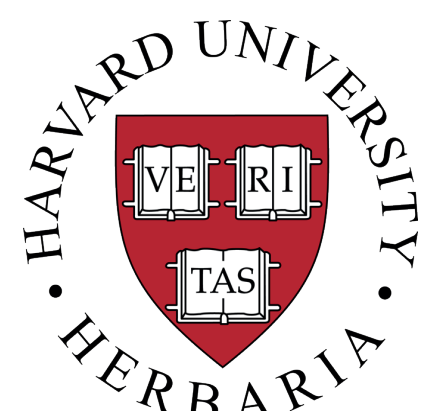


A new nematode trapping *Orbilia* from Puerto Rico

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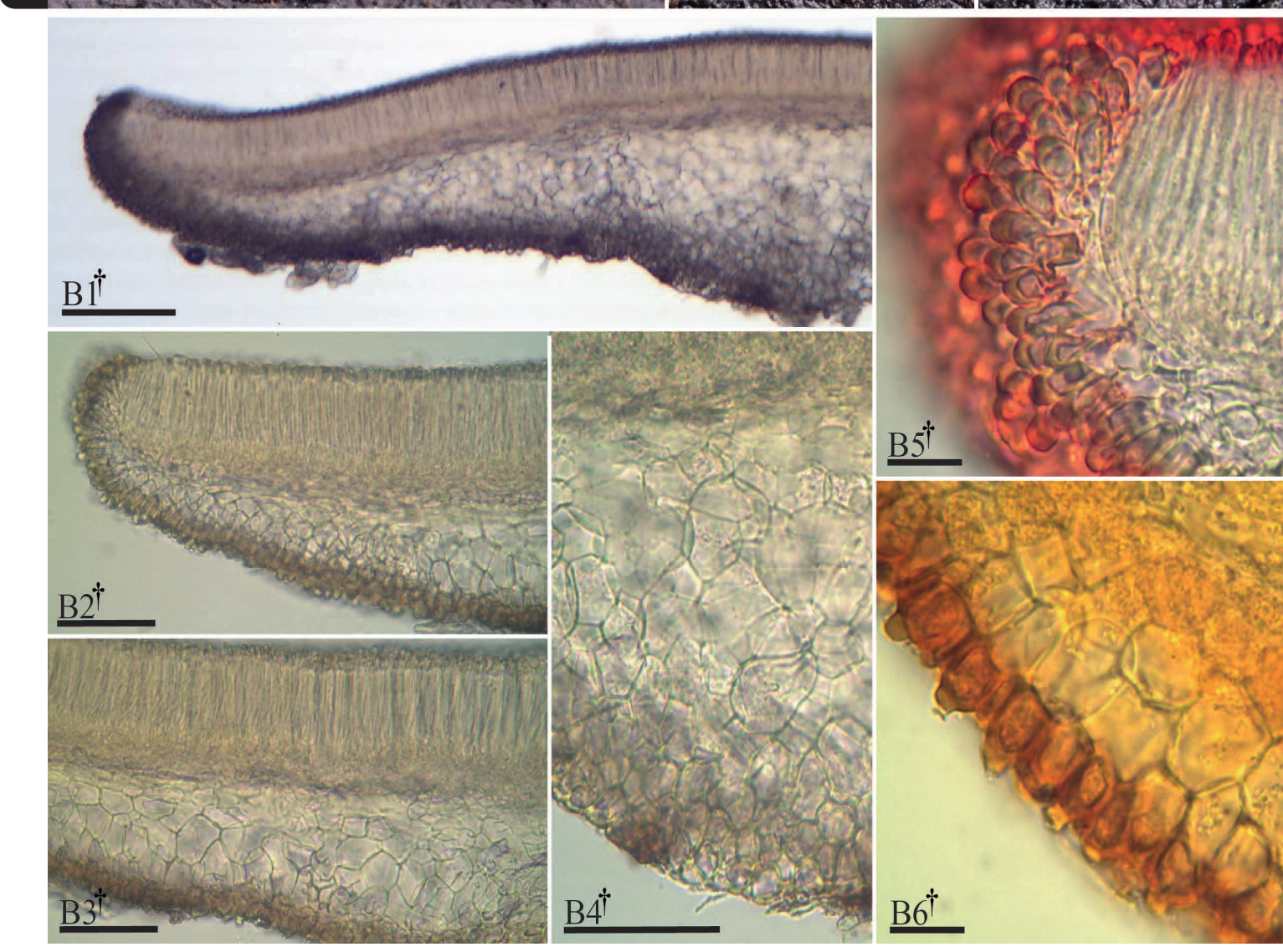
1. Farlow Herbarium, Harvard University, Cambridge, Massachusetts, United States of America. 2. Blaihofstr, Tübingen, Germany. 3. Plant Pathology Herbarium, Cornell University, New York, United States of America.



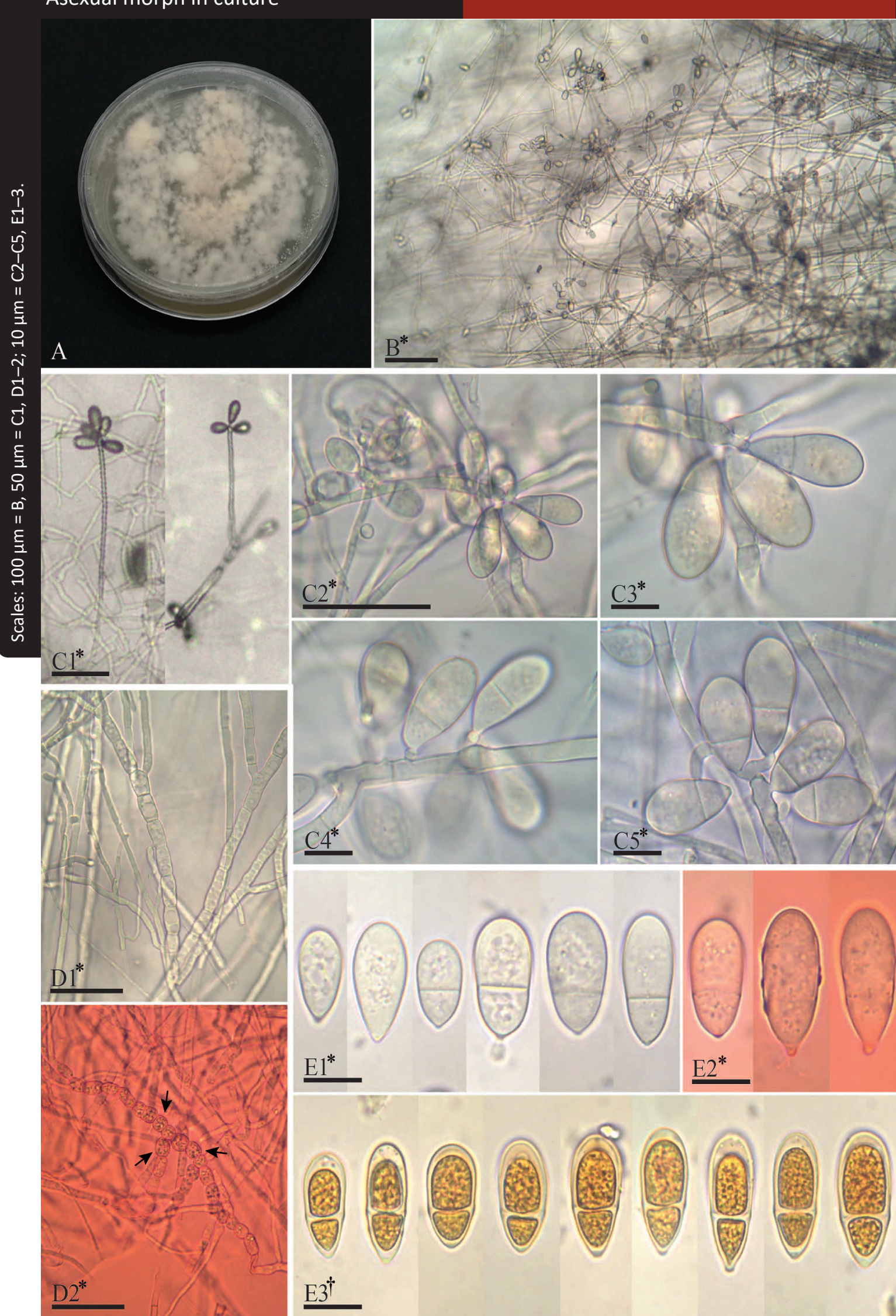
INTRODUCTION

Fungi are recognized worldwide as saprobes, parasites and symbionts, but a small percentage of Zygomycota, Basidiomycota and Ascomycota evolved as carnivores (Yang *et al.* 2012). Fungi with this lifestyle appear in microhabitats poor in nitrogen. In such situations, fungi with the ability to capture small animals become more competitive in colonizing the available substrates (*op. cit.*). Carnivorous Ascomycota can produce complex trapping structures – constricting and non-constricting rings, sessile or stalked adhesive knobs, and adhesive nets (*op. cit.*). All these types of traps can be found in the genus *Orbilia* Fr. (sexual morph, erected in 1836), but solely in section *Arthrobotrys* (asexual morph, Baral *et al.* 2018). Under the modern trend of genus splitting, however, the name *Arthrobotrys* is still used by some authors at the generic level. *Arthrobotrys* Corda 1839 was erected three years after *Orbilia* and is the oldest generic name applied to asexual trapping morphs of *Orbilia*. Although more than 100 spp. have earlier been recognized in *Arthrobotrys* for species with 1-septate conidia formed on swollen fertile nodes in roundish clusters, 20 years ago Scholler *et al.* (1999) accepted only 46 species by redefining the genus for those with three-dimensional adhesive networks, reclassified by Baral *et al.* (2018) as *Orbilia* series *Arthrobotrys*. Species of *Orbilia* section *Arthrobotrys* often have sexual morphs with narrow, subulate and curved ascospores with small spore bodies in the rounded apex. Fungal diversity in the Caribbean Islands is far from being fully documented, but this region is one of the world's biodiversity hotspots (Myers *et al.* 2000). Little is known about the diversity of Orbiliomycetes from Puerto Rico. Cantrell & Lodge (2008) compiled a list of fungi from Puerto Rico, and only mentioned four species of *Orbilia*: *O. andina*, *O. chrysocoma*, *O. delicatula* and *O. cf. gaillardii*. As far as we know, 14 species of Orbiliomycetes have been verified by Baral during his monographic work in the class (Baral *et al. ined.*). But some occurrences were missed in their list, for example: *O. blumenaviensis* and *O. pilosa* (Baral *et al. ined.*). On the other hand, Pfister collected other species that were never published, such as: *A. musiformis* (PR-98-20, culture #256) and *O. tenuissima* (PR-11) (personal notes of Pfister, identified by Baral). These five collections have *Arthrobotrys*-like asexual morphs and are so far the only reports of nematode trappers for the Caribbean region. During IMC11 (Puerto Rico, 2018), several collections of Orbiliomycetes were found in Julio Enrique Monagas Park during the field trip of the Ascomycete Workshop. These were deposited in FH (Farlow Herbarium). One such collection caught the attention of the first author due to its distinctive morphological features. This species is not related to any of the species reported by Cantrell & Lodge (2008), Pfister or Baral (*ined.*). The aim of this investigation is to describe a new species to science providing morphological and phylogenetical evidences for its inclusion in *Orbilia* series *Arthrobotrys*.

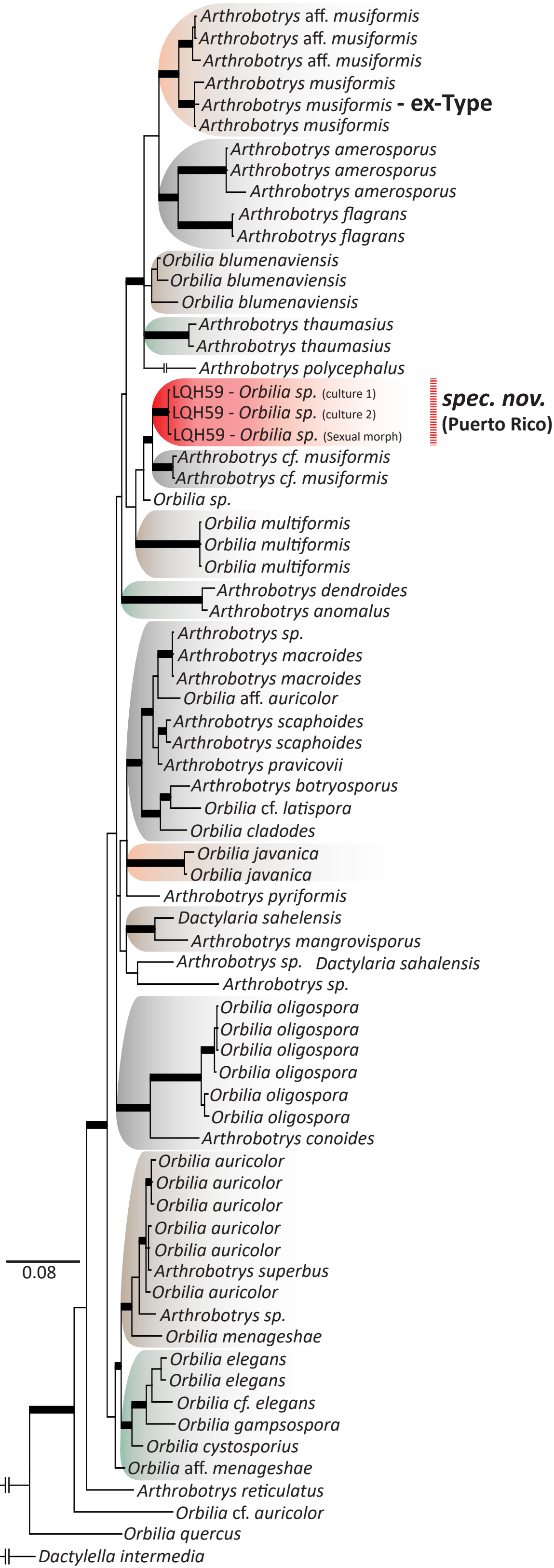
A1–6. Rehydrated apothecia on the substrate. B1. Transversal section of the apothecia. B2–4. Details of excipulum at margin, upper and lower flank. B5–B6. Cells of the cortical layer at margin and upper flank: B5. glassy processes stained in Congo red, B6. dextrinoid reaction or the cortical cells in MLZ.



A. Macromorphological aspects of the colony in PDA. B. General view of the vegetative hyphae, conidiophores and conidia. C1–5. Conidiophores with conidia attached. D1. Vegetative hyphae. D2. Chlamydospores.



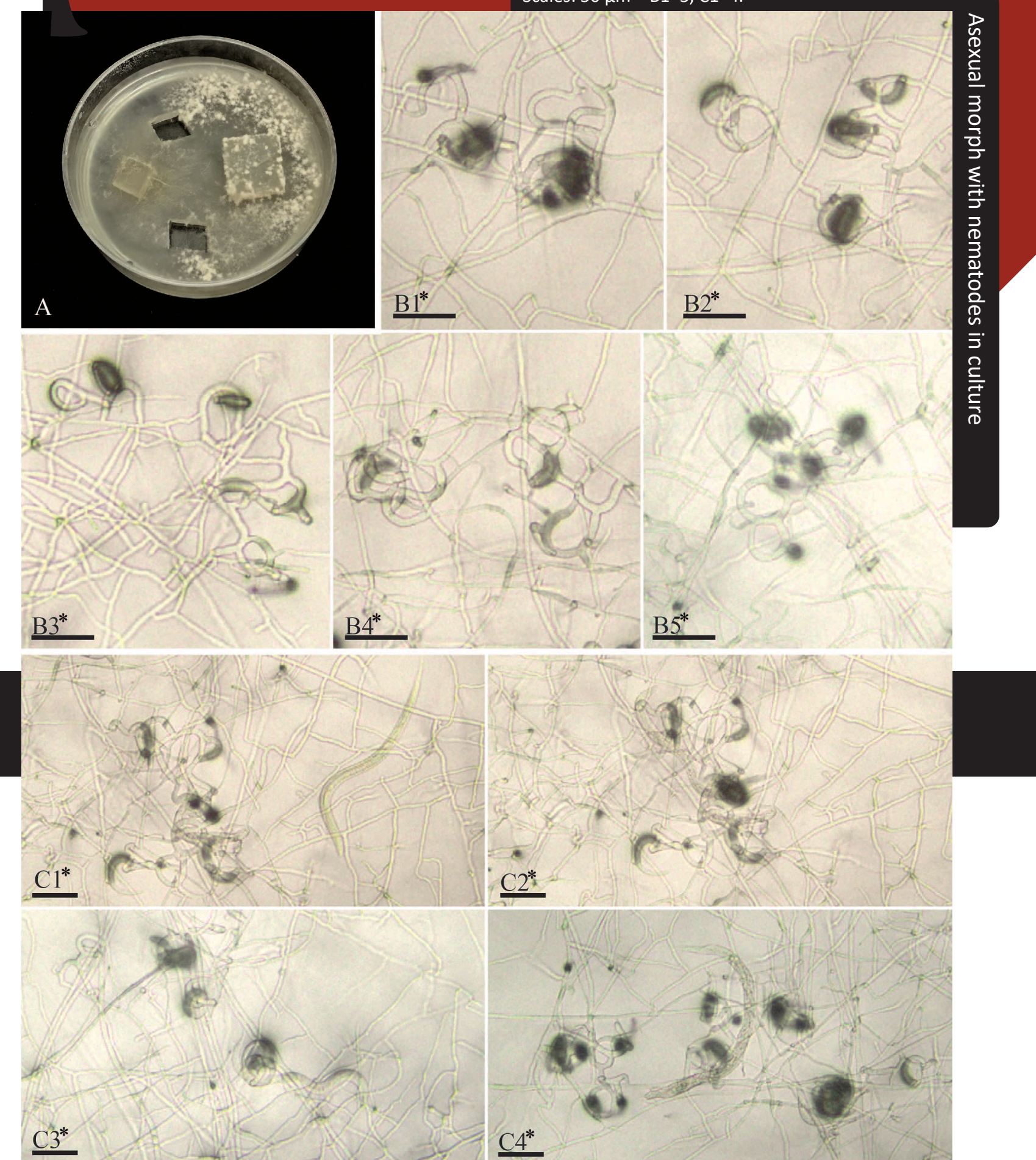
Bayesian majority-rule consensus tree based on concatenated ITS and LSU sequences. Bold branches are those which were well supported (> 0.95 BIPP) by BI methods



RESULTS

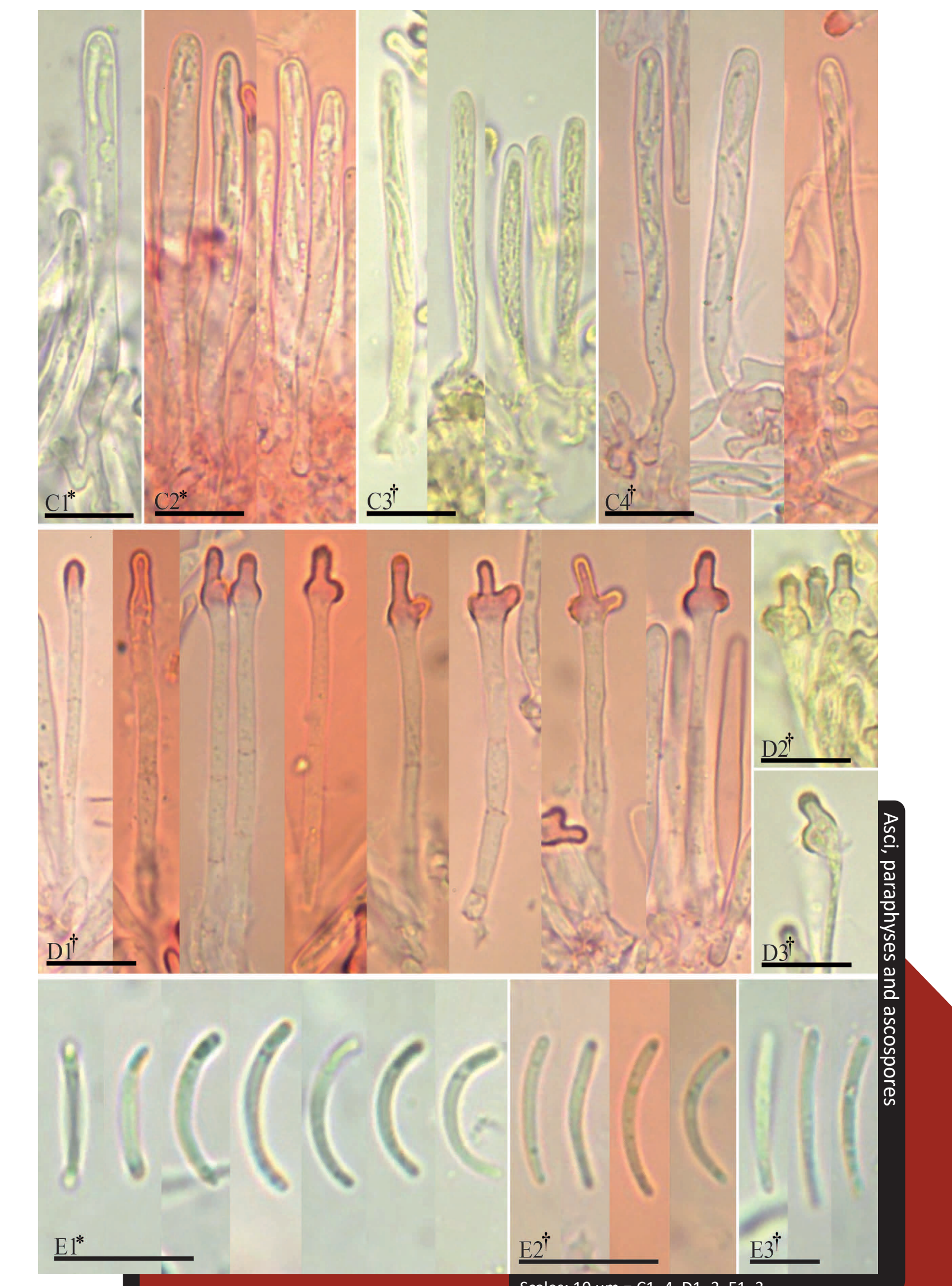
Using Baral's unpublished key for series *Arthrobotrys* based on the sexual morph, the most similar species are *Orbilia blumenaviensis*, *O. terrestris*, and the sexual morphs of *A. javanica* and *A. musiformis*. All these species have similar falcate spores. *O. blumenaviensis* is a subtropical to tropical species but differs in its lanceolate paraphyses and 1–3-septate conidia. *O. terrestris* is known by now only from the type collection and its sexual morph is on soil in mountainous central Asia; its ascospores are wider than ours ($\dagger 1.4\text{--}1.7\ \mu\text{m}$ vs. $\dagger 0.9\text{--}1.1\ \mu\text{m}$) and have a distinct tail. *A. javanica* and *A. musiformis* have similar conidia and ascospore morphology. The former has been reported from subtropical to tropical southeastern Asia, the latter is widespread in temperate and tropical ecosystems. Some biometric differences are noted between these two species and the collection from Puerto Rico. First, the ascospore length (*in situ*) of our collection ($\ast 10\text{--}11.8\ \mu\text{m}$) is larger than in the two species (*A. javanica* = $\ast 7\text{--}9\ \mu\text{m}$, *A. musiformis* = $\ast 7.7\text{--}10\ \mu\text{m}$). Second, the conidial length tends to be shorter while the width is in the middle: Puerto Rico = $\ast 14\text{--}26 \times 7.5\text{--}13\ \mu\text{m}$, *A. javanica* = $\ast 25\text{--}42.5 \times 7.5\text{--}15.5\ \mu\text{m}$, *A. musiformis* = $\ast 18.5\text{--}32.5 \times 6\text{--}8.5\ \mu\text{m}$ (the latter two measurements taken from Baral *et al. ined.*). None of the species mentioned have similar thick-walled cortical excipular cells with glassy caps in combination with paraphyses with beaked, partly branched apex, therefore we consider this as a species new to science.

B1–5. Three-dimensional adhesive networks formed after 1 day in contact with nematodes. C1. Living nematodes close to the traps. C2–3. Living nematodes trapped. C4. Dead nematode with hyphae inside.



MATERIALS & METHODS

Morphological methods and abbreviations were adopted from Baral (1992): * = living state; † = dead state. Both sexual and asexual morph were characterized in this study. PDA and CMA were inoculated using a piece of substratum bearing one apothecium. Once mycelia covered the media, subcultures were made and some of them were also used to extract DNA. Some of the plates were inoculated with nematodes (*Caenorhabditis elegans*) to induce the formation of traps. The culture was monitored over 24 hours to observe trap formation and the capture of nematodes, and to observe the different stages of the process. DNA was extracted using Qiagen QIAamp DNA Micro Kit from mycelia from cultures and from apothecia following Karakehian *et al.* (2019). A dataset with 72 sequences of ITS and LSU for *Orbilia* species with *Arthrobotrys*-like nematode traps was used to explore the phylogenetic relationships among them and the new Puerto Rican species following the methods in Quijada (2015).



C1–4. Living and dead asci. D1–3. Morphological variation of paraphyses, with one or three cylindrical beaks. E1–3. Living and dead ascospores.

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