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Rhynchostomatoid fungi occurring on Proteaceae

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Abstract: A new ascomycete fungus, with long-necked perithecia having central ostioles and striate ascospores, was isolated from flowerheads of *Protea burchellii* and *P. laurifolia* in South Africa and is described here as *Rhynchostoma proteae* sp. nov. Sequence data obtained from the small-subunit ribosomal DNA (SSU nrDNA) place this fungus with 100% bootstrap support in a clade containing the type species of *Rhynchostoma*, *R. minutum*. A similar fungus with verruculose ascospores also was observed on a member of the Proteaceae from Australia, *Lomatia polymorpha*, which is described here as *Rhynchomeliola lomatiae* sp. nov. These two species are illustrated and contrasted with a third species from Proteaceae, *Rhynchomeliola australiense*, known from *Grevillea* in Australia.

Key words: Ascomycetes, Chaetothyriomycetes, Fynbos, South Africa

INTRODUCTION

The Proteaceae, which existed before Gondwanaland began to break up 140 million years ago (Rebelo 1995), is known to be one of the most prominent flowering plant groups in the Southern Hemisphere, with the majority of genera found in South Africa and Australia. The family is divided into more than 60 genera containing about 1400 species (Rebelo 1995). *Protea* L. has flowerheads (or inflorescences) comprising many flowers with bracts, which often are mistaken for flowers. Some are open only a few weeks and then close and remain intact for at least a few years (serotinous) (Rebelo 1995). Studies on the mycota of flowerheads (Wingfield and van Wyk 1993, Marais and Wingfield 1994) revealed the presence of unique ophiostomatoid fungi and proved flowerheads to be a potentially rich niche for saprobic fungi.

While studying saprobic fungi in flowerheads of Proteaceae as part of a fungal biodiversity program, an unusual ascomycete with a long perithecial neck was discovered among ophiostomatoid fungi. The fungus was identified as a new member of *Rhynchostoma* P. Karst. A similar fungus coincidentally was found on a herbarium specimen of *Lomatia polymorpha* R. Br. (Proteaceae), which was identified as a new member of *Rhynchomeliola* Speg.

The genera *Rhynchomeliola* and *Rhynchostoma* were introduced separately to accommodate fungi with perithecia having prominent ostiolar necks, persistent unitunicate asci without apical apparati and two-celled, brown ascospores. In *Rhynchostoma*, ascomata are immersed to becoming erumpent in a stroma. *Rhynchomeliola* ascomata are superficial on a subiculum, or with a semi-immersed base. The hierarchical placement of these two genera varied from the Diatrypaceae (Müller and von Arx 1962), Sphaeriaceae (Müller and von Arx 1973), Bolineaceae (Barr 1990), Trichosphaeriaceae (Eriksson 1984, Hawksworth et al 1995) to the Rhynchostomataceae (Winka and Eriksson 2000, Eriksson et al 2001) and remains uncertain. In an attempt to clarify their morphology these fungi were studied along with the third species, *Rhynchomeliola australiensis* (Petr.) E. Müll., known from *Grevilla*. Furthermore, to elucidate phylogenetic relationship of the new species of *Rhynchostoma* from *Protea* with other genera, a phylogenetic analysis was done based on the SSU nrDNA sequence data.

MATERIALS AND METHODS

Isolates.—One- to 2-year-old flowerheads of serotinous Proteaceae were collected from Stellenbosch Mountains in South Africa's western Cape Province. The samples were inspected immediately for fungal structures and air-dried for further study. Air-dried samples were incubated in mois-

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ture chambers 2–3 d before examination. Single-spore isolations were carried out on fungal taxa that were encountered, and cultures were established on 5% malt-extract agar (MEA; Biolab, Gauteng, South Africa), supplemented with 0.04 g L^{-1} streptomycin sulphate. Cultural characteristics were rated in triplicate from MEA plates after 30 d of incubation at 25 C in the dark; colors were determined according to Rayner (1970). Microscopic observations, measurements and photography of characteristic structures were made either from structures mounted in clear lactophenol or directly from specimens under the dissecting microscope. The 95% confidence intervals were derived from 30 observations, whenever possible, with the extremes given in parentheses in the species descriptions. Sections of ascomata were made on a Leica CM1100 Cryostat microtome from material mounted with Jung tissue-freezing medium™ (Leica Instruments, Germany). Digital images were captured with a Nikon Digital Camera DXM 1200 mounted on a Nikon Eclipse E600 light microscope or a Nikon SMZ800 dissecting microscope. Herbarium specimens are lodged at PREM and BPI, and reference cultures are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

DNA extraction.—Genomic DNA was isolated respectively from mycelia and spore masses of *Rhynchostoma proteae*, following the method described Crous et al (2000). Three areas of the rDNA gene operon, namely part of the small subunit, part of the large subunit and the internal transcribed spacers along with the 5.8S rDNA gene, were selected for polymerase chain reaction (PCR) amplification. The small-subunit gene was amplified with primers NS1 and NS4 (White et al 1990) and a part of the large-subunit gene using primers ITS1 (White et al 1990) and LR5 (Vilgalys and Hester 1990). The primers ITS1 and ITS4 (White et al 1990) were used to amplify the 3′ end of the 18S (small-subunit) nrDNA gene (SSU nrDNA), the first internal transcribed spacer (ITS1), the 5.8S rDNA gene, the second ITS (ITS2) region and the 5′ end of the 28S (large-subunit) nrDNA gene. PCR amplifications were conducted in a GeneAmp® PCR System 2700 thermocycler (Applied Biosystems, Foster City, California, U.S.A.) using standard protocols in a final volume of 25 μL. PCR products were purified according to the manufacturer's instructions with a GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech Europe GmbH, Germany). Sequencing reactions were carried out with PCR primers and the ABI PRISM Big Dye Terminator Cycle version 3.0 Sequencing Ready Reaction Kit (Applied Biosystems), according to the manufacturer's recommendations. The reaction was analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Sequence and phylogenetic analyses.—Sequence data were analyzed with EditView 1.0.1 (http://www.appliedbiosystems.com). BLAST searches (Altschul et al 1997) were performed with the analyzed sequences to identify similar sequences for determination and confirmation of the isolate's taxonomic placement. The ITS, small-subunit and large-subunit sequences were deposited in GenBank (AY230153, AY230152 and AY230151, respectively). To detemine the phylogenetic placement of *Rhynchostoma proteae*, 30 small-subunit sequences were added from GenBank and manually aligned with the corresponding sequence from *R. proteae* by inserting gaps with Sequence Alignment Editor version 2.0 (Rambaut 2002).

Phylogenetic analyses, including maximum parsimony with 1000 random-addition replicates and neighbor joining with uncorrected "p", Jukes-Cantor and Kimura-2-parameter models, were done with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2000). *Saccharomyces cerevisiae* (V01335) and *Taphrina deformans* (U00971) were used as outgroups. For parsimony analysis, gaps were treated as a fifth character. Gapped regions caused by major insertions were excluded from the analysis. The robustness of the trees was evaluated by 1000 bootstrap replications (Hillis and Bull 1993). Other indices calculated for parsimony topology included tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC). Resulting trees were printed with TreeView version 1.6.6 (Page 1996).

RESULTS

Taxonomy.—Three species of the Chaetothyriomycetes collected from Proteaceae in South Africa and Australia, namely *Rhynchomeliola australiensis*, a new species of *Rhynchomeliola* from *Lomatia*, and a new species of *Rhynchostoma* from *Protea*, are treated below.

Rhynchostoma proteae S. Lee et Crous, sp. nov.

FIGS. 1–17

Stromata maxime reducta. Ascomata stromatica, perithecioidea, immersa in textura hospitali, distincta, gregaria, in sectione verticali globosa vel subglobosa, usque ad 185 μm alta, usque ad 155 μm lata, collo ostiolato. Collum ostiolatum plerumque centrale, solum, obliquum, periphysibus, cylindricum, contractum ad apicem, usque ad 970 μm altum, usque ad 115 μm latum base, usque ad 50 μm apice. Peridium constans ex stratis duobus, 22.5–30 μm diam, carbonaceum. Paraphyses hyalinae, non ramosae, filamentosae, flexuosae, septatae, abundae, 1–2 μm latae. Asci circa septum peritheciale enascentes, unitunicati, octospori, fusiformes, apedicellati, comparate persistentes, (27–)29–35 × 5–7 μm, nullus apparatus apicalis observatus. Ascosporae bicellulares, ellipsoideae vel fusiformes, constrictae septo, septum leniter submedium, extremum omne contractum vel extremum unum leniter apiculatum, brunnescentes maturitate, oblique striatae trans paginam sporae in directione una, porus germinis inconspicuus, (9–)9.5–10.5(–11.5) × 3(–4) μm.

Stromata reduced around the venter of the ascomata, up to 15 μm wide, comprising dark, thick-walled cells (FIG. 6). Ascomata stromatic, perithecioid, immersed in host tissue, scarcely becoming superficial, separate, gregarious, in vertical section globose to subglobose, up to 185 μm high and 155 μm wide, with an ostiolar neck (FIGS. 1–4). Ostiolar neck most-


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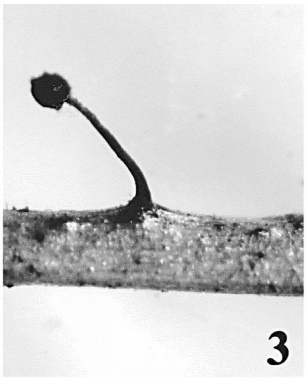
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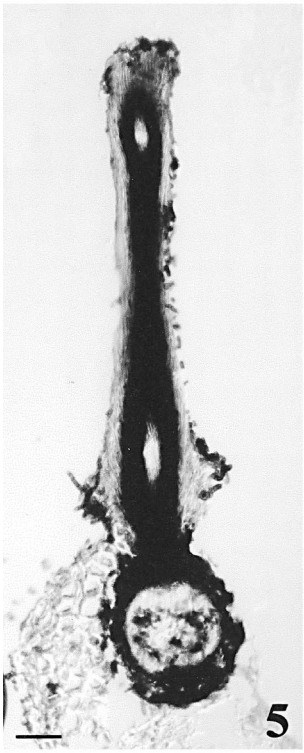
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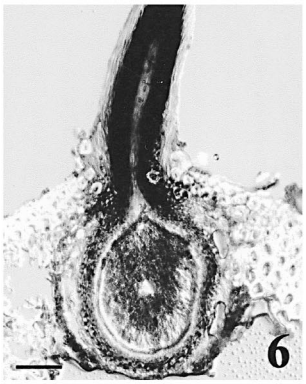
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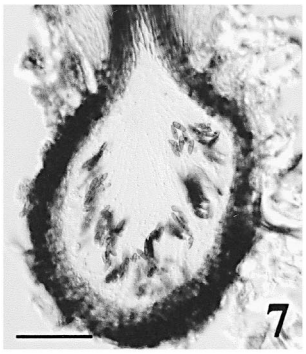
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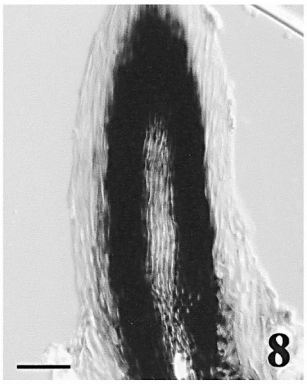
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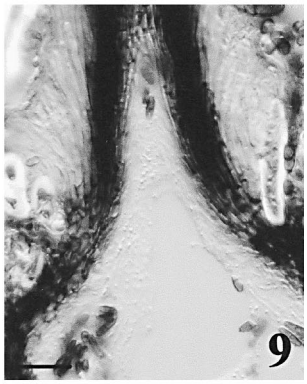
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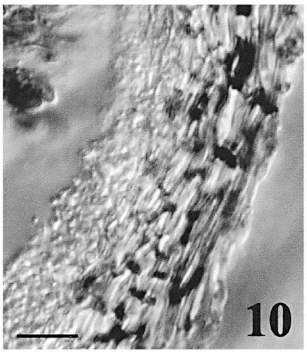
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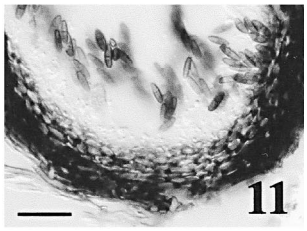
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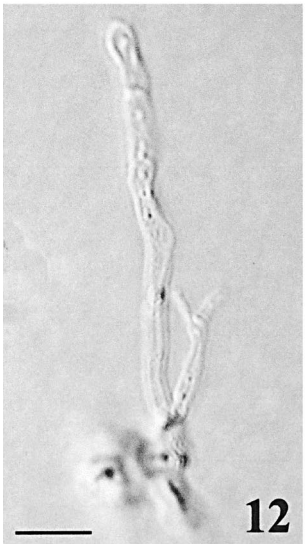
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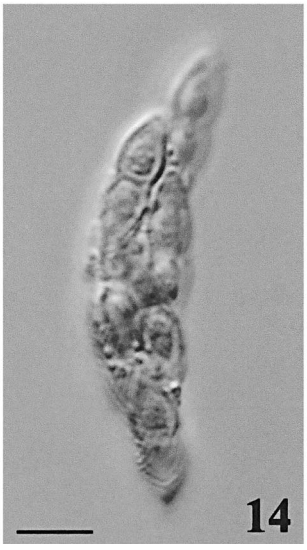
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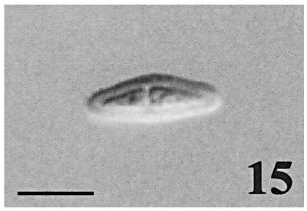
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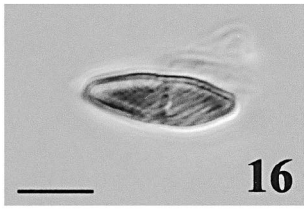
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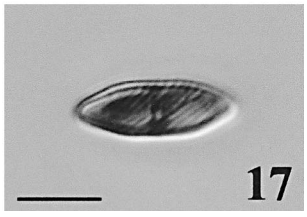
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ly central, single or rarely two, oblique, lined with periphyses, cylindrical, tapering off to the apex, consisting of longitudinally angular cells, up to 970 μm high, 115 μm wide at the base and 50 μm wide at the apex, with an outer amorphous crust (FIGS. 5, 7–9). Peridium consisting of two layers, 22.5–30 μm diam, carbonaceous, cells of outer layer angular, moderately thick-walled, brown, becoming paler toward the inside, cells of inner layer hyaline, angular, compressed (FIGS. 10, 11). Paraphyses hyaline, unbranched, filamentous, flexuose, septate, abundant, 1–2 μm wide (FIGS. 7, 12). Asci lining the perithecial wall unitunicate, octosporous, fusiform, apedicellate, relatively persistent, indistinguishable when ascospores are fully developed, (27–)29–35 × 5–7 μm, no apical apparatus observed (FIGS. 13, 14). Ascospores overlapping, multiseriate, bicellular, ellipsoidal to fusiform, constricted at the septum, septum slightly submedian, ratio of upper to lower cell 1–1.3:1, each end tapered or one end slightly apiculated, hyaline to pale brown when young, becoming medium brown at maturity, obliquely striated all over spore surface in one direction, striations already visible when immature, germ pore inconspicuous, (9–)9.5–10.5(–11.5) × 3(–4) μm (av. 9.9 × 3 μm) (FIGS. 15–17).

Colonies sterile, circular with entire margins, convex, lavender gray (43‴′f) to fuscous black (7‴k), reverse fuscous black (7‴k) with olivaceous buff margin (21″′d). Dense, velvety superficial mycelium; colonies 4.5 mm diam on MEA after 35 d at 25 C in the dark.

Etymology. In reference to the host genus, *Protea*.

Specimens examined. SOUTH AFRICA, WESTERN CAPE PROVINCE: Stellenbosch, Stellenbosch Mt. (Grid reference; S: 33°56.743′ E: 18°52.711′), on senescent flowerheads of *Protea laurifolia* Thunb. (Proteaceae), 24 May 2000, *F. Roets* SL39 [HOLOTYPE, PREM57498], ex-type culture CBS112051; 6 Jun 2000, *S. Lee* SL198 [ISOTYPE, BPI842141], *Protea burchellii* Staph (Proteaceae), 24 May 2000, *S. Lee* SL41 [PARATYPE, PREM57499]; 6 Jun 2000, *F. Roets* SL118 [PARATYPE, BPI842141].

Notes. Rhynchostoma proteae can be distinguished easily from *Rhynchostoma* species known to date. The type of *Rhynchostoma*, *R. minutum* P. Karst., has asci of similar dimensions (30 × 8 μm) but smaller ascospores (6–8 × 3–4 μm) (Saccardo 1882, Yue and Eriksson 1986, Winka and Eriksson 2000). Furthermore, *R. minutum* has a remarkably strong red pigment at the apex of the neck, which is absent in *R. proteae*. In contrast, *R. exasperans* P. Karst. has comparable ascospore dimensions (9–12 × 4–5 μm) but much larger asci (65 × 6 μm) (Saccardo 1882). Ascospores of *R. cornigerum* P. Karst. (8–10 × 3–4 μm) are similar to those of *R. proteae*, but the overall size of its ascomata, including the ostiolar neck (about 600 μm), is smaller than that of *R. proteae* (Saccardo 1882).

Rhynchomeliola australiensis (Petr.) E. Müll., *Beitr. Kryptogamenflora Schweiz* 11:595 1962. FIGS. 18–22
≡ *Rhynchostoma australiense* Petr., *Sydowia* 8:206 1954.

Ascomata nonstromatic, perithecioid, superficial among trichomes, with the base immersed, separate, gregarious, globose to subglobose, up to 80 μm high and 90 μm wide, with an ostiolar neck. Ostiolar neck central, single, cylindrical, tapering off toward the apex, consisting of longitudinally angular cells, up to 290 μm high and 30 μm wide at the base, and 23 μm wide at the apex (FIG. 18). Paraphyses hyaline, unbranched, filamentous, flexuose, septate, scanty, 1–2 μm wide. Asci lining the perithecial wall, unitunicate, octosporous, fusiform, apedicellate, relatively persistent, indistinguishable when ascospores are fully developed, 50–60 × 6–8 μm, no apical apparatus observed (FIG. 19). Ascospores overlapping, multiseriate, bicellular, ellipsoidal to fusiform, constricted at the septum, septum slightly submedian, ratio of upper to lower cell 1–1.2:1, each end tapered, hyaline to pale brown when young, becoming medium brown at maturity, obliquely striated all over spore surface in one direction, striations visible even when immature, germ pore inconspicuous, (12–)12.5–13(–14) × (3–)4(–4.5) μm (av. 12.9 × 4 μm) (FIGS. 20–22).

Specimen examined. AUSTRALIA, TASMANIA: Mount Franklin, on leaves of *Grevillea* sp. (Proteaceae), 20 Nov 1952 [HOLOTYPE, BPI618690].

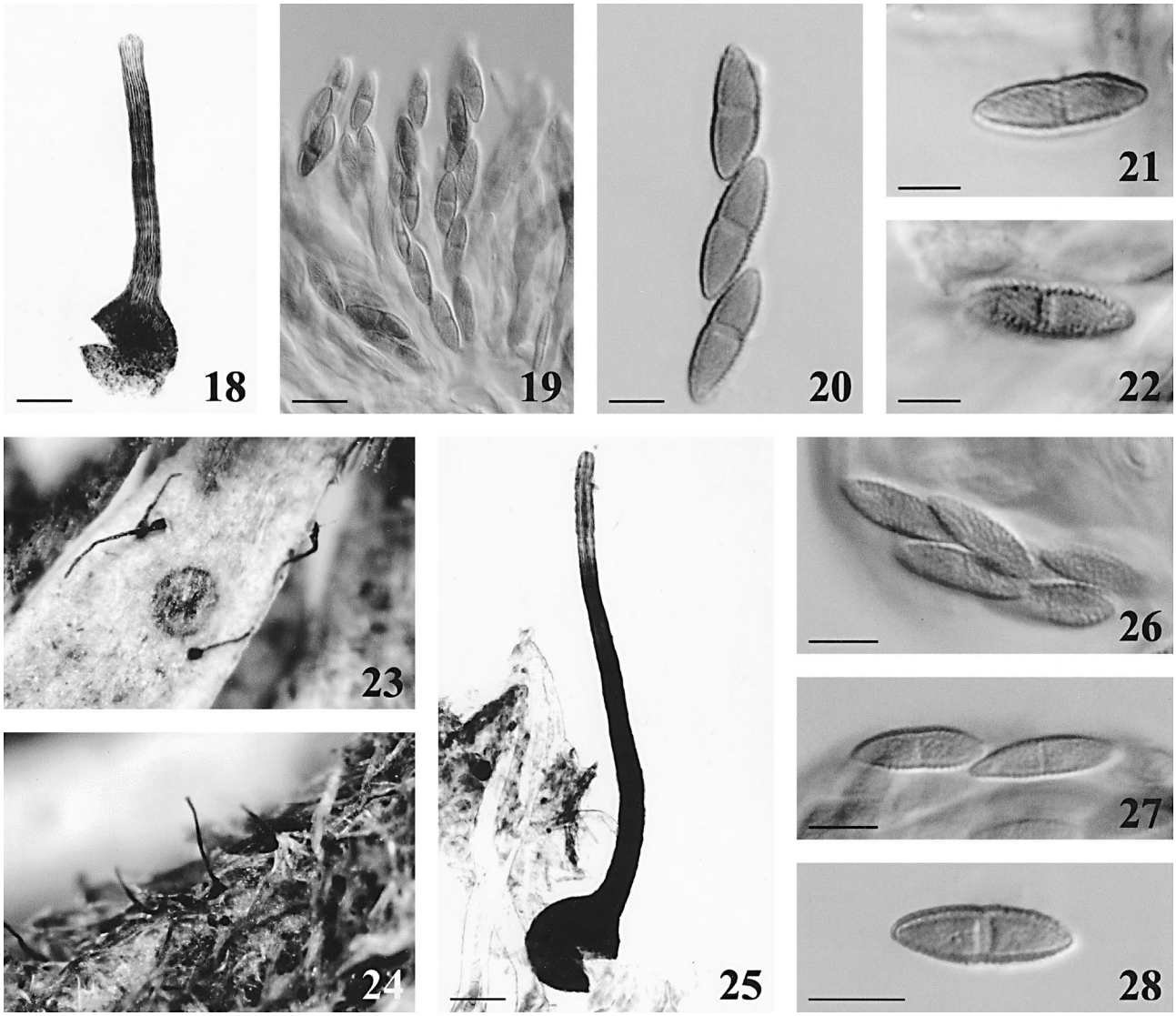
Notes. Paraphyses were sparse in this specimen, but they might have disintegrated with time. Although not mentioned in the original description (Petrak 1954), ascospores were found to be striate.

Rhynchomeliola lomatiae S. Lee et Joanne E. Taylor, sp. nov. FIGS. 23–28

Ascomata nonstromatica, perithecioidea, superficialia inter trichomata, base immersa, distincta, gregaria, globosa vel subglobosa, usque ad 100 μm alta, usque ad 95 μm lata, collo ostiolato. Collum ostiolatum centrale et solum, cylindricum, contractum ad apicem, usque ad 400 μm altum,

←

FIGS. 1–17. *Rhynchostoma proteae* (HOLOTYPE, PREM57498). 1–3. Perithecium on host surface with a long neck. 4. Perithecium squashed on a slide mount. 5, 6. Section through perithecium. 7. Asci among paraphyses. 8, 9. Section of neck showing an outer amorphous crust. 10, 11. Peridium. 12. Paraphysis. 13. Mature ascus with ascospores. 14. Ascus with immature ascospores. 15. Young ascospore. 16, 17. Ascospores showing oblique striations. Scale bars: 4 = 100 μm, 5–7 = 50 μm, 8, 9, 11 = 25 μm, 10 = 10 μm, 12–17 = 5 μm.



FIGS. 18–22. *Rhynchomeliola australiense* (HOLOTYPE, BPI618690), 23–28 *Rhynchomeliola lomatiae* (HOLOTYPE, BPI1110296). 18. Perithecium squashed on a slide mount. 19. Asci. 20–22. Ascospores showing oblique striations. 23, 24. Appearance of perithecia on the host surface. 25. Perithecium squashed on a slide mount. 26–28. Ascospores showing echinulated ornamentation. Scale bars: 18, 25 = 50 μm, 19 = 10 μm, 20–22, 26–28 = 5 μm.

usque ad 30 μm latum base, usque ad 20 μm latum apice. Paraphyses hyalinae, filamentosa, flexuosa, septatae, sparsae, 1–2 μm latae. Asci circa septum perithecialia enascentes, unitunicati, octospori, fusiformes, apedicellati, comparate persistentes, haud distincti ubi ascosporae plene effectae, 45–50 × 7–8 μm, nullus apparatus apicalis observatus. Ascosporae bicellulares, ellipsoideae, constrictae septo, septum leniter submedium, extremum omne contractum, brunnescentes maturitate, verruculosae, porus germinis inconspicuus, (8–)9–10(–11) × (2–)3(–4) μm.

Ascomata nonstromatic, perithecioid, superficial among trichomes, with the base immersed, separate, gregarious, globose to subglobose, up to 100 μm high and 95 μm wide, with an ostiolar neck (FIGS. 23, 24). Ostiolar neck central and single, cylindrical, tapering off toward the apex, consisting of longitudinally angular cells, up to 400 μm high, 30 μm wide at the base, and 20 μm wide at the apex (FIG. 25). Paraphyses hyaline, filamentous, flexuous, septate, sparse, 1–2 μm wide. Asci lining the perithecial wall, unitunicate, octosporous, fusiform, apedicellate, relatively persistent, indistinguishable when ascospores are fully developed, 45–50 × 7–8 μm, no apical apparatus observed. Ascospores overlapping multiseriate, bicellular, ellipsoidal, constricted at the septum, septum slightly submedian, ratio of upper and lower cell 1–1.2:1, each end tapered, hyaline to pale brown when young, becoming medium brown at maturity, verruculose, germ pore inconspicuous, (8–)

9–10(–11) × (2–)3(–4) μm (av. 9.5 × 3 μm) (FIGS. 26–28).

Etymology. In reference to the host genus, *Lomatia.*

Specimen examined. AUSTRALIA, TASMANIA: Mountfield National Park, on leaves of *Lomatia polymorpha* R. Br. (Proteaceae), 5 Mar 1951, type of *Apogloeum concinnum* and *Rhynchomeliola lomatiae* [HOLOTYPE, BPI1110296].

Notes. Rhynchomeliola lomatiae differs from *R. australiense* in ascospore ornamentation, verruculose in the former and striate in the latter. Another species with verruculose ascospores is *R. usteriana* (Speg.) Arx & E. Müll., which is similar to *R. lomatia* but has larger ascospores (13–18 × 3.5–5.5 μm) (Müller and von Arx 1962).

Sequence and phylogenetic analyses.—The small-subunit dataset contained 31 sequences, including two outgroups (TreeBASE study accession number S854). After the introduction of gaps, the alignment included 1375 nucleotide positions. Three areas with major insertions were excluded from the analysis. The first insertion (spanning characters 134–188), as well as the second insertion (characters 281–335), was present only in *Rhynchostoma minutum* AF242268. The third excluded region spanned characters 884 to 1296 and contained insertions present from *Rhynchostoma proteae* (263 nucleotides), *Rhynchostoma minutum* AF242268 (279 nucleotides) and *Ascolacicola austriaca* AF242263 (371 nucleotides). Of the remaining 852 characters, 573 characters were constant, 119 were parsimony uninformative and 160 characters were parsimony informative.

Neighbor-joining analysis of the dataset, using uncorrected (''p''), Jukes-Cantor (FIG. 29) and Kimura-2-parameter models, placed *Rhynchostoma proteae* and *Rhynchostoma minutum* with 100% bootstrap support as a sister group in the Chaetothyriales. Parsimony analysis of the small-subunit alignment yielded only two equally parsimonious trees (TL = 581 steps, CI = 0.623, RI = 0.761, RC = 0.474) and supported the placement of *Rhynchostoma proteae* as provided by neighbor-joining analysis (data not shown). The topology of the two parsimony trees were identical to the NJ tree, except for a polytomy involving *Pullularia prototropha* X91898 and *Capronia mansonii* X79318. Similar bootstrap values were obtained for both parsimony and NJ analyses. BLAST results of the ITS, large- and small-subunit sequences also confirmed the association between *Rhynchostoma proteae* and the Chaetothyriales.

DISCUSSION

Six species have been included in the genus *Rhynchomeliola* since its introduction. They are all foliicolous and distributed in the Southern Hemisphere, whereas the approximate 27 species of *Rhynchostoma* are mostly lignicolous or corticolous and have a worldwide distribution. Twenty of these species have been included in lists of Saccardo and Petrak, with three having been allocated to other genera.

Müller and von Arx (1962) placed *Rhynchostoma* in the Diatrypaceae (Sphaeriales) and *Rhynchomeliola* in the Sphaeriaceae (Sphaeriales), a family to which both genera later were allocated (Müller and von Arx 1973). The segregation between *Rhynchostoma* and *Rhynchomeliola* was based respectively on the presence or absence of stromatic tissue. Stromatic structures of *Rhynchostoma* had not been recognized before the treatment of Müller and von Arx (1962), who recognized immersed ascomata growing out of a stroma in the substratum. Species of *Rhynchostoma* vary from having immersed to superficial perithecia, while species of *Rhynchomeliola* form their ascomata superficially on a subiculum or on the leaf surface among trichomes, having a partly immersed base. Ascomata of *Rhynchomeliola lomatiae* and *R. australiense* are formed superficially among the trichomes, with the base slightly immersed (FIGS. 18, 23–25), while those of *Rhynchostoma proteae* mostly are found deeply imbedded between subepidermal cells (FIGS. 1–4). This feature is variable, however, because a few ascomata of *R. proteae* also were found to occur superficially on host tissue (FIG. 2).

Barr (1990) generally accepted the concept of Müller and von Arx (1962) and classified *Rhynchostoma* in the Bolineaceae (Xylariales), while *Rhynchomeliola* was omitted from her monograph of Hymenoascomycetes. Barr (1990) characterized *Rhynchostoma* as having ascomata immersed in stromata, forming numerous paraphyses and 1–2-celled ascospores with a minute germ pore at one end. The stromata of *Rhynchostoma* were described as poorly developed and reduced to a narrow, gelatinized outer layer over brownish, interwoven hyphae, which covered the widely erumpent and beaked ascomata (Barr 1990). Although the stromatic tissue around the venter of ascomata of *Rhynchostoma proteae* was not clearly visible, vertical sections through these perithecia revealed the stroma to have separated from the peridium, yet the peridium appeared to be complete (FIG. 6).

Hawksworth et al (1995) tentatively placed both *Rhynchostoma* and *Rhychomeliola* in the Trichosphaeriaceae (Trichosphaeriales), of which the genera are characterized by nonstromatic perithecial ascomata with a periphysate ostiole, presence of paraphyses and cylindrical asci with a nonamyloid apical ring and hyaline to brown, sometimes septate ascospores. Eriksson (1984, 1999) placed both genera in the Trichosphaeriaceae (Trichosphaeriales) but questioned the placement of *Rhynchostoma* in this family.

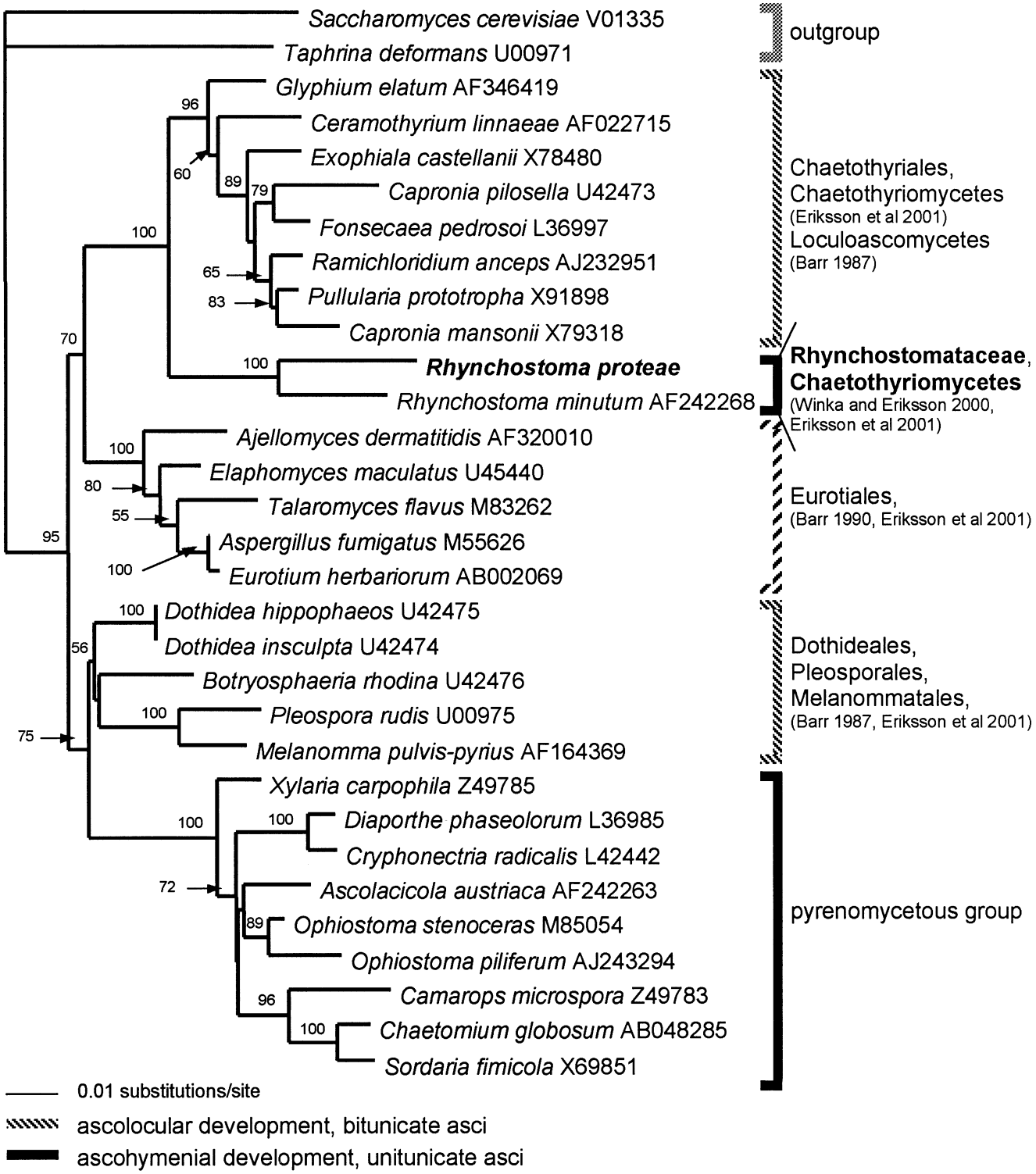


FIG. 29. Phylogenetic placement of *Rhynchostoma proteae* (in bold) inferred from SSU nrDNA sequence data. The distance tree was generated using neighbor-joining analysis with Jukes-Cantor distance correction. The numbers at the branch nodes indicate the confidence value (values higher than 50%) obtained from bootstrap analysis using 1000 replications. Vertical bars indicate morphological affinity.

Both *Rhynchomeliola* and *Rhynchostoma* share one or more characteristics that fit into the hitherto mentioned families. However, they do not exactly correspond to them in stromatic structure, ascal shape and the presence of an ascospore germ pore.

Analyses of sequence data of SSU nrDNA of *Rhynchostoma proteae* revealed an unexpected result, which placed this species as a sister clade to the Chaetothyriales (Chaetothyriomycetes), along with *R. minutum*, with 100% bootstrap support (FIG. 29). The taxon is distant from any pyrenomycetous clades in which *Rhynchostoma* is supposed to belong in the sense of Müller and von Arx (1962, 1973), Barr (1990) and Hawksworth et al (1995). Sequence data of large-subunit and ITS regions confirmed the relatedness of *Rhynchostoma* to the Chaetothyriales. This result confirms a similar finding based on a phylogenetic analysis of SSU nrDNA sequence data of the type species, *R. minutum*, which clustered within the Chaetothyriomycetes clade, providing the foundation for the new family Rhynchostomataceae Winka & O.E. Erikss. (Winka and Eriksson 2000).

The phylogenetic status of *Rhynchomeliola* based on molecular data could not be investigated because both cultivation and direct DNA extraction from herbarium specimen were unsuccessful.

The order Chaetothyriales Bat. & Cif. ex M.E. Barr, traditionally known as a member of loculoascomycetous fungi, was restored to accommodate the fungi that occasionally form locules in a stroma and have a hamathecium composed of short periphysoids and bitunicate asci (Barr 1987). In contrast, perithecia of both *Rhynchostoma proteae* and *R. minutum* form in a rudimentary stromatic structure, have distinct paraphyses observed in an early stage of ascomatal development, which sometimes extend over the asci, and have thin-walled, unitunicate asci. The appearance of *Rhynchostoma* species with the Chaetothyriales reflects the sister-group relationship of the Chaetothyriomycetes and the Eurotiomycetes, which lacks any morphological support (Winka et al 1998, Liu et al 1999, Tehler et al 2000). Lindemuth et al (2001) suspected the possibility of long-branch attraction with an accelerated substitution rate in the sister-group relationship of the Chaetothyriomycetes and Eurotiomycetes, but statistical data rejected their hypothesis. They eventually concluded that additional morphological research was required in these two groups. To resolve the puzzle of *Rhynchostoma*, a multiple gene molecular study, combined with ultrastructural and developmental studies of perithecia in *Rhynchostoma* would be required to shed further light on this alien placement.

Eriksson et al (2001) maintained the Rhynchostomataceae in the Chaetothyriomycetes as a family of uncertain position, while leaving *Rhynchomeliola* as a member of the Trichosphaeriaceae in the Sordariomycetes. Kirk et al (2001) also mentioned the Chaetothyriales connection of *Rhynchostoma* and *Rhynchomeliola* in the Sordariomycetidae. In this paper, the genera are treated *sensu* Müller and von Arx (1962), with the separation of *Rhynchostoma* and *Rhynchomeliola* based on stromatic structures. The affinity between two genera based on molecular data could not be examined due to the lack of information on *Rhynchomeliola*. It is apparent, however, that the two genera are almost identical in morphology, having rostrate ascomata, thin-walled unitunicate asci, paraphyses and ascospores with surface ornamentation. The only difference thus lies in their habit, being imbedded in a stroma or on a subiculum. Fresh collections on *Rhynchomeliola* species with molecular data might help further investigation of its generic relationship with *Rhynchostoma* and familial placement.

Mature ascospores of *Rhynchostoma proteae* emerge through the ostiolar neck and gather at the tip or around the neck in an outer amorphous crust that acts like glue (FIGS. 3, 5). This is accepted as a mechanism of insect-dispersed fungi, in which they make spores available for dissemination. Many insects are known to inhabit the inflorescences of *Protea* species (Coetzee and Giliomee 1987a, b, Coetzee 1989, Wright 1990, Visser 1992), but at this stage it still is unknown which insects might act as vectors. No red stain was seen at the apex of perithecial necks, as reported for *R. minutum* (Yue and Eriksson 1986). It is suspected that this pigment might play a role in insect attraction (O. E. Eriksson, pers comm). The availability of nutrients and the protective and moist environment, make flowerheads an ideal niche for flourishing fungal populations. Finding the insect vectors of these fungi will add greatly to our current meager knowledge of *Protea* flowerhead ecology and to the ecological processes within the fynbos biome as a whole. It is interesting to note that we could find *Rhynchostoma proteae* only in the Stellenbosch Mountains (Stellenbosch, South Africa) and in two *Protea* species, whereas ophiostomatoid fungi were found in several other, far distant, areas and in various proteas. The role of *Rhynchostoma proteae* in the relationship between fungal and insect communities within this niche and the microclimate within the inflorescences might be another exciting area for study.

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