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Ultrastructural studies on freshwater ascomycetes, *Fluminicola bipolaris* gen. et sp. nov.

Sze-Wing Wong[1], Kevin D. Hyde[1*] and E.B. Gareth Jones[2]

[1] Fungal Diversity Research Project, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong; * email: kdhyde@hkucc.hku.hk
[2] Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

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Fluminicola bipolaris is described based on a specimen found on submerged wood collected in a tropical river in the Philippines. The taxon is similar to species of *Annulatascus,* but differs in having ascospores with bipolar bifurcate or cup-like appendages. The structure of these appendages is unique and is illustrated at the light, scanning and electron microscope levels. *Fluminicola bipolaris* is compared with species of *Annulatascus* and *Proboscispora*.

Key words: appendages, Annulatascaceae, freshwater fungi, ontogeny, taxonomy.

Introduction

A number of new ascomycete genera, e.g. *Annulatascus* K.D. Hyde (Hyde, 1992), *Proboscispora* S.W. Wong and K.D. Hyde (Wong and Hyde, 1999) and *Submersisphaeria* K.D. Hyde (Hyde, 1996) have recently been reported from tropical rivers. These genera have several characters in common, including dark brown to black, beaked ascomata, wide and tapering paraphyses, and long cylindrical, unitunicate asci, provided with a relatively massive, refractive, apical ring. The Annulatascaceae has recently been introduced to accommodate these genera (Wong, Hyde and Jones, 1998a). In a collection of freshwater fungi from the Philippines, and subsequently several other countries, we have collected an ascomycete possessing characters typical of the Annulatascaceae, but which differ in having ascospores possessing bipolar bifurcate or cup-like appendages. *Fluminicola bipolaris* gen. et sp. nov. is therefore introduced to accommodate this taxon which is illustrated with interference contrast and electron micrographs. *Fluminicola bipolaris* is compared with other genera in the Annulatascaceae. The ascospore appendages are also compared at the ultrastructural level with those of *Lanspora coronata* K.D. Hyde and E.B.G. Jones (Hyde and Jones, 1985).

Materials and methods

Wood submerged in rivers in the Philippines, was collected and subsequently incubated in moist plastic boxes. The wood was examined periodically to check for the presence of fruiting structures. Preparation of specimens for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) follow those of Wong, Hyde and Jones (1998b).

Taxonomy

Fluminicola S.W. Wong, K.D. Hyde and E.B.G. Jones, gen. nov.

Etymology: *Flumen* and *cola* meaning "river" and "dweller" respectively.

Ascomata globosa, subglobosa vel ellipsoidea, immersa vel semi-immersa, coriacea, solitaria. *Asci* 8-spori, unitunicati, cylindrici, apparatu apicali praeditae. *Ascosporae* fusiformes, hyalinae, 3-septatae, appendicibus bipolaribus bifurcatisque praeditae.

Ascomata globose, subglobose or ellipsoidal, immersed or semi-immersed, coriaceous, solitary and beaked. *Necks* brown or black, periphysate. *Peridium* comprising several layers of brown walled compressed cells. *Paraphyses* wide at base, septate and tapering distally. *Asci* 8-spored, unitunicate, cylindrical, with a relatively massive refractive, bipartite (at TEM level) apical ring. *Ascospores* uniseriate or overlapping uniseriate, fusiform, hyaline, 3-septate, with mesosporial outgrowths which form verruculose ornamentations and irregular bifurcate or cup-like bipolar appendages.

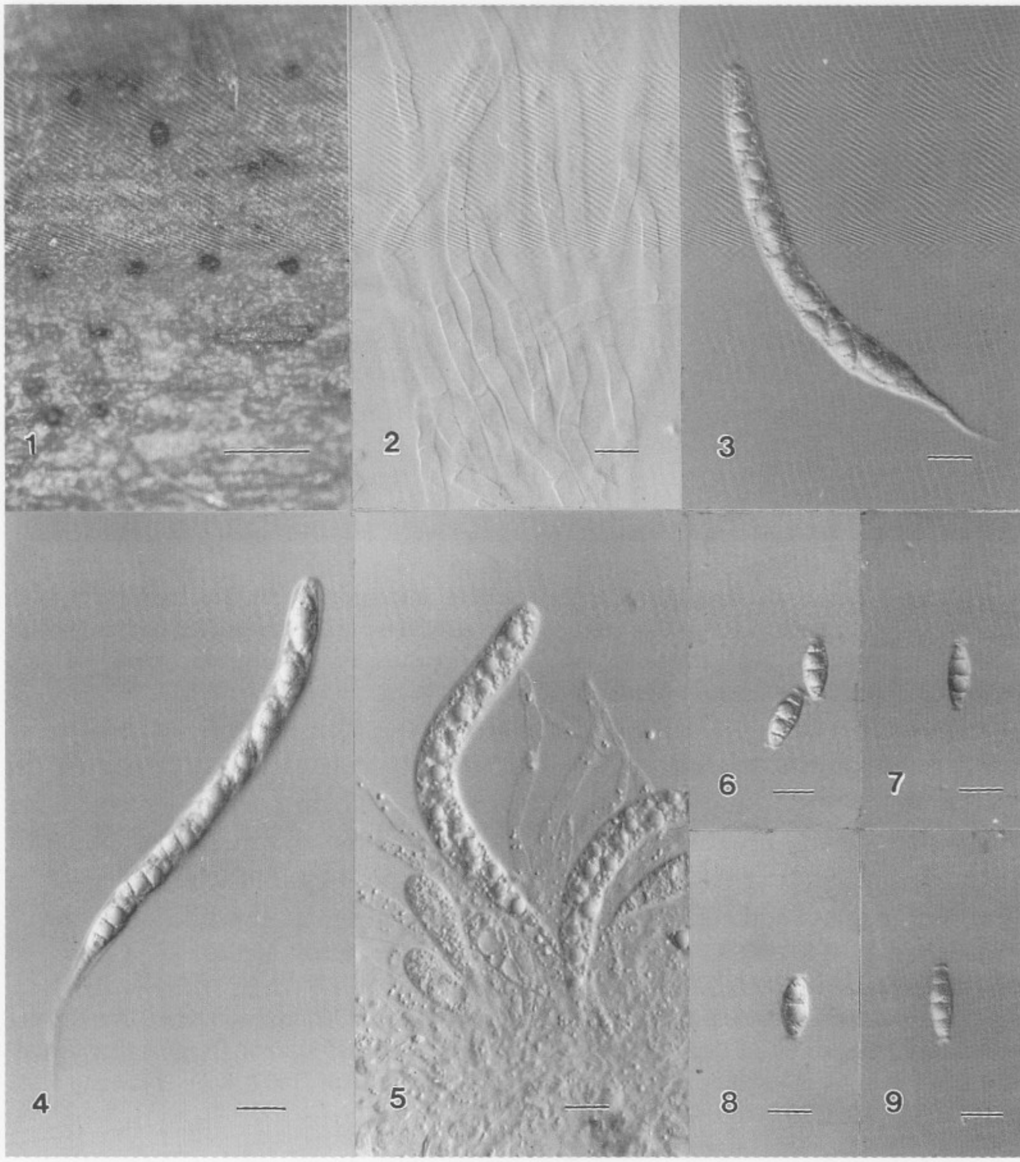
Type species: *Fluminicola bipolaris* S.W. Wong, K.D. Hyde and E.B.G. Jones sp. nov.

Fluminicola bipolaris S.W. Wong, K.D. Hyde and E.B.G. Jones, sp. nov.
(Figs. 1-16)

Etymology: *bipolaris,* referring to the appendages at each end of the ascospore.

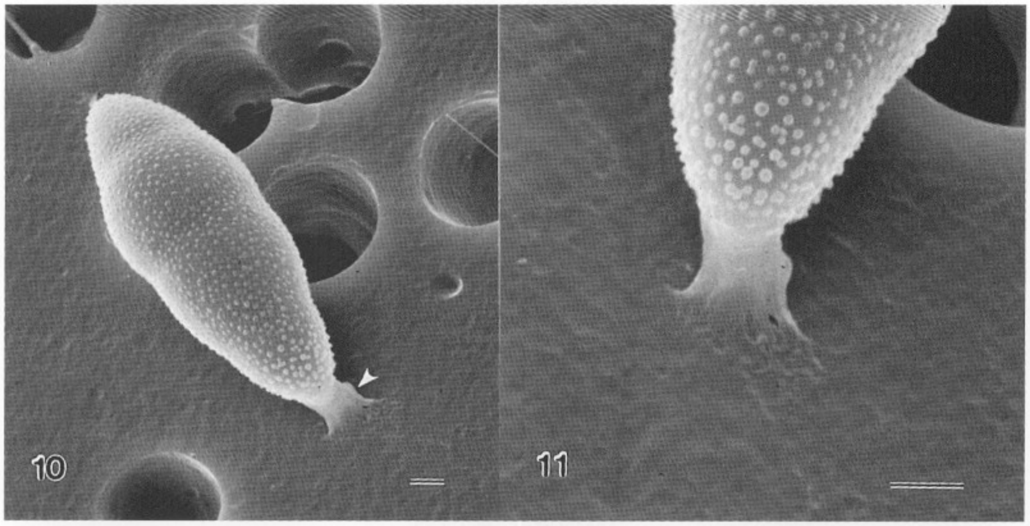
Ascomata 75-177 µm longa, 39-80 µm diam., immersa vel semi-immersa, globosa, subglobosa vel ellipsoidea, coriacea, solitaria. *Asci* 107-192 µm longi latique 9-12 µm, 8-spori, cylindrici, apparatu apicale 3-6 µm longi latoque 1.5-3 µm praediti. *Ascosporae* 15-21 µm longae lataeque 6-9 µm, fusiformes, hyalinae, 1-3-septatae, appendicibus bipolaribus bifurcatisque praeditae.

Ascomata 75-177 µm long, 39-80 µm diam., immersed or semi-immersed, globose, subglobose or ellipsoidal, black, coriaceous, solitary, beaked (Fig. 1). *Neck* short, central or lateral, black. *Peridium* comprising several layers of brown walled compressed cells. *Paraphyses ca* 11 µm wide at base, up to 5 µm at the apex, tapering, hyaline and septate (Fig. 2). *Asci* 107-192 × 9-12 µm ($\bar{x}$ = 145 × 10 µm, n = 25), 8-spored, cylindrical, base tapering to a point, with a



Figs. 1-9. *Fluminicola bipolaris.* Light micrographs. **1.** Immersed or semi-immersed ascomata on wood surface. **2.** Wide septate paraphyses. **3, 4.** Asci, note the relatively massive refractive apical ring. Ascospores are arranged uniseriately or overlapping uniseriately in the ascus. **5.** Immature ascus with a small apical ring. **6-9.** Ascospores with bipolar bifurcate or cup-like appendages. Bars: 1 = 100 µm; 2-9 = 10 µm.

relatively massive refractive apical ring (3-6 µm long, 1.5-3 µm wide) (Figs. 3-5). *Apical ring* bipartite and derived from the inner ascus wall (at EM level). *Ascospores* 15-21 × 6-9 µm ($\bar{x}$ = 18 × 7 µm, n = 50), uniseriate or overlapping



Figs. 10, 11. *Fluminicola bipolaris.* Scanning electron micrographs. **10.** Fusiform ascospores which are slightly constricted at the septa, with verruculose wall ornamentations and polar appendage (arrowed). **11.** Irregular ascospore appendages closely appressed to the polycarbonate membrane. Bars = 1 μm.

uniseriate, fusiform, hyaline, 1-3-septate, slightly constricted at the septa (at EM level), with mesosporial outgrowths which form verruculose ornamentations and irregular bifurcate or cup-like bipolar appendages (Figs. 6-9). *Appendages* at first flattened against the ascospore tip, but when released in water, they expand and curl backwards to form an irregular cup-like structure.

Holotype: PHILIPPINES, Mindanao, Bukidnon, Impalutao, Natigbasan Creek, on submerged wood, Jan. 1994, K.D. Hyde (HKU(M) 3127).

Other materials examined: PHILIPPINES, Mindanao, Bukidnon, Impalutao, Natigbasan Creek, on submerged wood, Jan. 1994, K.D. Hyde (HKU(M) 3103); *ibid.*, Negros Occidental, Bario Alegria, Lupit River, Lot 1320, on submerged wood, 27 Apr. 1997, K.D. Hyde and V.A. Arimas (HKU(M) 5175).

Mode of life: Saprobic on wood submerged in freshwater.

Known distribution: The Philippines.

Scanning electron microscopy

Mature ascospores were fusiform, slightly constricted at the septa and had bipolar appendages (Fig. 10). The appendages of free ascospores were constricted near the ascospore and wide at their ends (Fig. 11). These irregular appendages appeared to be sticky in nature as they were closely appressed to the polycarbonate membrane (Fig. 11). Ascospores were verruculose with evenly distributed wall ornamentations (Fig. 11).

Transmission electron microscopy

Mature ascospores were 3-septate and fusiform (Fig. 12). The central cells contained one large, and several smaller lipid globules, whereas the polar cells contained several smaller globules. The ascospore wall was verruculose, covered with numerous dome-shaped electron-dense structures and surrounded by remnants of the membrane complex (Figs. 12, 13). The polar appendages were electron-dense and amorphous (Fig. 14), and connected to the ascospore apex at the ascospore pole (Fig. 12). The bipolar appendages were initially appressed to the ascospore tip (Fig. 12). The upper surface of the appendage was covered with a tri-lamellate membrane, which was absent on the lower surface. This membrane comprised an outermost electron-dense layer; an intermediate thick electron-transparent layer; and an innermost electron-dense layer which was appressed to the appendage (Fig. 14).

Once the ascospore had been released into water, the part of the appendage closest to the ascospore expanded and separated from the ascospore wall (Fig. 12). However, the expansion of the upper part was restricted by the tri-lamellate membrane. Subsequently, the appendage curled backwards (towards the tri-lamellate membrane) to form an irregular structure, while the base remained connected to the ascospore apex (Figs. 13, 14). Some internal electron-transparent zones (cavities?) occurred at the basal part of the appendages (Fig. 13).

The wall of mature ascospores comprised an electron-dense episporium (*ca* 25 nm) and a thicker electron-transparent mesosporium (*ca* 55 nm) (Fig. 13). The episporium elaborated to form the dome-shaped structures (i.e. verruculose wall ornamentations) (Fig. 13). The episporium was discontinuous at the region where the polar appendage were connected to the ascospore poles (Fig. 13).

Two distinct layers could be recognised in the ascus wall (Figs. 15, 16): an outer electron-dense layer (*ca* 150 nm thick); and an inner thick electron-transparent layer (0.35-1.75 µm thick). The inner ascus wall appeared to fuse with (or differentiate into?) the apical ring (Fig. 15). The mature apical ring was cylindrical and appeared to be derived from the inner ascus layer, whereas the outer ascus wall was absent at the apex (Figs. 15, 16). The apical ring comprised two parts: (i). an electron-dense upper part; and (ii). a lower more electron-dense part with electron-dense granular deposits (Fig. 16). A less electron-dense, outwardly convex plug was located at the opening of the apical ring (Fig. 15).

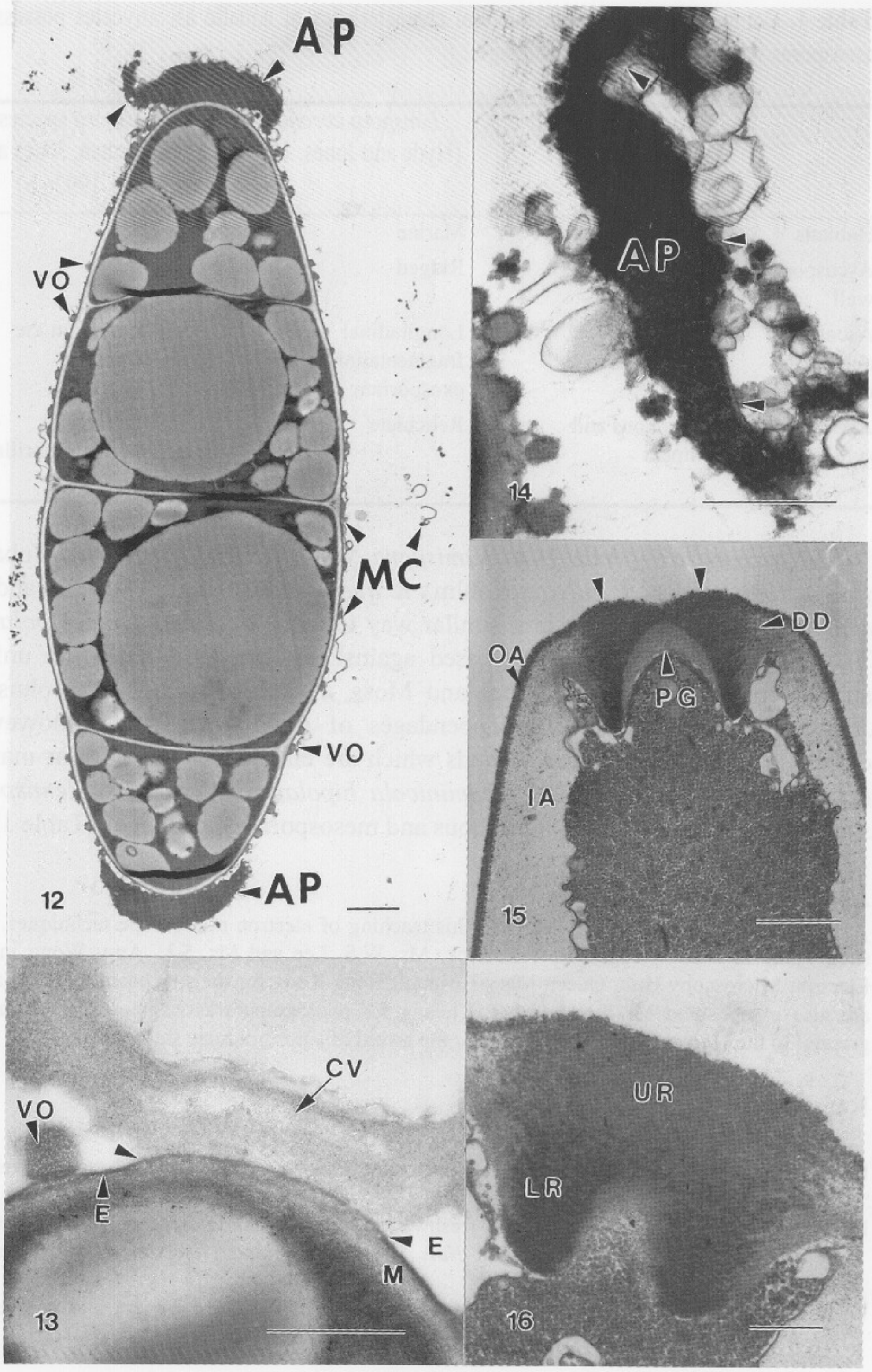
Discussion

Fluminicola bipolaris differs from species in other genera in the Annulatascaceae e.g. *Annulatascus* (Hyde, 1992, 1995), *Proboscispora* (Wong and Hyde, 1999) and *Submersisphaeria* (Hyde, 1996), in having ascospores with a unique appendage ontogeny. We believe that this character is sufficient to warrant the erection of a new genus. *Fluminicola* belongs in the Annulatascaceae (Wong *et al.*, 1998a).

The ascospore appendages in *Fluminicola bipolaris* are initially covered by a tri-lamellate membrane (membrane complex?), which remains flattened and appressed closely to the wall at the ascospore apex. Once in water, the appendage absorbs water and expands. The presence of the tripartite membrane allows the appendage to curl backwards and become bifurcate or cup-like. The cup-like form of the appendages may be helpful in the adhesion and entrapment of the ascospores to substrata (Rees and Jones, 1984; Jones, 1994).

The ascospores of *Fluminicola bipolaris* are similar to those found in the unrelated species, *Lanspora coronata* (Hyde and Jones, 1985), as they possess bipolar crown-like appendages. At the ultrastructure level, however, *L. coronata* differs from *Fluminicola bipolaris* in having long radiating appendage segments which have a reticulate substructure (Table 1). In *Fluminicola bipolaris*, the polar appendages have no radiating appendage segments and the texture is amorphous and compact. Appendages of *L. coronata* are formed by longitudinal fragmentation of the exosporium, whereas the appendages in *Fluminicola bipolaris*, are formed as an elaboration of the mesosporium. In addition, in *Lanspora bipolaris* ascospores possess ridged walls and lack verruculose ornamentations.

Figs. 12-16. *Fluminicola bipolaris*. Transmission electron micrographs. **12.** Longitudinal section of a mature ascospore illustrating the bipolar appendages (AP). The upper appendage is beginning to curl upwards (arrowed) while the lower appendages is still appressed to the ascospore wall. Note the remnants of membrane complex (MC) covering the verruculose ornamentations (VO) on the ascospore wall. **13.** Longitudinal sections of an unfurled polar appendage. Note the base which is attached to the mesosporium (M). The episporium (E) is discontinuous at the site of appendage connection (arrowed). Verruculose ornamentations (VO) are elaborations of the episporium (E). Note the electron-transparent zones or cavities (CV) within the appendage base. **14.** Higher magnifications of ascospore appendage illustrating the tri-lamellate membrane (arrowed), which is external of the polar appendages (AP). **15, 16.** Longitudinal sections illustrating an apical ring comprising an upper part (UR) with less electron density and a lower elongated part (LR) containing electron-dense granular deposits (DD). The inner ascus wall (IA) appears to fuse with the apical ring. The outer wall (OA) is absent at the apex of the apical ring (arrowed). A less electron-dense, outwardly convex plug (PG) is located at the opening of the apical ring. Bars: 12 = 1 μm; 13-16 = 0.5 μm.


AP
VO
MC
VO
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12
AP
14
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UR
LR
16

Table 1. Comparison of the ultrastructural characteristics of aquatic ascomycetes possessing ascospores with polar cup-like appendages.

	Fluminicola bipolaris	*Lanspora coronata* (Hyde and Jones, 1985)	*Remispora* species (Manimohan, Jones and Moss 1993a,b)
Habitats	Freshwater	Marine	Marine
Ascospore wall	Verruculose	Ridged	Smooth
Ascospore appendage ontogeny	Mesosporial elaborations	Longitudinal fragmentation of the exosporium	Radiations from the episporium
Appendage substructure	Amorphous and compact	Reticulate	Radiating strands embedded in a fibrillar matrix

The ascospore appendages of *Remispora crispa* Kohlm., *R. galerita* Tubaki, *R. maritima* Linder, *R. pilleata* Kohlm., *R. quadriremis* (Höhnk) Kohlm. and *R. stellata* Kohlm., also form in a similar way to those of *Fluminicola bipolaris.* The appendages are first compressed against the ascospore wall and unfurl subsequently (Hyde, 1985; Jones and Moss, 1980; Johnson, 1980, Johnson, Jones and Moss, 1984). The appendages of *Remispora* species, however, comprise radiating episporial strands which are embedded in a fibrillar matrix (Manimohan *et al*., 1993a,b). *Fluminicola bipolaris* differs from *Remispora* species in having compact, amorphous and mesosporial appendages (Table 1).

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