hillis & Bull 1993). Bayesian analyses were performed using MrBayes v 3.2.6 (Huelsenbeck & Ronquist 2001, 2005) under the above model on the CIPRES Portal 2.0 (Miller et al. 2010). Constant characters were included and 10 million generations with trees sampled every 1000th generation were run, resulting in 10,000 total trees. The first 2500 trees were discarded as burn-in, and Bayesian posterior probabilities (BPP) were determined from a consensus tree generated from the remaining 7500 trees using PAUP* 4.0b10 (Swofford 2002). Clades with BPP ≥ 95% were considered significant and strongly supported (Alfaro et al. 2003; Larget & Simon 1999). Source and GenBank accession numbers for all taxa included in the LSU analyses are shown after taxa names in Figure 1. Based on the LSU tree results, alignments of ITS sequences were attempted for the most closely related taxa in each of the two subclades containing the three proposed new species. However, taxon sampling for this locus was so poor for these taxa that phylogenetic analyses were abandoned. ITS sequences for *Capronia austrocedri* (MH809169), *Capronia capucina* (MH809170) and *Capronia rubiginosa* (MH809171) were deposited in GenBank.

Results

The final LSU alignment consisted of 610 bp after removal of missing data and ambiguous regions. The ML tree generated with PhyML based on this LSU dataset is shown in Figure 1. A second ML analysis using RAxML produced a similar tree with no significant differences in topology from the PhyML tree (BV from RAxML shown in Fig. 1). Overall, the relationships among taxa were similar to those shown in previous trees based on LSU data (Crous et al. 2007; Feng et al. 2014; Seyedmousavi et al. 2014; Teixeira et al. 2017; Untereiner & Naveau 1999; Untereiner et al. 2008). *Capronia capucina* and *C. rubiginosa* formed a weakly supported clade that occurred in a highly supported clade containing *Cladophialophora chaetospira* and *Chaetothyriales sp*. ITS sequence divergence between *Capronia capucina* and *C. rubiginosa* was 29 bp (5.1%) out of 573 bp after ignoring a large ~350 bp intron in the 5' end of *C. rubiginosa*. *Capronia austrocedri* occurred in an unsupported clade containing *Herpotrichiellaceae sp.*, *Thysanorea papuana* and *Veronaea japonica*.

Taxonomy

Capronia austrocedri R.M. Sánchez, A.N. Mill. & Bianchin., sp. nov. (Figs 2, 4)

MycoBank MB 827442

Diagnosis: similar to *Capronia mansonii* but phylogenetically very distant and distinguished by its wider ascomata and asci.

Type: Argentina, Chubut, Parque Nacional Los Alerces, 42°46′22.2″S, 71°43′54.94″W, 551 m, on decorticated branches of *Austrocedrus chilensis*, 24 Oct 2006, *M. V. Bianchinotti & R. M. Sánchez* (BBB 204, holotype designated here!).

Description. Ascomata perithecioid, erumpent to superficial, subperidermal, solitary, seated on sparse, brown mycelium, with dark brown to black setae only in upper third of ascomata and sinuous brown septate hyphae at base, papillate, ostiole circular, black when dry, yellowish when mounted in water, 60–245 µm high, 60–325 µm diam. Papilla 30–60 µm high. Setae of two types: smaller ones forming a crown around ostiole, usually sinuous at base, black, 8–28 µm long, 5–6 µm basal diam, 2–3 µm apical diam; larger ones distributed in upper half of ascomata up to papilla, usually straight, sometimes with

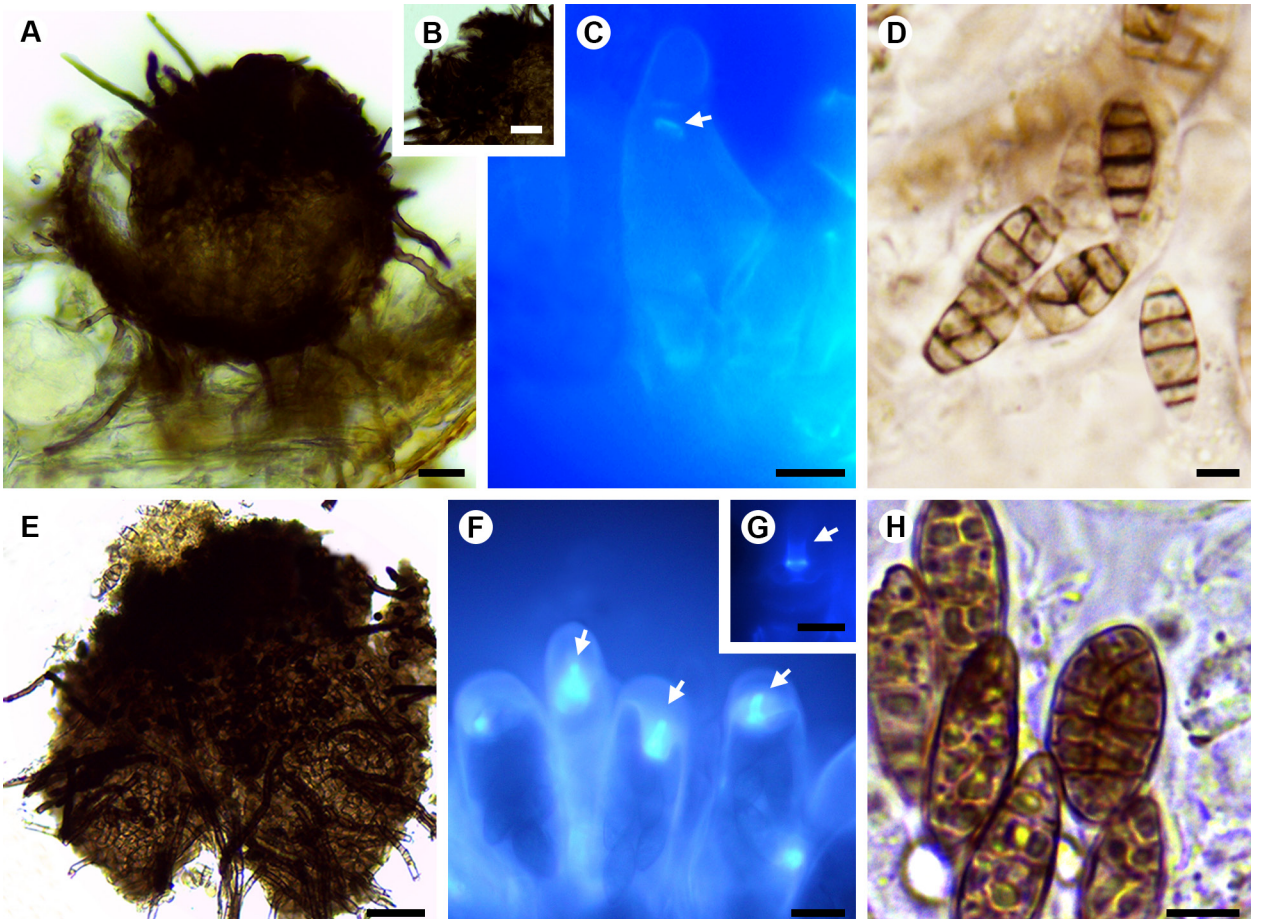

Figure 2. *Capronia austrocedri* sp. nov. A – ascoma; B – view of crown of short setae around ostiole; C – ascus viewed with fluorescence microscopy, arrow points to apical ring; D – ascospores (BBB 204). *Capronia capucina* sp. nov. E – ascoma; F – asci viewed with fluorescence microscopy, arrows point to apical cap-shaped apparati; G – enlarged view of cap-shaped apparati; H – ascospores (BBB 561). A, B, D, E, H = tap water. C, F, G = calcofluor 0.5%. Scales: A, E = 20 µm, B–C, F–G = 10 µm, D, H = 5 µm.

irregular outline, with acute apices, walls 1 µm wide, 0–3 septa, dark brown, 30–122 µm long, 4–9 µm basal diam, 2–4 µm apical diam. Basal hyphae formed by 4–10 cells, sinuous, with irregular outline, with rounded apices, up to 113 µm long, 3–5 µm diam. Ascomatal wall of *textura angularis*, 10–25 µm thick, two-layered in longitudinal section, outer layer composed of 2–3 rows of angular, thick-walled, dark brown cells, 4–11 × 4–7 µm, inner layer composed of 4–5 rows of cylindrical, thin-walled, hyaline cells, 2–10 × 1–3 µm. Pseudoparaphyses absent. Periphysoids hyaline, 5–9 × 1–3 µm. Periphyses hyaline. Asci bitunicate, clavate, with small, fluorescent apical ring, IKI-, 8-spored, 61–85 × 16–27 µm. Ascospores biseriate, fusiform, apical end acute, basal end rounded, 3–7 transverse septa, 0–3 longitudinal septa, oblique septa present, brown, 13–25 × 5–10 µm ($\bar{x}$ = 19 × 7). Asexual morph not known.

Etymology. Named after the host on which it was found growing.

Ecology and distribution. This species is currently known only from the type and one other specimen, which was found growing on decorticated branches of *Austrocedrus chilensis* from a cold temperate forest in Parque Nacional Los Alerces, Argentina.

Other Specimens examined. ARGENTINA: Chubut, Parque Nacional Los Alerces, 42°46′22.2″S, 71°43′54.94″W, 551 m, on decorticated branches of *Austrocedrus chilensis*, 24 Oct 2006, *M. V. Bianchinotti & R. M. Sánchez 206* (BBB).

Notes. This species resembles *Capronia mansonii* in most features of the ascospores and in the presence of a ring in the apex of the ascus (Untereiner 1997). However, *C. mansonii* has smaller ascomata (90–160 µm high × 70–125 µm diam.) and asci (32.9–45 × 11–23.1 µm), according to Schol-Schwarz (1968) and Untereiner (1997). Untereiner (op. cit.) described *C. mansonii* ascospores as strongly constricted at the septa. *Capronia austrocedri* is morphologically similar to *C. semiimmersa*, but in this last the ascospores are shorter (Untereiner & Naveau 1999). Based on our LSU tree results (Fig. 1) these three taxa are not closely related. In our phylogenetic analyses, *C. austrocedri* is related to two conidial species with unknown sexual morphs (*Thysanorea papuana*, *Veronaea japonica*) and to *C. parasitica*. An asexual morph is unknown in *Capronia austrocedri*, and the sexual morph is quite different from *C. parasitica*, which has smaller ascomata (120–150 µm), sometimes with a short stromatic stalk at the base, setae reduced to dark protruding cells, and smaller ellipsoid to fusoid ascospores (9–11 × 3–4 µm) with only three transverse septa (Barr 1972, Müller et al. 1987).

Capronia capucina R.M. Sánchez, A.N. Mill. & Bianchin., sp. nov. (Figs 2, 4)

MycoBank MB 827443

Diagnosis: similar to *Capronia epimyces* but distinguished by its oval, muriform ascospores with basal ends sometimes rounded but usually obtuse, and by the absence of long unicellular setae on the surface of ascomata.

Type: Argentina, Neuquén, Parque Nacional Nahuel Huapi, on National Route 40, one kilometer before arriving to the intersection with Provincial Route 65 when traveling from Villa La Angostura to Villa Traful, 40°37′5.41″S, 71°38′48.79″W, 892 m, on pieces of bark of *Nothofagus antarctica*, 16 May 2007, *M. V. Bianchinotti & R. M. Sánchez* (BBB *561*, holotype designated here!).

Description. Ascomata perithecioid, superficial, subperidermal, solitary, setose with setae more densely distributed in upper half, sinuous brown hyphae at base, papillate, ostiole circular, black when dry, brown when mounted in water, 100–245 µm high and diam. Papilla 30–50 µm high. Setae usually curved or sinuous at base, apices rounded, rough wall 1 µm thick, 0–2 septa, dark brown, 10–75 µm long, 3–8 µm basal diam, 1–4 µm apical diam. Basal hyphae formed by 4–7 cells, sinuous, with irregular outline, with rounded apices, up to 80 µm long and 5 µm diam. Ascomatal wall of *textura angularis*, 15–85 µm thick, two-layered in longitudinal section, outer layer composed of 3–5 rows of polyhedral to globose, thick-walled, dark brown cells, 5–7.5 × 3–7 µm, inner layer composed of 3–4 rows of cylindrical, thin-walled, pale brown cells, 10–14 × 2–3 µm. Pseudoparaphyses absent. Periphysoids hyaline, 10–25 × 2 µm. Periphyses hyaline. Asci bitunicate, clavate, with fluorescent apical cap-shaped apparatus, IKI-, 8-spored, 55–82 × 12.5–20.5 µm. Ascospores biseriate, oval, ends usually obtuse, sometimes rounded at basal end, muriform, 3–8 transverse septa, 0–5 longitudinal septa, 0–4 oblique septa, each cell with a green drop, pale brown, 15–27.5 × 5–11 µm ($\bar{x}$ = 20 × 8). Asexual morph not known.

Etymology. Referring to the ascal apparatus with hood or cowl form.

Ecology and distribution. This species is currently known from the type and three other specimens, which were found growing on pieces of bark of *Nothofagus antarctica* and *N. alpina*. The collections were obtained from Parque Nacional Nahuel Huapi and Parque Nacional Lanín, two nature reserves in the Andean Patagonian forest of Argentina.

Other Specimens examined. ARGENTINA: Neuquén, Parque Nacional Nahuel Huapi, on National Route 40, one kilometer before arriving at the intersection with Provincial Route 65 when traveling from Villa La Angostura to Villa Traful, 40°37′5.41″S, 71°38′48.79″W, 892 m, on pieces of bark of *Nothofagus antarctica*, 16 May 2007, *M. V. Bianchinotti & R. M. Sánchez 560*; *562* (BBB); Parque Nacional Lanín, access path to the Cascada Chachín waterfall, 40°8′25.29″S, 71°40′3.84″W, 757 m, on pieces of bark of *N. alpina*, 17 May 2007, *M. V. Bianchinotti & R. M. Sánchez 588* (BBB).

Notes. This species resembles *Capronia epimyces* in the size of ascomata (80–200 µm diam) and in ascospore characteristics such as size (18–27 × 7.5–12 µm), number of septa (5–9 transverse, 1–3 longitudinal) and color (dull greyish brown). However, *C. epimyces* presents short protruding cells and very long unicellular setae (26–120 µm) on the surface of ascomata, and the ascospores are ellipsoid with tapered to obtuse ends, without the presence of oblique septa. Based on our LSU tree results (Fig. 1) these two taxa are not closely related. However, *C. capucina* is related to *C. rubiginosa* and the conidial species *Cladophialophora chaetospira*. No asexual morph was found associated with the ascomata of *C. capucina*. *Capronia rubiginosa* has larger, oval to fusiform, reddish brown ascospores (18–35 × 7–15 µm) with acute ends and more septa [8–10(–12) transverse septa and 1–10 longitudinal septa] and an apical ring in the asci instead of a cup-shaped apparatus.

Capronia chlorospora (Ellis & Everh.) M.E. Barr, Mycotaxon 41(2): 426. 1991. (Figs 3, 4)

Complete synonymy and description in Barr (1991).

Ecology and Distribution. This species was originally found in the USA on *Quercus* sp. (Ellis & Everhart 1892), but later found on *Acer saccharum*, *Acer* sp., *Ailanthus* sp., *Carya* sp., *Lemaireocereus thurberi*, *Stenocereus thurberi* and old *Hypoxylon* sp. on *Fagus* sp. (Barr 1991). In 1997 it was recorded for the first time in Europe by Réblová and Svrcek (1997) on rotten wood often beneath peeling bark of *Carpinus betulus* from the Czech Republic. Recently it was reported in Norway on *Ulmus glabra* (Vetlesen 2017), and it is recorded here on bark of *Nothofagus obliqua* from Argentina.

Specimen examined. ARGENTINA: Neuquén, Parque Nacional Lanín, access path to Cascada Chachín waterfall, 40°8′25.29″S, 71°40′3.84″W, 757 m, on pieces of bark of *N. obliqua*, 17 May 2007, *M. V. Bianchinotti & R. M. Sánchez 592* (BBB).

Notes. The species was described by Ellis and Everhart (1892) as *Teichospora chlorospora* on *Quercus* sp. Barr (1991) then transferred it to *Capronia* and redescribed it from new material because the holotype was missing. The description of the Argentine specimen mostly agrees with both descriptions. The differences that we found are related to non-diagnostic characteristics. The ascomatal setae are slightly longer (30–85 vs. 25–65 µm) than those described by Barr (1991) and sometimes possess a septum, but they are similar in size (50–80 µm) to those mentioned by Ellis and Everhart (1892). The asci are slightly wider (18–23 vs. 15–20 µm diam.) than those described by Ellis and Everhart (1892). The ascospores are reddish brown instead of greenish or olivaceous brown as described by Ellis and Everhart (1892) or dull grayish brown or olivaceous as described by Barr (1991). We also recorded more longitudinal septa (2–10) and oblique septa (5–6) in the mature ascospores, and the presence of a fluorescent apical ring in the asci.

A search of published fungal lists and online fungaria shows that this is the first record of *Capronia*

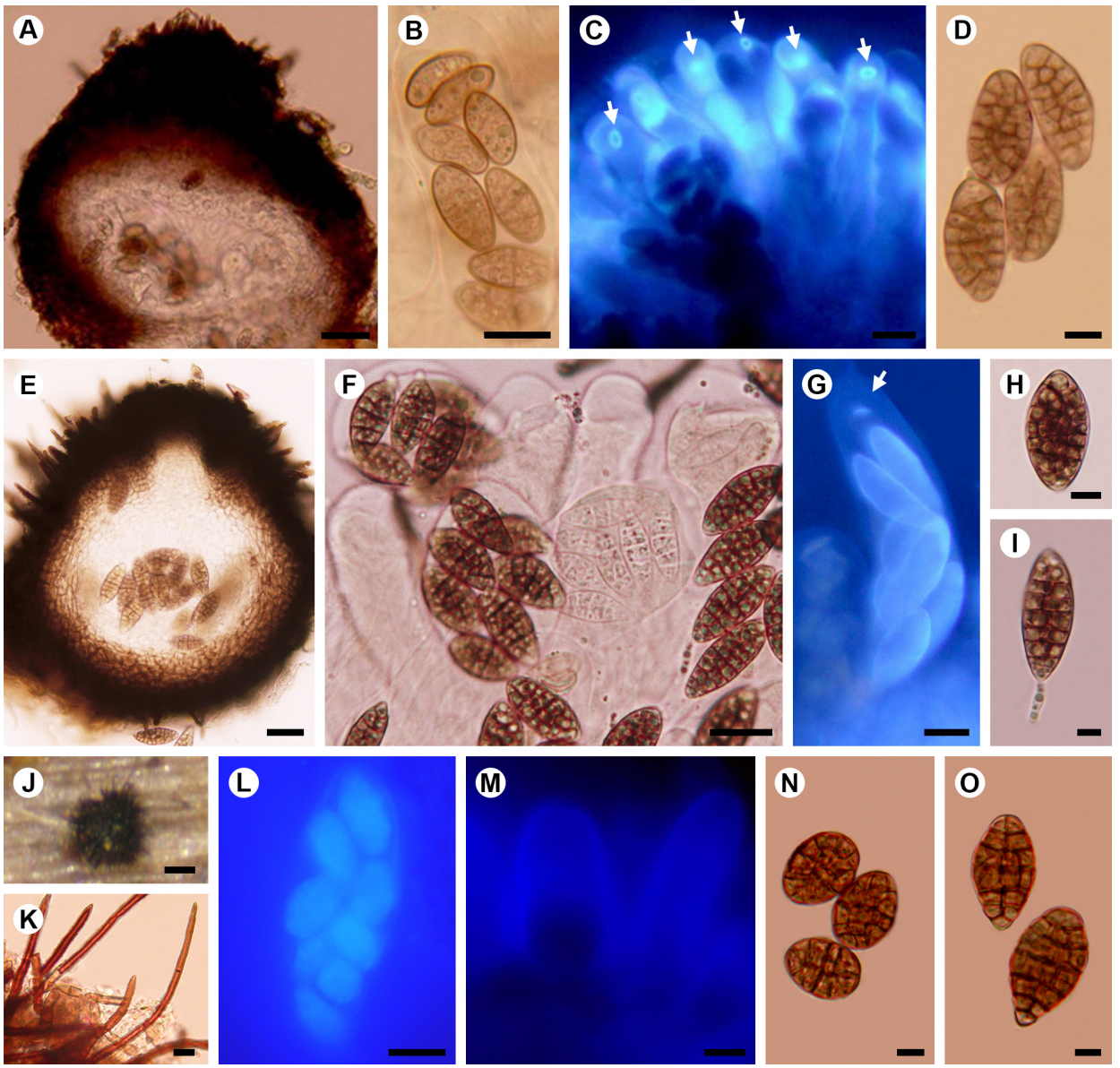

Figure 3. *Capronia chlorospora*. A – longitudinal section through ascoma; B – ascus with immature ascospores; C – asci viewed with fluorescence microscopy, arrows point to apical rings; D – ascospores (BBB 592). *Capronia rubiginosa* sp. nov. E – longitudinal section through ascoma; F – Asci; G – ascus viewed with fluorescence microscopy, arrow points to apical ring; H – ascospore; I – germinated ascospore (BBB 536). *Capronia acutiseta*. J – ascomatal. K – ascomal setae; L, M – ascus viewed with fluorescence microscopy; N, O – ascospores (PDD 34068). A, B, D–F, H–K, N, O = tap water. C, G, L, M = calcofluor 0.5%. Scales: J = 100 µm, A, E = 20 µm, B, C, F, G, K, L = 10 µm, D, H, I, M–O = 5 µm.

chlorospora in South America (Farr 1973; Etayo 2003; Iturriaga & Minter 2006; Minter & Peredo López 2006; Minter & Silva 2007; Etayo & García Sancho 2008; Rodriguez-Flakus et al. 2016; INCT Herbário Virtual da Flora e dos Fungos 2019; MyCoPortal 2019). The material in this collection is insufficient for molecular studies.

Capronia rubiginosa R.M. Sánchez, A.N. Mill. & Bianchin., sp. nov. (Figs 3, 4)

MycoBank MB 827444

Diagnosis: similar to *Capronia acutiseta* but distinguished by its broad, oval to fusiform ascospores, with more transverse septa, and asci with a fluorescent apical ring.

Type: Argentina, Chubut, Parque Provincial Lago Baggilt, in *Nothofagus pumilio* forest on the sides of the road arriving at Baggilt Lake, 43°15′59.76″S, 71°39′2.52″W, 1079 m, on branches of *Nothofagus pumilio*, 15 May 2007, *M.V. Bianchinotti & R.M. Sánchez* (BBB *536*, holotype designated here!).

Description. Ascomata perithecioid, erumpent to superficial, subperidermal, globose, sometimes ovoid, setose, papillate, ostiole circular, dark brown, 112–220 µm high, 100–210 µm diam. Papilla ~85 µm high. Setae usually straight, sometimes with irregular outline, with acute apices, walls 1 µm wide, 0–3 septa, dark brown, 25–65 µm length, 3–8 µm basal diam, 1–4 µm apical diam. Ascomatal wall of *textura angularis*, 35–50 µm thick, two-layered in longitudinal section, outer layer composed of 5–7 rows of angular, thick-walled, dark brown cells, 5–10 × 3–8.5 µm, inner layer composed of 4–5 rows of flattened to angular, thin-walled, pale brown cells, 8–10 × 4–6 µm. Pseudoparaphyses absent. Periphysoids hyaline, 8 × 2–3 µm. Periphyses hyaline, 15 × 2.5 µm. Asci bitunicate,

clavate to globose, with small, fluorescent apical ring, not always visible, IKI-, 8-spored, 71–85 × 18–28 µm. Ascospores biseriate, oval to fusiform, ends acute, bilaterally asymmetrical, muriform, (6–)8–10(–12) transverse septa, 1–10 longitudinal septa, 0–3 oblique septa, each cell with a green drop, reddish ochraceous when young, reddish brown when mature, 18–35 × 7–15 µm ($\bar{x}$ = 26 × 11). Asexual morph not known.

Etymology. Referring to the reddish hue of the ascospores.

Ecology and distribution. This species is currently known from the type specimen and one other specimen, which was found growing on bark of branches of *Nothofagus pumilio* from the Andean Patagonian forest of Argentina.

Other specimens examined. ARGENTINA: Chubut, Parque Provincial Lago Baggilt, on the road to Baggilt Lake, 43°15′59.76″S, 71°39′2.52″W, 1079 m, on branches of *Nothofagus pumilio*, 15 May 2007, *M.V. Bianchinotti & R.M. Sánchez 535* (BBB). ***Capronia acutiseta***. NEW ZEALAND: Westland, Saltwater State Forest, *Dacrydium cupressinum*, 3 May 1974, *G.J. Samuels s.n.*, (PDD 34068 – type!).

Notes. This species differs from *Capronia acutiseta* (Figs. 3J-O, 4O-P) by the latter having smaller, ellipsoidal ascospores (14–27 × 11–14 µm) with fewer transverse septa (4–5) and by the absence of apical rings in the asci. Some ascospores were seen with a few cells germinating, and brownish red mycelium was seen nearby the ascomata. Based on the LSU tree results (Fig. 1) these two taxa are not closely related. However, *C. rubiginosa* is related to *C. capucina* and the conidial species *Cladophialophora chaetospira.* An asexual morph was not found near the ascomata of *C. rubiginosa* on the substrate. *Capronia capucina* has smaller, oval, pale brown ascospores (15–27.5 × 5–11 µm) with obtuse ends and fewer septa (3–8 transverse septa and 0–5 longitudinal septa) and has an apical cup-shaped apparatus in the asci instead of an apical ring.

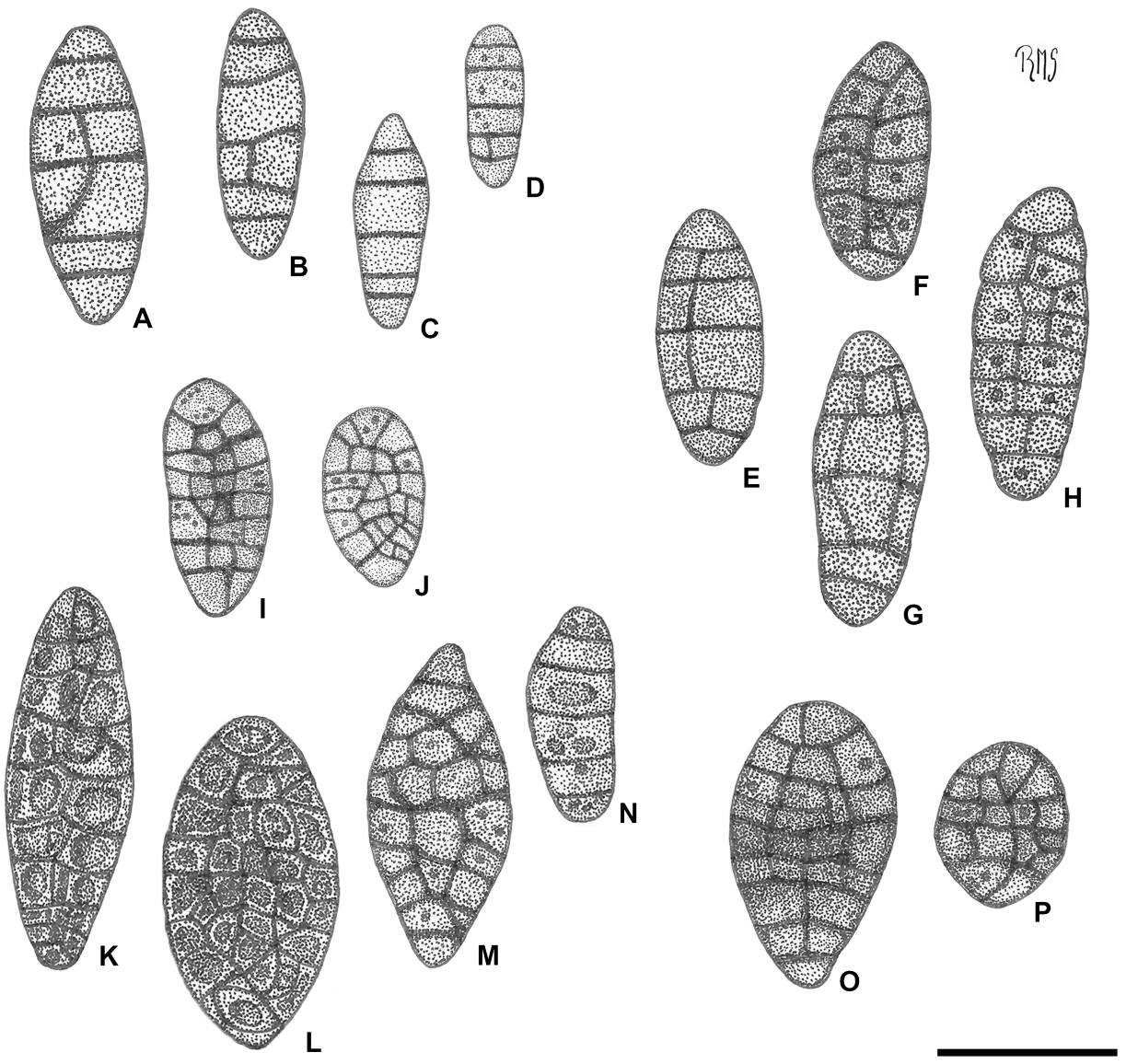

Figure 4. Ascospore comparison. *Capronia austrocedri*. A–D – Mature ascospores. *C. capucina*. E–H – Mature ascospores. *C. chlorospora*. I, J – Mature ascospores. *C. rubiginosa*. K–M – Mature ascospores. N – Immature ascospore. *C. acutiseta*. O, P – Mature ascospores. Scales: A–P = 15 µm.

Key to *Capronia* species present in Argentina

1 Ascomata with a crown of short setae around the ostiole 2
Ascomata without a crown of short setae around the ostiole 3

2(1) Longer setae scattered over the entire ascomata 4
Longer setae only in upper half of ascomata 5

3(1) Longer setae only in upper half of ascomata; ascospores with 5–7 transverse septa and 1–2 longitudinal septa, (12–)13.5–15.5(–20) × 3.6–5 µm . . . . . ***C. nigerrima***
Longer setae scattered over the entire ascomata 6

4(2) Ascospores with 3–7 transverse septa and 1–2 longitudinal septa; ascal apical ring present. . . ***C. pulcherrima***
Ascospores with 1–3 transverse septa; ascal apical ring absent 7

5(2) Ascospores fusiform, with asymmetrical ends, 0–3 longitudinal septa and 0–3 oblique septa present, 7–25 × 4–10 µm . . . . . ***C. austrocedri***
Ascospores ovoid to ellipsoid, with obtuse ends, 2–10 longitudinal septa and 5–6 oblique septa, 11–20 × (5.5–)7–9(–10) µm . . . . . ***C. chlorospora***

6(3) Ascal apparatus ring-shaped; ascospores oval to fusiform, reddish brown, with obtuse ends, 6–12 transverse septa, 1–10 longitudinal septa and 0–3 oblique septa, 18–35 × 7–15 µm . . . . . ***C. rubiginosa***
Ascal apparatus cup-shaped; ascospores oval, pale brown, with usually obtuse ends and sometimes rounded bottom ends, 3–8 transverse septa, 0–5 longitudinal septa and 0–4 oblique septa, 15–27.5 × 5–11 µm . . . ***C. capucina***

7(4) Ascospores elliptic ovate, with obtuse ends, 11–15(–16) × 3–5(–6) µm . . . . . ***C. pilosella***
Ascospores ellipsoid to fusiform, with rounded ends, (10–)11.5–13.5(–15.5) × (2–)4–5 µm . . . . ***C. coronata***

Discussion

The ascomata of *Capronia* species are so small that they are very difficult to recognize on darkened substrates often colonized by other microfungi (Untereiner 2000). As Eriksson (1981) remarked, these species are typically found only when mycologists examine collections in depth to find other fungi. In our case, the specimens were found when studying ascomycete biodiversity on wood and bark of native trees from different nature reserves of the Andean Patagonian forest in Argentina. The discoveries of these *Capronia* species were made 1–5 months after the samples were collected. Although several attempts were made to obtain axenic cultures, unfortunately it was not possible even when the asci appeared turgid and some ascospores were seen germinating. Untereiner (2000) stated that obtaining cultures from direct plating of substrates or ascomata is usually unsuccessful. Only a few ascomata were found in each sample and there was no asexual morph associated with them, a feature common in several *Capronia* species (Barr 1991; Etayo & Diederich 1996, 1998; Etayo et al. 2013; Friebes 2011; Réblová 1996, 1997; Vetlesen 2017). Besides the morphological studies, the identity of the novel species was confirmed through phylogenetic studies using DNA extracted directly from the ascomata. Although it is a highly destructive method, it turns out to be the only option when the specimens cannot be cultivated (Jayasiri et al. 2015; Promputtha & Miller 2010; Zeng et al. 2018).

Our findings increase the number of *Capronia* species known in Argentina to eight. Unlike the species recorded here from the southernmost forest of Argentina, the species previously mentioned for the country were collected at lower latitudes, in places with tropical to subtropical climate: *C. coronata*, *C. nigerrima* and *C. pulcherrima* in the Yungas (northwestern region of Argentina), the former also recorded from Misiones Province, and *C. pilosella* from Buenos Aires (Carmarán et al. 2002; Catania & Romero 2008; Gallo et al. 2014). All eight species in Argentina were found growing on wood or bark of standing trees or fallen branches, and no asexual morph was found associated with any of them. *Capronia chlorospora* appears to be the most polyphagous species, as it also grows on a *Cactaceae* (*Stenocereus thurberi*) in the USA. However, in other temperate countries the other species grow on substrates similar to those mentioned in Argentina. This probably means little from an ecological perspective, since investigations of micro-ascomycetes in Argentina are mostly patchy, and it is unwise to make general assumptions about niche preferences without more data. We should not forget, however, that these species are generally not host-specific.

The three new species described here were compared genetically to all *Capronia* species and their anamorph species with valid sequences in the *Herpotrichiellaceae*. *Capronia austrocedri* has similarities with *C. mansonii* and *C. semiimmersa*, *C. capucina* resembles *C. epimyces*, and *C. rubiginosa* reminded us of *C. acutiseta*, but all of the Patagonian species can be distinguished morphologically from species in Europe, North America and New Zealand. This is also supported by the phylogenetic analyses, which confirmed the uniqueness of the South American species. Nevertheless, it is important to bear in mind that *Capronia* is polyphyletic and that its type species, *C. sexdecimspora*, has not been sequenced and little is known about it. This raises a series of questions about the circumscription of the genus, especially about which clade truly represents *Capronia*; that may only be resolved when the actual type species (or an epitype) has been sequenced.

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