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A new species of *Camarops* and phylogenetic analysis of related taxa in the Boliniaceae

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Abstract: *Camarops rogersii* is described as a new species in the family Boliniaceae (Order Boliniales) from Puerto Rico, distinguished by confluent, monostichous, soft, brightly-colored stromata. It is most similar to *Camarops flava* and *Mollicamarops stellata*. These taxa differ in ascospore morphologies, distant geographic distributions and their occurrence in distinct habitats. *Camaropella pugillus* is reassessed based on a collection from North Carolina and compared to *Camaropella lutea.* Phylogenetic analysis of nuclear 28S large subunit (LSU) DNA sequences supports the recognition of these taxa.

Key Words: Ascomycota, *Camaropella*, LSU, systematics

Introduction: The Boliniaceae was established by Rick in 1931 to include the single genus, *Camarops* P. Karst. Historically, it has been thought to have a close relationship with the Xylariaceae based on the stromatic ascomata and brown ascospores (Munk 1953; Dennis 1960; Wehmeyer 1975) but others have suggested it has affinities with the Sordariaceae (Doi and Nunomura 1980; Barr 1990). Molecular data has supported its close relationship with members of the Sordariales into which it was transferred (Andersson et al. 1995). More recently, the family has been elevated to the order level by Cannon (Kirk et al. 2001) and has been shown to be closely related to the more narrowly defined Sordariales in several recent phylogenetic studies (Huhndorf et al. 2004; Miller and Huhndorf 2005; Zhang et al. 2006). The Boliniales currently consists of two families, Boliniaceae and Catabotrydaceae, and over 30 species divided into nine genera including *Apiocamarops* Samuels & J.D. Rogers, *Camaropella* Lar. N. Vassiljeva, *Camarops*, *Catabotrys* Theiss. & Syd., *Cornipulvina* Huhndorf, A.N. Mill., F.A. Fernández & Lodge, *Endoxyla* Fuckel, *Mollicamarops* Lar. N. Vassiljeva, *Neohypodiscus* (Lloyd) J.D. Rogers, Y.M. Ju & Læssøe, and *Pseudovalsaria* Spooner. The order represents an ecologically important group of fungi with many members commonly occurring throughout temperate and tropical regions as saprobes on wood and bark. Although several new species have recently been described based on morphology, their phylogenetic relationships remain unknown (Samuels and Rogers 1987; Rogers and Samuels 1988; Catania and Romero 2005; Rogers et al. 2006).

Surveys of wood-inhabiting Sordariomycetes in tropical and temperate areas have led to the discovery of new species in various groups (Huhndorf et al. 2003, 2005; Miller et al. 2007). Several collections of Boliniaceae were collected as part of pyrenomycete surveys in Puerto Rico and North Carolina in 1997-98 and 2006 respectively. Morphological and molecular studies were conducted to assess their placement in the group.

Materials and methods

Taxon sampling and morphological characterization

Three specimens were sequenced for inclusion in this study, ILLS 58462, SMH3846 and SMH3001. Nine species in the Boliniaceae were included in analyses along with representatives of several orders within the Sordariomycetes. Two species in the Xylariales were used to root trees based on previous phylogenetic analyses (Huhndorf et al. 2004; Miller and Huhndorf 2005). GenBank accession numbers for all taxa are given after taxon names in Figure 1.

Ascomata were mounted in water which was then replaced with lactophenol containing azure A. Measurements were made and images were captured of material in both mounting fluids. Images were captured using photomacrography, bright field (bf), phase contrast (ph) and differential interference microscopy (dic) and photographic plates were produced following the methods of Huhndorf and Fernández (1998). Voucher specimens are deposited in the Field Museum Mycology Herbarium (F) or the Illinois Natural History Survey Mycology Herbarium (ILLS).

DNA extraction, PCR amplification, sequencing and sequence alignment

Methods for DNA extraction, PCR amplification and sequencing of the LSU gene along with procedures for the alignment of LSU sequences have been fully described elsewhere (Huhndorf *et al*. 2004; Miller *et al.* 2007). DNA was extracted directly from ascomata.

Phylogenetic analyses

Maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Portions of the 5' and 3' ends of LSU were excluded from all analyses due to missing data in

most taxa. A single spliceosomal intron in *Linocarpon appendiculatum* K.D. Hyde was excluded from all analyses as were six ambiguously aligned regions. The remaining unambiguously aligned characters were subjected to a symmetrical stepmatrix to differentially weight nucleotide transformations using STMatrix ver. 2.2 (François Lutzoni and Stefan Zoller, Biology Dept., Duke University), which calculates the costs for changes among character states based on the negative natural logarithm of the percentages of reciprocal changes between any two character states. Unequally weighted MP analyses were performed with 10 000 random addition heuristic searches, TBR branch-swapping, MULTREES option in effect, zero-length branches collapsed, constant characters excluded, and gaps treated as missing. Branch support was estimated by performing 1000 bootstrap replicates (Felsenstein, 1985) each consisting of 100 random addition heuristic searches as above.

Results

Phylogenetic analyses

A 1257 bp fragment of the 5' end of LSU, which consisted of 245 informative characters, was analyzed using MP. A single most parsimonious tree was generated in the MP analysis (Fig. 1). The Boliniaceae is weakly supported as a monophyletic group and is composed of two smaller clades, neither of which are supported. *Camarops rogersii* occurs as a sister taxon to *Camarops ustulinoides*. Both morphological and molecular data support the establishment of *C. rogersii* as a new species in the Boliniales.

Taxonomy

Camaropella lutea (Alb. & Schwein.) Lar.N. Vassiljeva, Fungal Diversity 25: 225 (2007). Figs. 2-6.

≡ *Camarops lutea* (Alb. & Schwein.) Nannf., Svensk Botanisk Tidskrift 66: 363 (1972).

Stromata immersed, valsoid with a prominent erumpent, ostiolar cushion, composed of yellow-colored, loose, hyphal tissue around the ascomata and brown-colored, denser hyphae around the ostioles. Ascomata perithecial, valsoid, in small to large clusters (ca. 20-50 perithecia per stroma, but highly variable), polystichous, obpyriform to subglobose with long necks converging in an erumpent, flat-topped cushion with more-or-less vertical sides. Ascomal walls dark brown, composed of pseudoparenchymatic cells. Paraphyses long, tapering above the asci. Asci clavate, stipitate, 50-60 X 5.5-6.5 µm, with 8 uniseriate ascospores. Ascospores ellipsoid to oblong, one-celled, brown, 5.5-6.5 X 2.5-3.0 µm, with inconspicuous pore at one end.

Material examined. UNITED STATES. North Carolina, Haywood Co., Great Smoky Mountains National Park, Cataloochee, Rough Fork Trail, 869 m, [35.6165, -83.1209], on wood fragment on ground, 23-V-2006, L.N. Vasilyeva, ILLS 58462 (GenBank EU481407).

Camaropella pugillus (Schwein.) Lar.N. Vassiljeva, Mikol. Fitopatol. 31(1): 6 (1997). Figs. 7-10.

≡ *Camarops pugillus* (Schwein.) Shear, Mycologia 32: 549 (1940).

Stromata immersed, valsoid with a distinct, erumpent, short or tall ostiolar cushion, hyphal tissue indistinct around the ascomata, dense, yellow-colored around the short ostiolar cushions (Fig. 8) and white-colored around the tall ostiolar cushions; a bluish-purple color sometimes present on the ostioles and surrounding substrate from masses of discharged ascospores. Ascomata perithecial, valsoid, in small to large clusters (ca. 15-30 perithecia per stroma, but highly variable), polystichous, subglobose with long necks converging in an erumpent, flat-topped cushion. Ascomal walls dark brown, composed of pseudoparenchymatic cells. Paraphyses long, tapering above the asci. Asci clavate, stipitate, spore bearing part 35-40 x 4-6 µm, stipe 14-17 µm

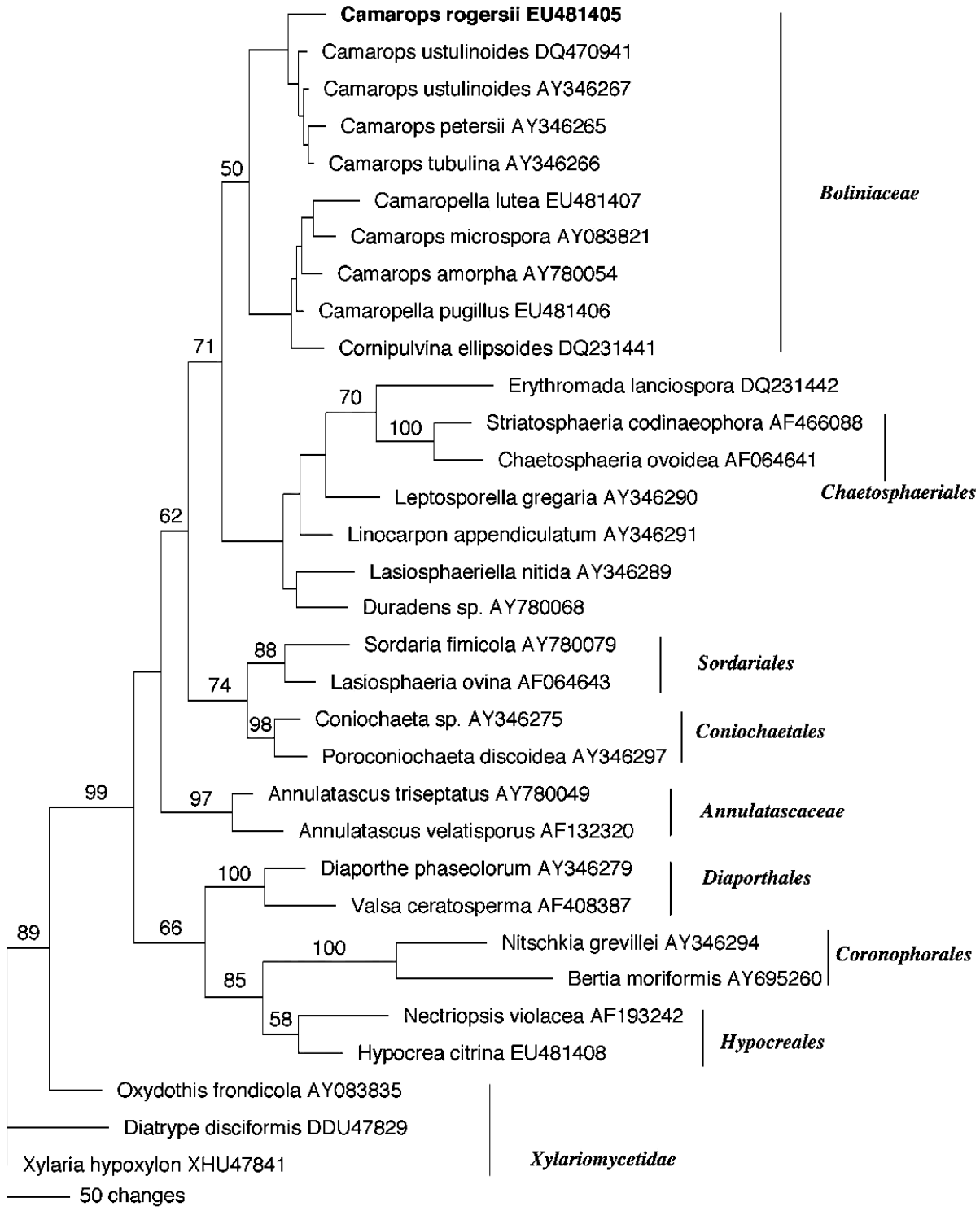
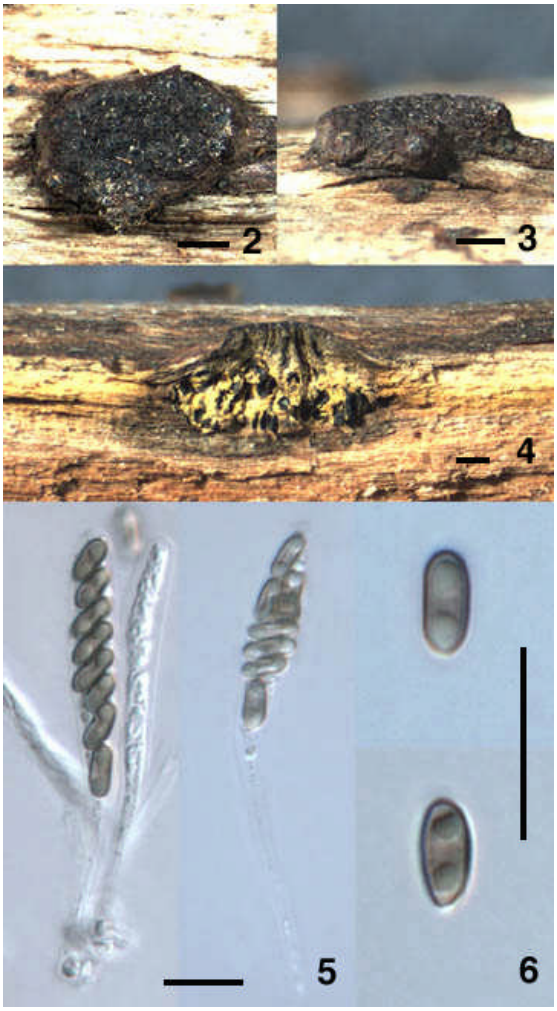
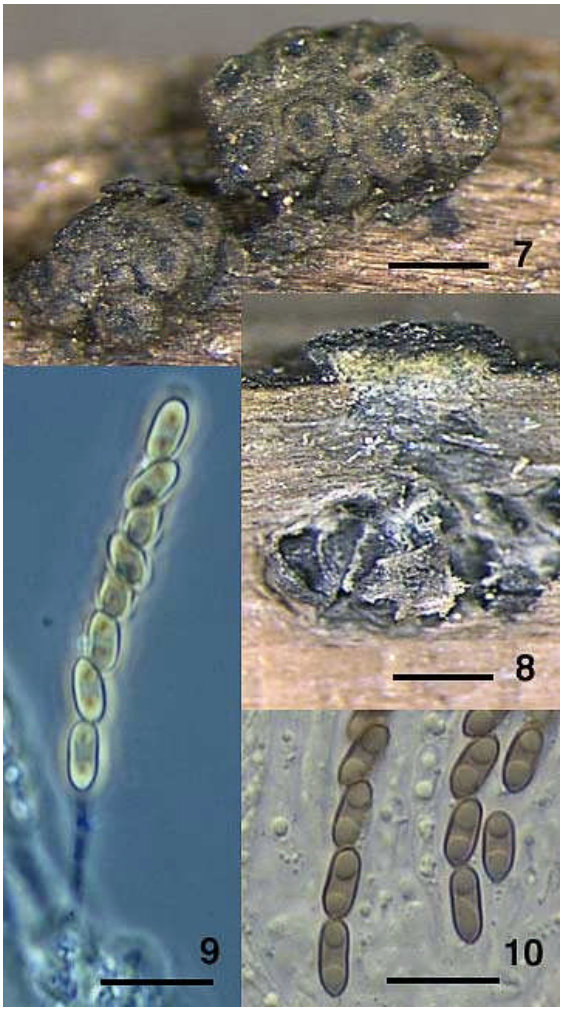


Fig. 1. The single most parsimonious tree (length = 1829.04; CI = 0.469; RI = 0.556; RC = 0.261) generated from a maximum parsimony analysis of 32 LSU sequences. Numbers above branches refer to maximum parsimony bootstrap values. *Xylaria hypoxylon* and *Diatrype disciformis* are outgroups.



Figs. 2-6. *Camaropella lutea*. 2-4. Stroma on the substrate. 5. Asci, dic. 6. Ascospores, dic. Bar lines: 2-4 = 1 mm, 5,6 = 10 µm.



Figs. 7-10. *Camaropella pugillus*. 7,8. Stromata on the substrate. 9. Ascus, ph. 10. Ascospores, dic. Bar lines: 7,8 = 500 µm, 9,10 = 10 µm.

long, with 8 uniseriate ascospores. Ascospores oblong, one-celled, brown, 6.3-7 x 2.5-3.5 µm, with inconspicuous pore at one end.

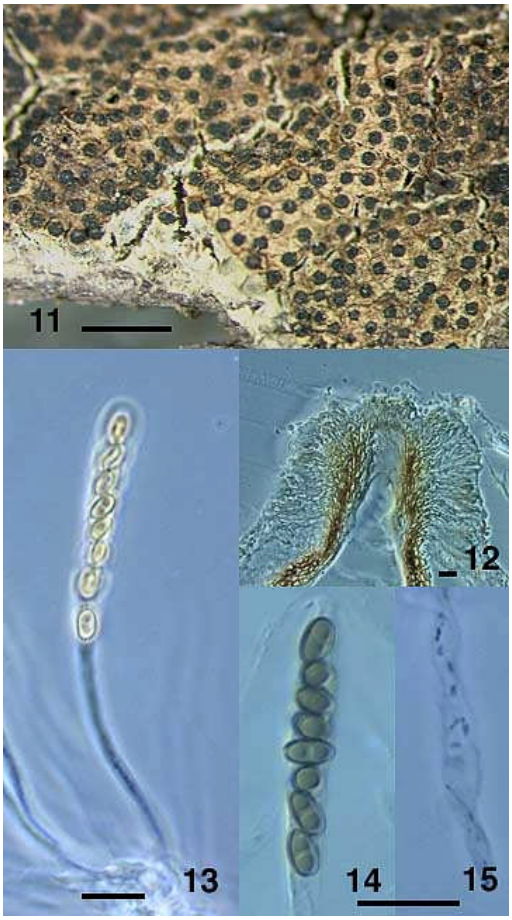
Material examined. UNITED STATES. North Carolina, Macon Co., Blue Valley, Highlands, 1000 m, [35.0192, -83.2736], on 10 cm branch in water, 30-VII-1998, F. A. Fernández, SMH3846 (F, GenBank EU481406).

Camarops rogersii Huhndorf & A.N. Mill. sp. nov. Figs. 11-15.
Mycobank: 511985

Etymology. The species is named in honor of Jack Rogers, an authority on the Boliniaceae.

Stromata superficialia, late effusa, flava vel fusca, mollia, ostiolares papillae. Ascomata obpyriformia, conferta, 425-450 µm alta, 325-350 µm diam. Asci clavati, longi-stipitati, 65-85 x 5-6 µm. Ascosporae ellipsoideae, brunneae, unicellulares, 4.8-5.3 x 2.5-3.5 µm.

Stromata superficial, widely effused, cream-to-yellow colored, becoming brown, soft-textured, composed of loose, hyphal tissue, easily disintegrating, with short, protruding, dark



Figs. 11-15. *Camarops rogersii.* 11. Stroma on the substrate. 12. Ascomal neck, ph. 13. Ascus, ph. 14. Ascospores, dic. 15. Paraphyses, ph. Bar lines: 11 = 500 µm, 12-15 = 10 µm.

ostiolar papillae. Ascomata perithecial, monostichous, densely packed, elongate-obpyriform, sometimes horizontally collapsing, 425-450 µm high, 325-350 µm diam, short beaked, ostioles round, surrounded with thickened stromatic tissue composed of a perpendicular palisade of elongate, hyaline, hyphal cells, ca. 45 µm thick. Ascomal walls dark brown, 15-20 µm thick, composed of pseudoparenchymatic cells. Paraphyses long, tapering above the asci. Asci clavate, long stipitate, spore bearing part 27-40 x 5-6 µm, stipe 38-45 µm long, with 8 uniseriate ascospores. Ascospores ellipsoid, one-celled, brown, 4.8-5.3 x 2.5-3.5 µm, with inconspicuous pore at one end.

Material examined. UNITED STATES. Puerto Rico, El Verde Research Area, Luquillo Mts., 16-ha Grid, 350 to 425 m, [18.3167, -65.8167], on trunk above the ground, 16-I-1997, S. M. Huhndorf & F. A. Fernández, SMH3001 (F, holotype designated here, GenBank EU481405).

Discussion: All the members of the Boliniaceae form a monophyletic group although without good support. The taxa separate into two sister groups, again however without support. *Camarops rogersii* groups with the *Camarops* Karst. species that form typical erumpent stromata: *C. ustulinoides* (Henn.) Nannf., *C. petersii* (Berk. & M.A. Curtis) Nannf. and *C. tubulina* (Alb. & Schwein.) Shear. *Camaropella pugillus* and *C. lutea* cluster with *C. microspora* (P. Karst.) Shear and *C. amorpha* (Boedijn) Nannf. *Camarops microspora* has stromata that are similar to *C. lutea* in being immersed in the wood with erumpent necks but *C. amorpha* has large, erumpent, yellow stromata with polystichous, long-necked ascomata. There is some trend toward long necks and yellow coloration in this clade. *Cornipulvina ellipsioides* Huhndorf, A.N. Mill., F.A. Fernández & Lodge, with soft stromata and long necks is also in this clade. It differs from *Camarops* and *Camaropella* by having beaked necks that entirely project from the clustered, stromatic ascomata and having hyaline, ellipsoid ascospores. The taxon was placed in the Boliniaceae when it was described (Huhndorf et al. 2005).

Camarops rogersii from a lowland tropical forest in Puerto Rico is morphologically very similar to two other described species - *Mollicamarops stellata* Lar. N. Vassiljeva, which was described from far eastern Russia from a deciduous forest habitat (Vasilyeva 2007), and *Camarops flava* Samuels & J.D. Rogers which was found growing on the hymenial surface of a polypore in New

Zealand (Samuels and Rogers 1987). In creating the genus *Mollicamarops,* Vasilyeva (2007) emphasized the distinctive stromatal structure. All three species share the features of thin, soft, brightly-colored stromata and similar stipitate asci and ascospores. The asci and ascospores are similar to those seen in other members of the family. *Camarops rogersii* differs from *M. stellata* by having smaller asci and ascospores. *Camarops flava* differs from the other two species by having coarsely striate ascospores and *M. stellata* differs from the other two by having stellate ostioles. They are also recognized as separate species based on their large geographic distance and differing habitats. Molecular data from the latter two species would be useful in assessing the distinction between all three species.

Camarops pugillus was given its own genus, *Camaropella* (Vasilyeva 1997) and a second species, *C. lutea* was recently transferred (Vasilyeva et al. 2007). The segregation of these species into a separate genus was based on the stromata having the characteristics of clustered ascomata with long beaks (Eutypelloid) and being immersed in wood (eutypoid or valsoid in configuration) (Vasilyeva et al. 2007), not erumpent to superficial as is found in *Camarops* species such as *C. ustulinoides*. There was some speculation that these two species might be as close as varieties (Shear 1940) but the molecular data does not seem to indicate that. Even from geographically close localities, the two species can be distinguished both morphologically and molecularly. *Camaropella lutea* forms stromata immersed in the wood with a yellow entostroma and composed of numerous perithecial ascomata. The necks converge into a wide, flat, mesa-like cushion that is erumpent on the wood surface. *Camaropella pugillus* forms immersed perithecial ascomata with reduced stromatal tissue in the collection examined. The ascomatal necks converge in a valsoid fashion and are erumpent from the wood surface, with yellow entostroma confined to the area surrounding the necks when the necks are small (Fig. 8). In the taller ostiolar cushions, the yellow color is reduced to the outer edges and the entostroma surrounding the ostioles is white. In the collection examined, the necks do not elongate greatly beyond the wood surface, a characteristic that is reported in collections from Europe (Lundqvist 1987), however the boundaries of the individual necks are clearly delineated (Fig. 7).

Acceptance of the segregation of species into the separate genera, *Mollicamarops* and *Camaropella*, remains to be assessed with additional molecular data. With additional collections and other genes, an assessment can be made whether *C. microspora* and *C. amorpha* should be transferred to *Camaropella* and whether *Cornipulvina* stands as a separate genus. Not enough species are included in this analysis to draw adequate conclusions and part of the difficulty lies in the lack of molecular data for the type species *Camarops polyspermum* (Mont.) J.H. Mill. If molecular data followed a placement based on morphology, *C. polyspermum* should cluster with *Camarops tubulina*. Greater taxon sampling and additional genes would help clarify relationships within this group.

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