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## New species of *Aporomyces*

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**Abstract:** Three new species of *Aporomyces* (Laboulbeniomyces) parasitic on beetles from Ecuador are described. These are *A. aequatorialis*, parasitic on *Byrrhinus* near *B. plenus* (Limnichidae); *A. benjaminii*, parasitic on *Plagiogramma* sp. (Histeridae); and *A. maximus*, parasitic on *Byrrhinus* cf. *maculatus*. A key to all the species in the genus *Aporomyces* is given.

**Key words:** *Aporomyces*, Ascomycota, Ecuador, Histeridae, Laboulbeniomyces, Limnichidae

### 1. Introduction

The genus *Aporomyces* Thaxt. consists of 8 species: *A. byrrhini* R.K. Benjamin, *A. lurochi* R.K. Benjamin, *A. perpusillus* (Speg). I.I. Tav., *A. physemi* R.K. Benjamin, *A. subulatus* Thaxt., *A. szabo* Bánhegyi, *A. trinitatis* Thaxt., and *A. uniflagellatus* Thaxt. (Spegazzini, 1917; Thaxter, 1931; Bánhegyi, 1944; Tavares, 1981; Benjamin, 1989). Seven of these species are parasitic on beetles of the family Limnichidae, while the only *A. perpusillus* was found on Staphylinidae. Five species are dioecious, being characterized by large female thalli bearing perithecia, paired with tiny male thalli bearing antheridia. In 2 other species, *A. byrrhini* and *A. subulatus*, no differentiated antheridium was observed on the “diminutive” thalli, and, therefore, the dioecious nature of these last 2 species could not be confirmed. As to *A. perpusillus*, diminutive thalli associated with perithecial ones, which are present in all the other species in the genus, were not observed. Tavares (1985) suggested that this species could be monoecious.

In the present paper 3 new species of *Aporomyces* parasitic on beetles (Coleoptera) from Ecuador are described. One of these, *A. maximus*, bears an antheridium on the diminutive thallus. In a second species, *A. aequatorialis*, no differentiated antheridium is present. The third species, *A. benjaminii*, is closely allied with *A. perpusillus*, and like the latter lacks antheridia and the whole diminutive thallus; these 2 species are the only ones not associated with the Limnichidae.

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The 3 new species described here bring to 93 the number of Laboulbeniales reported from Ecuador (Rossi, 2009; Rossi, 2010; Rossi and Santamaría, 2010; Rossi, 2011; Rossi and Santamaría, 2012; Barragán et al., 2013; Goldmann et al., 2013; Rossi et al., 2013). This figure is relatively high: as to the American continent, higher numbers of Laboulbeniales have only been recorded in 3 much larger states (Argentina, Brazil, and United States).

### 2. Materials and methods

Insects were collected in the field by a mouth-operated aspirator and placed in 75% ethyl alcohol for transport to the laboratory, where they were carefully examined with a dissecting microscope. The parasitized specimens were transferred to a small petri dish on a bed made of various layers of soft and humid paper. The parasitic fungi were removed from the hosts by means of an entomological pin (# 3) and temporarily transferred to a small drop of Hoyer's medium (Arabic gum, 30 g; chloral hydrate, 200 g; glycerol, 16 mL; distilled water, 50 mL) on a clean slide. Permanent slides were then prepared, broadly following the method described by Benjamin (1971) with a few modifications.

The thalli were placed upside down in a very tiny drop of Hoyer's medium. A small drop of Amann's solution (phenol, 20 g; lactic acid, 16.5 mL; glycerol, 32 mL; distilled water, 20 mL) was centered on an 18 × 18 mm square cover slip, which was inverted and carefully lowered upon the

thalli and held in place by the viscous Hoyer's medium. The slide was placed in a warming chamber for 3–4 days, during which the Hoyer's medium completely diffused into the Amann's solution and the thalli regained their natural size. The cover slip was sealed with transparent nail polish; a second coat of nail polish was laid down after a few days.

Photomicrographs were made with a Jenoptik ProgRes 10 Plus digital camera on a Leica DMR microscope equipped with differential interference contrast optics (DIC).

Permanent slides are deposited in the in the Botanical Museum in Florence (FI) and in the Mycotheca of the Autonomous University of Barcelona (BCB).

### 3. Results

#### Taxonomic observations

**3.1. *Aporomyces aequatorialis* W. Rossi et Santam. sp. nov.** (Figures 1a, b)

MycoBank no. MB 809162

**Type:** Ecuador, Pichincha, La Union del Toachi, Otongachi Reserve, small swamp near the river Toachi, alt. 800 m, on the elytra of *Byrrhinus* sp. (near *B. plenus* Wooldridge) (Limnichidae), 29.VI.2007, W. Rossi, (holotype: FI 3849).

**Etymology:** from *Aequatoria*, the Latin name of Ecuador.

**Diagnosis:** see keys 1, 7, 9, 10.

**Description:** Female thallus with the basal cell chestnut brown; the 2 cells above it and the lower portion of the perithecium are pale brown, while the rest of the fungus is almost hyaline. Receptacle below the perithecium funnel-shaped, consisting of 3 cells, of which the basal is strongly constricted near the base and distinctly longer than maximum width; the other 2 are distinctly broader than long. Receptacle above the perithecium consisting of only cell III, which is elongate and almost straight. Appendage broken above its lower cell in all the examined specimens. Perithecium broadest at the base, its axis in line with that of the lower receptacle, with a distinctly inflated venter, a long, slender, and tapering tip, and an elongate unicellular upgrowth extended for up to 14  $\mu\text{m}$  beyond the ostiole. Secondary stalk-cell persistent, usually trapezoidal in outline. Length from foot to perithecial apex 173–213  $\mu\text{m}$ . Perithecium 115–147  $\times$  29–34  $\mu\text{m}$ .

Male (?) thallus 30–43  $\mu\text{m}$  long, distinctly curved at the base, consisting of a linear series of 6 cells, gradually longer, paler and slenderer from below upwards. Differentiated antheridia not observed: there is no evidence that the distal cell is a phialide.

**Additional specimens examined (paratypes):** same data as the type, FI 3814, BCB SS-E568. A total of 18

mature and as many immature or incomplete thalli were examined.

**Remarks:** *Aporomyces aequatorialis* is allied with *A. subulatus* Thaxt. and *A. byrrhini* R.K. Benjamin. These 3 species are similar in the general habitus and share the same host genus, *Byrrhinus*. However, the new species differs from *A. subulatus* in having the long axis of the perithecium in line with that of the lower receptacle, and from *A. byrrhini* by the much more inflated perithecial venter; it is further distinguished from the other 2 species by the distinctly tapered lower receptacle composed of strongly unequal cells, and the larger dimensions.

The occurrence of a “diminutive” thallus lacking antheridia paired with thalli bearing perithecia is unusual among the Laboulbeniales, but it has already been observed, for example, in a few species of *Laboulbenia* parasitic on endogean ground beetles (Rossi and Santamaria, 2008). The function of these apparently useless thalli remains unclear.

**3.2. *Aporomyces benjaminii* W. Rossi et Santam. sp. nov.** (Figure 1c).

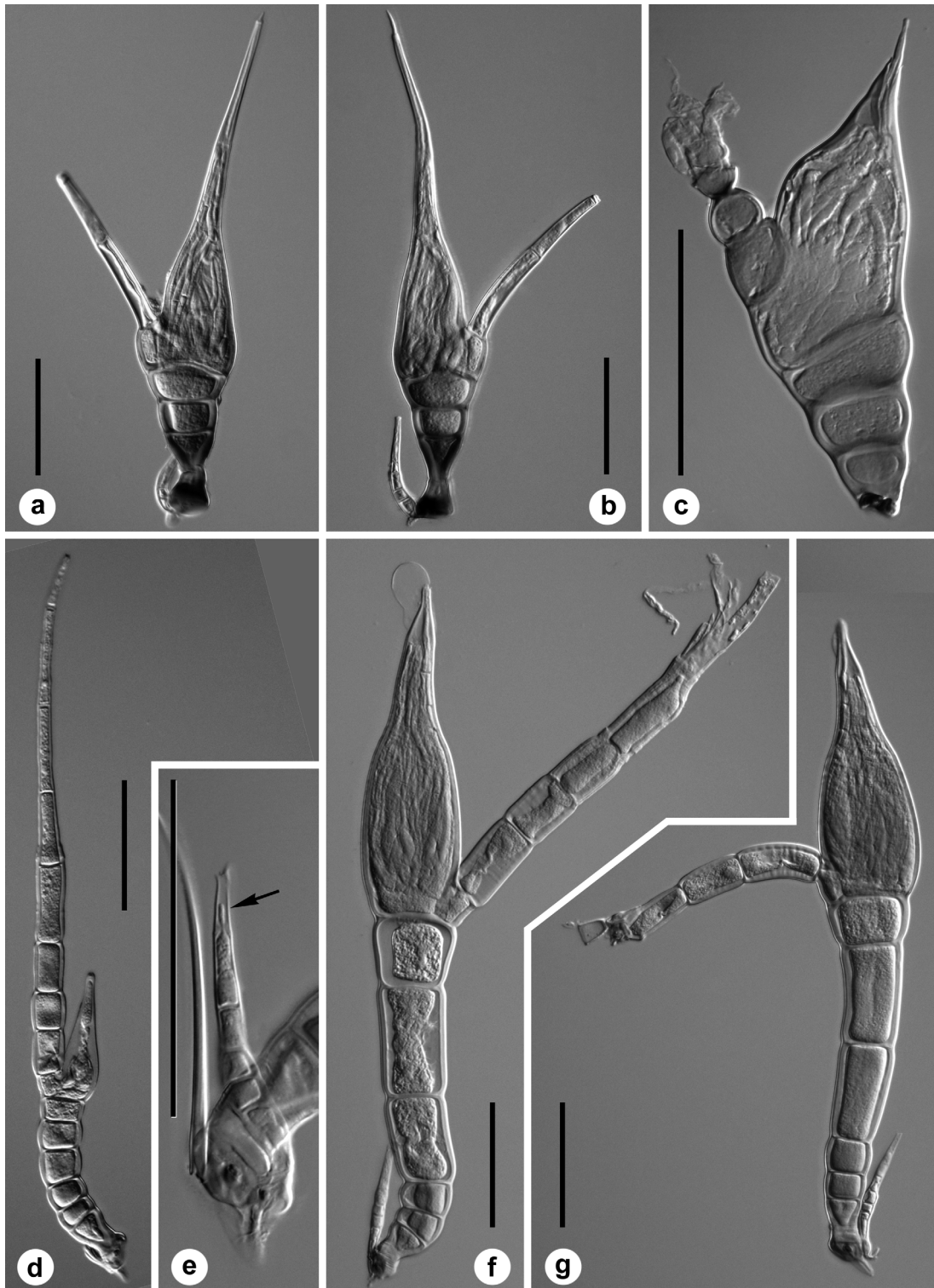
MycoBank no. MB 809163

**Type:** Ecuador, Orellana, P. N. Yasuní, Estacion Científica Yasuní, on the clubs of the antennae of *Plagiogramma* sp. (Histeridae) collected under the bark of a fallen tree, 15.IX.2013, W. Rossi (holotype: FI 3844b).

**Etymology:** named after the late Richard K. Benjamin (1922–2002), distinguished student of the Laboulbeniales, who first found this new species or a very similar one (Benjamin, 1989, p. 358).

**Diagnosis:** see keys 1, 7, 8.

**Description:** Thallus pale amber yellow, with the perithecial venter almost hyaline. Receptacle below the perithecium subconical, not abruptly tapering downward, consisting of 3 superimposed cells of nearly the same length but gradually broadening from below upwards and externally rounded on the ventral side. Receptacle above the perithecium consisting of 2 cells, the lower broadly cordiform derived from cell II, superimposed by cell III; the latter almost rounded and distinctly shorter than maximum width. Primary appendage broken near the base in all the examined thalli. Perithecium diverging for about 45° from the axis of the receptacle, stout, asymmetrical, with the dorsal margin more convex than the ventral, broadest at the base, strongly tapered distally, with an elongate, slender upgrowth projecting beyond the ostiole for up to 9  $\mu\text{m}$ . Secondary stalk-cell (cell VII) not persistent. Total length from the foot to the perithecial tip 82–101  $\mu\text{m}$ . Perithecium 51–61  $\times$  29–35  $\mu\text{m}$ .



**Figure 1.** *Aporomyces aequatorialis*: a- WR3814, mature thallus; b- WR3849, mature thallus. *Aporomyces benjaminii*: c- WR3829, mature thallus. *Aporomyces maximus*: d- WR2983, immature thallus with undamaged appendage; e- WR2982, male thallus (arrow indicates the antheridium with a spermatium in the neck); f- WR2982, mature thallus; g- WR2983, mature thallus. Scale bars = 50  $\mu$ m.

**Additional specimens examined (paratypes):** same data as the type, FI 3829 & 3850a. A total of 14 mature thalli were examined.

**Remarks:** *Aporomyces benjaminii* is closely allied to *A. perpusillus* (Speg.) I.I. Tav., which was described (as *Ecteinomyces perpusillus* Speg.) from Argentina (Spegazzini, 1917) on the antennae of *Rhopalophorus gestroi* Bernhauer (Staphylinidae). The latter fungus is much smaller (up to 40 µm only, according to Benjamin, 1989), has a cell III distinctly longer than broad, and the lower receptacle is smaller and more tapered below. Both these parasites lack antheridia and the whole diminutive thallus. As stated before, Tavares suggested that *A. perpusillus* is a monoecious species, but did not exclude the possibility that "...antheridial production has been dispensed with in this highly evolved taxon" (Tavares, 1985, p. 139). We think that the second hypothesis is more likely. On the other hand, in a few other Laboulbeniales antheridia are entirely lacking, as in some of the species of *Rickia* parasitic on mites (Thaxter, 1926).

The occurrence of the new species restricted to the tip of the antennae witnesses for frequent and intense contact between its host-insects through these organs. It is well known that antennation behavior is relatively frequent in insects, often associated with courtship leading to copulation. Because the transmission of the Laboulbeniales is carried out mainly through the direct contact between their hosts (De Kesel, 1995), the presence of Laboulbeniales on certain parts of the body of the insects gives information about the behavior of the same. Among the very many possible examples are the clusters of thalli observed on the mouthparts of the fly *Acrosticta apicalis* Williston, demonstrating the occurrence of trophallaxis (exchange of food) during courtship (Rossi and Ponziani, 2008) and information about mating behavior of the fly *Anastrepha striata* Schiner obtained through the Laboulbeniales *Stigmatomyces aciuræ* Thaxt. and *S. verruculosus* Thaxt (Hedström and Monge-Nájera, 1998).

**3.3. *Aporomyces maximus* W. Rossi et Santam. sp. nov.** (Figures 1d–g)

Mycobank no. MB 809164

**Type:** Ecuador, Cotopaxi, Canton Sigchos, San Francisco de Las Pampas, Bosque Integral Otonga, alt. 1700 m, on pro-, meso-, and metasternum of *Byrrhinus* cf. *maculatus* Wooldridge, collected at the edge of a small stream flowing into Rio Esmeraldas, a few meters outside the border of the protected area, 7–10 July 2006, W. Rossi (holotype: FI 2983).

**Etymology:** *maximus* = largest, because the size of the new species is larger than any other in the same genus described so far.

**Diagnosis:** see keys 1, 2.

**Description:** Female thallus pale amber colored, with darker perithecial venter and lower cells of the receptacle. Receptacle below the perithecium usually consisting of 6 superimposed cells, which are strongly unequal in mature specimens: the basal is small and distinctly longer than maximum width, the following 2 are also small but slightly wider than long, the 4th and 5th are large and up to 3 times longer than broad, the 6th large and subquadrate, or slightly wider than long. Receptacle above the perithecium consisting of 3 elongate cells, the series terminated by a slender cell III. Appendage simple, consisting of up to 7 cells gradually slenderer from below upwards, straight and erect in young thalli but displaced laterally in mature specimens by the growth of the perithecium. Secondary stalk-cell (cell VII) relatively small, slightly longer than broad, persistent, subtending the upper receptacle beside the base of the perithecium. Perithecium with the venter symmetrically elliptical in outline, strongly tapering in a rostrate distal portion, which ends with a very acute apex. Length from foot to perithecial apex 255–275 µm; longest appendage 330 µm; perithecium 110–130 × 40 µm.

Male thallus consisting of a linear series of 4–5 strongly unequal cells followed by an elongate antheridium. Total length 51–56 µm.

**Additional specimens examined (paratypes):** same data as the type, FI 2982, BCB SS-E570a. A total of 12 female thalli, 4 of which mature, and 6 male thalli were examined.

**Remarks:** The new species differs from all the others in the same genus by the large size and the shape of the perithecium. It bears a very superficial resemblance in the general habitus with *Aporomyces physemi* R.K. Benjamin, parasitic on *Pysemus* spp. (Limnichidae) from the US and Central America; however, the latter parasite is much smaller, with a darker and stockier perithecium and the receptacle consisting of almost flattened cells not strongly unequal in length (Benjamin, 1989).

In a single immature specimen the receptacle below the perithecium is composed of 7 cells, and the appendage is repeatedly branched above cell III.

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A KEY TO THE SPECIES OF *APOROMYCES*

1	Receptacle below the perithecium consisting of an uniseriate row of more than 3 cells .....	2
1	Receptacle below the perithecium consisting of 3 superimposed cells .....	7
2	Cells of the receptacle above the basal cell strongly unequal in length .....	<i>maximus</i>
2	Cells of the receptacle above the basal cell subequal in length .....	3
3	Receptacle above the perithecium consisting of an elongate axis of 9–20 cells .....	<i>lutrochi</i>
3	Receptacle above the perithecium consisting of an axis of 2–5 cells .....	4
4	Perithecial apex distinctly tapered .....	<i>physemi</i>
4	Perithecial apex blunt .....	5
5	Secondary appendages not formed; primary appendage often elongate, soon deciduous .....	<i>uniflagellatus</i>
5	Secondary appendages formed .....	6
6	Upper receptacle consisting of 3–4 cells below cell III .....	<i>szaboi</i>
6	Upper receptacle consisting of only 1 cell below cell III .....	<i>trinitatis</i>
7	Thallus small, less than 105 µm in length .....	8
7	Thallus larger, exceeding 105 µm in length .....	9
8	Cell III almost twice as long as broad (on Staphylinidae) .....	<i>perpusillus</i>
8	Cell III distinctly shorter than maximum width (on Histeridae) .....	<i>benjaminii</i>
9	Long axis of the perithecium forming an angle of about 45° with that of the lower receptacle; cell III curved .....	<i>subulatus</i>
9	Long axis of the perithecium in line with that of the lower receptacle; cell III straight or almost so .....	10
10	Perithecium very slender .....	<i>byrrhini</i>
10	Perithecium with a distinctly inflated venter .....	<i>aequatorialis</i>

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