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A NEW GENUS OF LABOULBENIALES (ASCOMYCETES) ON A SPECIES OF *PHALACRICHUS* (COLEOPTERA: DRYOPOIDEA; LIMNICHIDAE), WITH A NOTE ON MIRROR-IMAGE ASYMMETRY IN THE ORDER

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ABSTRACT

A new genus of Laboulbeniales, with two species, *Phalacrichomyces normalis* (type) and *P. anomalus*, is described from *Phalacrichus diligens* (Coleoptera: Dryopoidea; Limnichidae). *Phalacrichomyces* is placed in the Stigmatomycetinae of the Laboulbeniaceae where it appears to be most nearly related to *Stemmatomyces* and *Synandromyces* among the other 39 genera of the subtribe. The new taxa are characterized and salient features of the structure and development of their ascomata are summarized and illustrated with photographs and line drawings. The ascomata of associated pairs of *P. anomalus* display a remarkable degree of mirror-image asymmetry. This phenomenon, which appears to be a characteristic, although inconspicuous, feature of the ascomata of other Laboulbeniales, is discussed briefly.

Key words: Ascomycetes, Coleoptera, fungi, insect parasites, Laboulbeniales, Limnichidae, morphology, *Phalacrichomyces, Phalacrichus,* taxonomy.

INTRODUCTION

Limnichid beetles are mostly very small (1–3 mm in length), oval, convex, brownish to blackish, sometimes metallic insects, which may be invested with dense, fine, golden or grayish hairs. They are essentially riparian, often occurring in and around litter, among rocks, or on mudflats near the waterline.

Few Laboulbeniales have been described thus far on Limnichidae. These include *Cantharomyces bordei* Picard (1912), on *Limnichus sericeus* (Duft.) from Algeria, and seven of eight known species of *Aporomyces* Thaxter (1931). *Aporomyces* and its included species were the subjects of a recent detailed study by myself (Benjamin 1989), and this work may be consulted for specifics regarding these parasites and their hosts.

Among several unnamed Laboulbeniales in my collection that parasitize Limnichidae are two related forms that cannot be accommodated in any genus of the order. They were found on a species of *Phalacrichus* Sharp (1902) and belong to the subtribe Stigmatomycetinae as defined by Tavares (1985). My purpose here is to characterize and illustrate these fungi and place them in a new genus.

MATERIALS AND METHODS

The host of the fungi being described was originally received in 1972 from Dr. David P. Wooldridge, Department of Biology, The Pennsylvania State University, Ogontz Campus, Abington. The beetles had been collected in Venezuela in 1969, and had been forwarded to Wooldridge by Dr. Paul J. Spangler, U.S. National Museum of Natural History, Washington, D.C. The lot included several hundred parasitized individuals preserved in 70% ethyl alcohol. The insects, which at the time had not been identified, were later described as *Phalacrichus diligens* Wooldridge (1982:389).

The fungi were carefully removed from the host and mounted on slides in glycerine containing a trace of cotton blue or acid fuchsin by techniques given previously (Benjamin 1971:101, 1986:247).

Direct observations, measurements, and photomicrographs were made using a Leitz Dialux microscope equipped with differential interference contrast optics. Drawings were made with the aid of a camera lucida. Photographs were taken on 4 in. × 5 in. Kodak Technical Pan Film #2415. The camera used was a WILD 15/11 Semiphotomat with an ASA setting of 125. The film was developed for 8 minutes in Kodak HC-110, Dilution F, at 20 C. The scanning electron micrographs were prepared at the University of Florida by Dr. Gerald L. Benny.

Terms and abbreviations are defined in the text but, with a few exceptions, they are those of Tavares (1985:431–434).

TAXONOMY

Phalacrichomyces Benjamin, gen. nov.

Receptaculum cellularum trium superpositum constans appendicem liberum et perithecium gignens. Cellulae basilaris (I) et subbasilaris (II) receptaculi parallelae; cellula I postica cellulam III receptaculi subtenens; cellula III receptaculi appendicem subtenens. Appendix cellularum trium superpositarum constans; unaquaeque cellula appendicis antheridium singulum gignens; antheridium terminalem spinosem. Trichogyna bicellularis; cellula distalis trichogynae prominentiis terminalibus lobatis brevibus vel elongatis formans. Perithecium cellulis basilaribus tribus persistentibus et cellulis parietis externis in quatuor ordinibus longitudinalibus numusquique quinque cellularium; cellula ascogena unica; ascosporae 1-septatae.

Receptacle consisting of three superposed cells bearing posteriorly a free appendage and anteriorly a perithecium. The basal cell (I) and the subbasal cell (II) of the receptacle parallel to one another; cell I posterior to cell II and subtending the terminal cell (III) of the receptacle; cell II, which extends nearly to the foot formed at the base of cell I, giving rise to a stalked perithecium. Appendage, subtended by cell III of the receptacle, consisting of three superposed cells; each cell, including the basal, giving rise on the outside to a simple antheridium; the terminal antheridium spinose. Trichogyne two celled; the upper cell forming terminal, lobate, short or elongate outgrowths. Perithecium with three persistent basal cells and four vertical rows of outer wall cells of five cells each; ascogenic cell single; ascospores 1-septate.

Type species.—*Phalacrichomyces normalis* Benjamin.

Etymology.—From the host genus + *myces,* fungus.

A KEY TO THE SPECIES OF *PHALACRICHOMYCES*

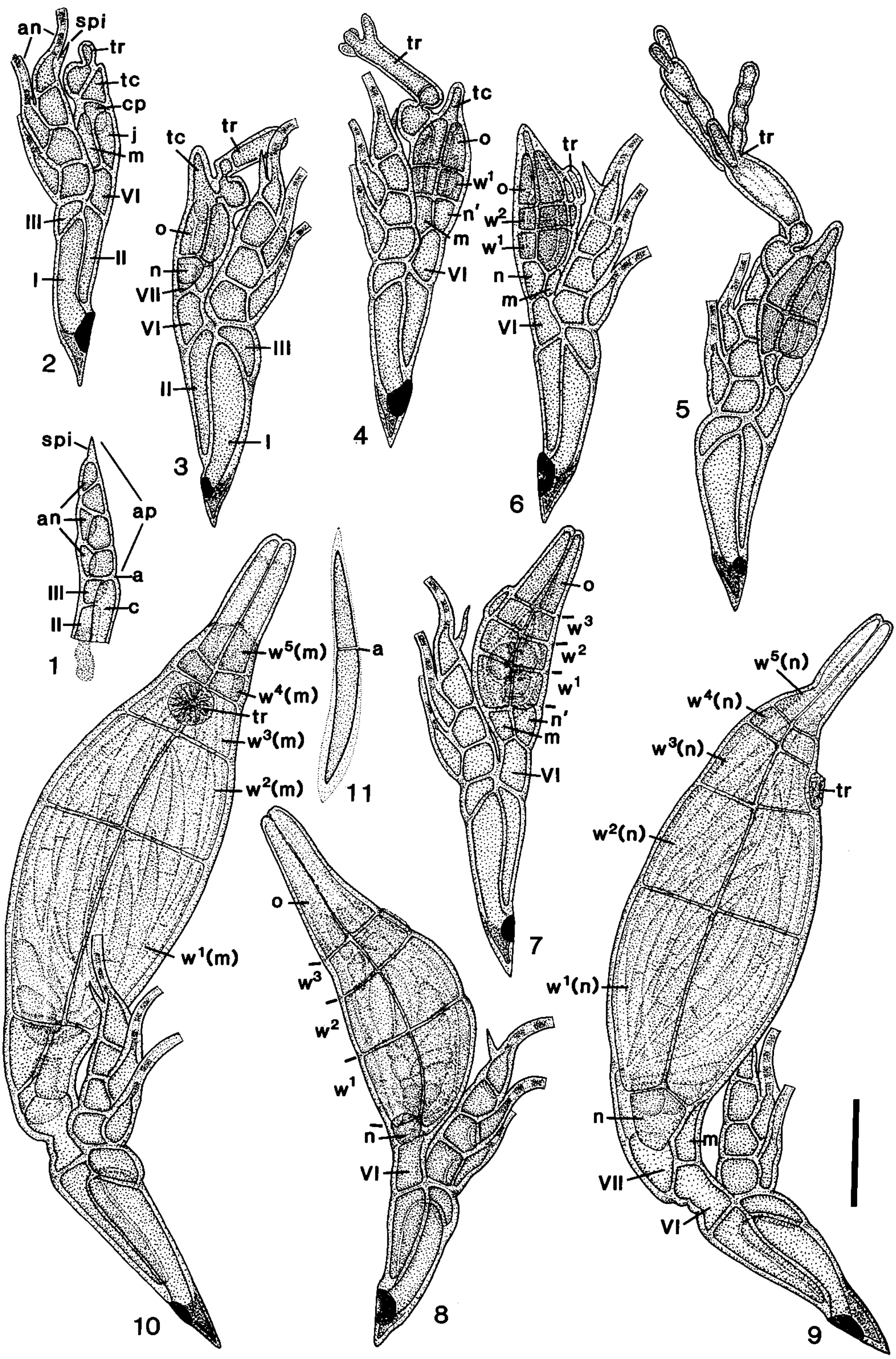
A. Perithecial stalk cell arising distally from cell II of the receptacle; basal cells and outer wall cells unmodified; without perithecial appendages . . . . *P. normalis*

– Perithecial stalk cell arising from the base of cell II of the receptacle; some of the lower basal cells and outer wall cells highly modified, with greatly thickened walls; perithecial appendages present . . . . *P. anomalus*

Phalacrichomyces normalis Benjamin, sp. nov. Fig. 1–9, 12–16

Ascoma luteola, plus minusve ascendens, fere recta vel flexa, tota 145–200 μm longa ad apicem ostioli. *Receptaculum* elongatum, triangulare, 40–50 μm longum, 15–20 μm latum in parte distalibus; cellula I 35–45 × 8–11 μm; cellula II 23–29 × 6–10 μm, cellulam VI perithecii subtenens; cellula III 9–14 × 7–11 μm. *Appendix* recta, 43–50 × 15–20 μm; cellulae basales et medianae subaequales, ca. 7–10 μm altae et latae, antheridia singulare ascendentes gignentes; cellula distalis 8–11 μm longa, 7–10 μm lata, antheridium singulum spinosum gignens; spina 5–7 μm longa; antheridia plus minusve sigmoideum, 18–26 μm longum, collos 10–16 μm. *Perithecium* inflatum, externe convexum, 90–130 μm latum ad medium attenuatum, tenuirostre; rostrum 20–22 × 8–10 μm; ostiolum simplex. *Ascosporae* hyaline, 50–60 × 3.5–4 μm. Typus *RKB 2818B*; RSA.

Ascoma: Uniformly pale yellow except for the ±blackened foot; ±ascending from the surface of the host, nearly straight or bent in the region of the basal and stalk cells, the perithecium then ±inclined backward over the appendage. Total height from tip of foot to tip of ostiole 145–200 μm. *Receptacle:* Triangular, 40–50 μm long, 15–20 μm wide distally, tapered downward to the acute tip, which extends beyond the opaque point of attachment to the host; the basal (I) and subbasal (II) cells elongate, parallel; cell I, 35–45 × 8–11 μm, posterior to cell II, slightly convex externally, subtending cell III, which is ±rounded externally, slightly longer than wide, 9–14 × 7–11 μm, and extends downward obliquely on the outside along the upper end of cell I; cell II, 23–29 × 6–10 μm, the upper end subtending the primary stalk cell of the perithecium (VI) and in contact on the inside with cell III, otherwise overlapped internally throughout its length by cell I. *Appendage:* Straight, usually inclined ±outward relative to the long axis of the receptacle, 43–50 μm long to the tip of the upper antheridium, 15–20 μm in greatest width; the axis consisting of three superposed cells; the basal and median cells subequal, ±pentagonal in lateral view, ca. 7–10 μm high and wide, bearing on the outside single upwardly directed antheridia with adnate venters and free efferent tubes; the upper cell slightly longer than wide, 8–11 × 7–10 μm, bearing distally a free antheridium, the venter of which bears on its inner margin a spine 5–7 μm long; antheridia usually sigmoid, 18–26 μm long, venters 5–7 μm wide, tubes slightly curved, 10–16 × 3 μm. *Perithecium:* Primary stalk cell (VI) relatively short, slightly longer than broad, 11–16 × 8–12 μm, with a well-developed median constriction, which is more pronounced on the outer margin than the inner when viewed laterally; secondary stalk cell (VII) 11–16 × 8–12 μm, lying above cell VI, from which it is separated below on the inside by a diagonal septum, its outer margin often strongly convex, its upper end surrounded by the perithecial basal cells (*m, n, n'*); basal cells relatively short, constituting only ca. 14% of the total height of the perithecium above the stalk cells, only slightly enveloping the base of the ascigerous cavity; body relatively large, 90–130 μm long, inflated, 30–44 μm wide at the middle; the inner margin somewhat abruptly concave at the level of the prominent trichogyne remnant, which is ca. 8–11 μm in diameter at the base and straddles two tier-three wall cells of the vertical rows derived from basal cells *m* and *n,* the outer margin strongly concave some distance below the tip, the distal two thirds of the terminal outer wall cells (w^5) forming a slender rostrum 20–22 × 8–10 μm; the basal tier of outer wall cells (w^1) ca. twice the height of the subbasal tier (w^2), these two tiers constituting ca. 60% of the total height of the body above the basal cells; the median tier of outer wall cells (w^3) about three fourths the height of the subbasal tier; the subterminal tier (w^4) very short, only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one fifth the height of the terminal tier (w^5), which equals ca. 26% of the total height of the body; the tip proper unmodified. Ascospores hyaline, 50–60 × 3.5–4 μm.

Etymology.—From *normalis* (L.) in reference to the unremarkable morphology of the ascoma.

Type.—VENEZUELA: Guárico; 12 km S of Calabozo, Est. Biologica los Llanos, 6–12 Feb 1969, P. & P. Spangler coll. (blacklight); on the lower surface of the thorax and abdomen, legs, and, rarely, elytra of *Phalacrichomyces diligens* (holotype: *RKB 2818B*; RSA [designated slide]; isotypes: RSA).

Other specimens examined.—VENEZUELA: Guárico; Calabozo, 7 Feb 1969, P. & P. Spangler coll. (blacklight); on left middle and anterior legs and outer margin of left elytron of *P. diligens* (*RKB 2822, 2823A*; RSA).—Aragua; Ocumare de la Costa, 19–20 Feb 1969, P. & P. Spangler coll. (blacklight); on middle inner margin of right elytron of *P. diligens* (*RKB 2824*; RSA).

Of some 70 mature or nearly mature and 30 immature specimens of *P. normalis* recovered for study, most were growing apparently at random on the ventral surface or legs of the host; however, a few were scattered on the elytra. This contrasts with *P. anomalus,* which was found only in various positions on the elytra or pronotum of the insect.

Phalacrichomyces anomalus Benjamin, sp. nov. Fig. 17–40

Ascoma luteola, applanata, adpressa, apice perithecii elongato ad angulum 45° ascendenti, tota 140–180 μm longa e basi rotundata ad apicem rostri. *Receptaculum* elongatum, externe rotundatum, 30–40 × 10–18 μm; cellula I 27–34 μm longa; cellula II 15–20 μm longa, externe convexa, cellulam VI perithecii e basi productens; cellula III 5–7 × 10–13 μm, appendicem subtenens. *Appendix* recta, 32–38 μm longa ad apicem spinae, 10–12 μm lata; cellulae basilares (6–8 × 10–12 μm) et medianae (7–9 × 8–11 μm) antheridia singulare perpendiculares vel parum ascendentibus gignentes; cellula distales 9–11 × 7–10 μm antheridium singulum spinosum gignens; spina terminalis, 5–6 μm longa; antheridia 25–30 μm longae collis 18–20 × 3–3.5 μm plus minusve curvatis. *Perithecium* applanatum, supra convexum, infra complanatum, 40–70 μm latum ad medium, attenuatum tenuirostre, appendicibus duo plus minusve curvatis, liberis, gracilibus, attenuatis, 30–50 μm longis infra rostrum; rostrum 45–55 μm longum, basi 10–12 μm, 4–6 μm latum ad apicem; ostiolum simplex; basis perithecii late rotundata, curvata, extensa transpedem, 70–85 μm lata. *Ascosporae* hyaline 50–65 × 3.5–4 μm. Typus *RKB 2818A*; RSA.

Ascoma: Uniformly pale yellow except for the blackened foot; dorsiventrally flattened, the receptacle, appendage, and perithecium closely appressed to the surface of the host except for the elongate perithecial tip, which projects upward at an angle of ca. 45°. Total length from bottom of rounded base to tip of ostiole 140–180 μm. *Receptacle:* More or less broadly rounded externally above the acute blackened foot, which is opaque around the point of attachment to the host, 30–40 × 10–18 μm; the basal (I) and subbasal (II) cells elongate, parallel; cell I, 27–34 μm long, posterior to cell II, ±convex externally, subtending cell III, which is

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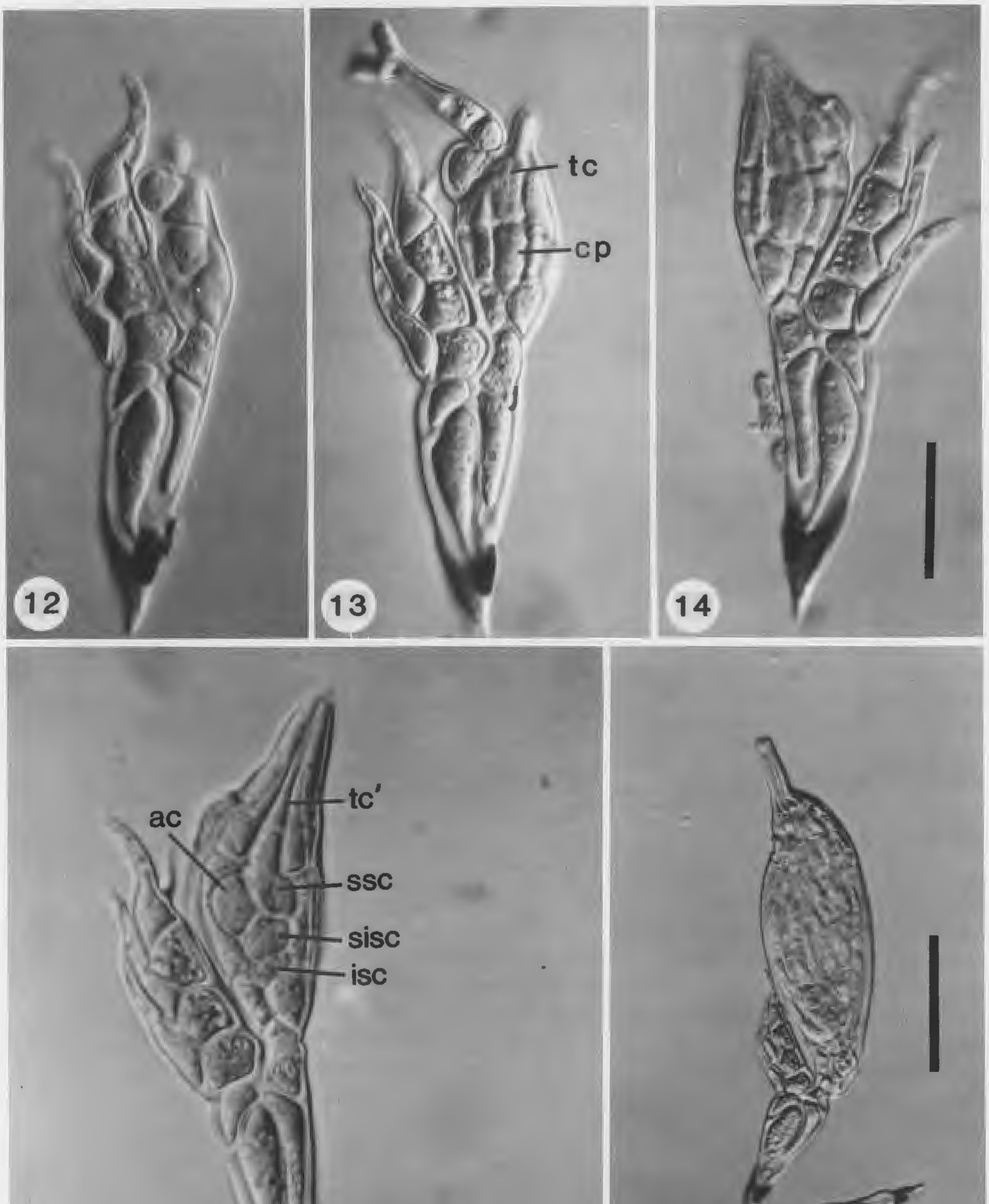
Fig. 1–11. *Phalacrichomyces normalis (RKB 2818B).*—Fig. 1–8. Stages of development of the receptacle, appendage, and perithecium.—Fig. 9–10. Two mature individuals; the perithecium of the ascoma shown in Figure 10 is rotated ca. 90° relative to the position of the one shown in Figure 9. Note the position of the trichogynic remnant (*tr*) in relation to two of the tier-three wall cells derived from basal cells *m* and *n.*—Fig. 11. Ascospore. Details and terminology are given in the text. (All figures, bar at lower right = 20 μm.)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relatively small, about twice as wide as high, 5–7 × 10–13 μm, and separated from cell I by a transverse often slightly diagonal septum; cell II, 15–20 μm long, the upper end, together with cell III subtending the basal cell of the appendage, otherwise overlapped throughout its length by cell I. *Appendage:* Straight, 31–38 μm long to the tip of the spine of the terminal antheridium, 10–12 μm wide; the axis consisting of three superposed cells; the basal and median cells subequal, rectangular or ±pentagonal in lateral view, 6–8 × 10–12 μm and 7–9 × 8–11 μm respectively, bearing on the outside single antheridia, 25–30 μm long, including the only partially adnate venters and wholly free ±curved efferent tubes 18–20 × 3–3.5 μm, which may be nearly perpendicular to the long axis of the appendage or only slightly ascending; the upper cell slightly longer than wide, 9–11 × 7–10 μm, subtending a free antheridium with the distally rounded venter and terminal spine in line with the long axis of the appendage and bearing on the outside a nearly straight or curved discharge tube like that of the other antheridia; spine 5–6 μm long. *Perithecium:* Primary stalk cell (VI) relatively short, slightly longer than broad, 9–11 × 7–9 μm, positioned at the base of cell II and nearly perpendicular to the long axes of the receptacle and body of the perithecium; secondary stalk cell (VII) smaller than and in line with cell VI, ±isodiametric, inconspicuous; basal cells *m* and *n'* about equal in size to cell VII, lying immediately above this cell on opposite sides of the dorsiventrally flattened perithecium, which is broadly convex above and flattened below where in direct contact with the host; basal cell *n* and one of the two tier-one wall cells (w^1) derived from cell *n* becoming greatly enlarged, growing downward and laterally around the base of the receptacle; the body of the mature perithecium broadly rounded at the base, 70–85 μm in overall width, curving upward around and beyond the foot; this modified basal cell spanning slightly more than one half the total length of the body, its outer wall varying from 10 to 18 μm in thickness, hyaline, faintly longitudinally striate; cell *n* attenuate toward each extremity, broadest below cells *m* and VII, 40–58 × 10–16 μm, united internally throughout its length with the recurved base of the modified basal wall cell; the lowermost tier-one wall cell (w^1) derived from basal cell *n'* 40–68 × 12–18 μm, lying adjacent to the appendage, with a free acuminate tip 4–13 μm long, externally rounded, its outer wall 10–12 μm thick; body above the modified basal cells abruptly rounded in the region of the relatively short tier-two (w^2) and tier-three (w^3) outer wall cells, narrowest immediately above the persistent trichogyne remnant, which is 9–10 μm in diameter, at the base of the

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Fig. 12–16. *Phalacrichomyces normalis (RKB 2818B).*—Fig. 12–14. Photographs of the immature ascomata depicted in Figures 2, 4, and 6, respectively. The young perithecium of the specimen in Figure 13 is shown in optical section; note the relationship of the carpogenic cell (*cp*) and trichophoric cell (*tc*).—Fig. 15. Immature ascoma with perithecium photographed in optical section to show the relationship of the five cells constituting a young centrum—derived from fused trichophoric and carpogenic cells (see example in *P. anomalus,* Fig. 25, *cp-tc*)—prior to the formation of ascal initials from the ascogenous cell (*ac*). Note position of the inferior supporting cell (*isc*), secondary inferior supporting cell (*sisc*), superior supporting cell (*ssc*), and re-formed trichophoric cell (*tc'*). The lower part of the *ssc* is in contact below with the *sisc* and laterally on the left with the *ac*; it extends upward between the developing inner wall cells (*p*) and contacts the base of the *tc'*, which is being compressed between the developing inner and outer wall cells (*p* and *o*).—Fig. 16. Mature individual (above); receptacle and appendage with perithecium missing (below). (Fig. 12–15, bar with Fig. 14 = 20 μm; Fig. 16, bar = 50 μm.)

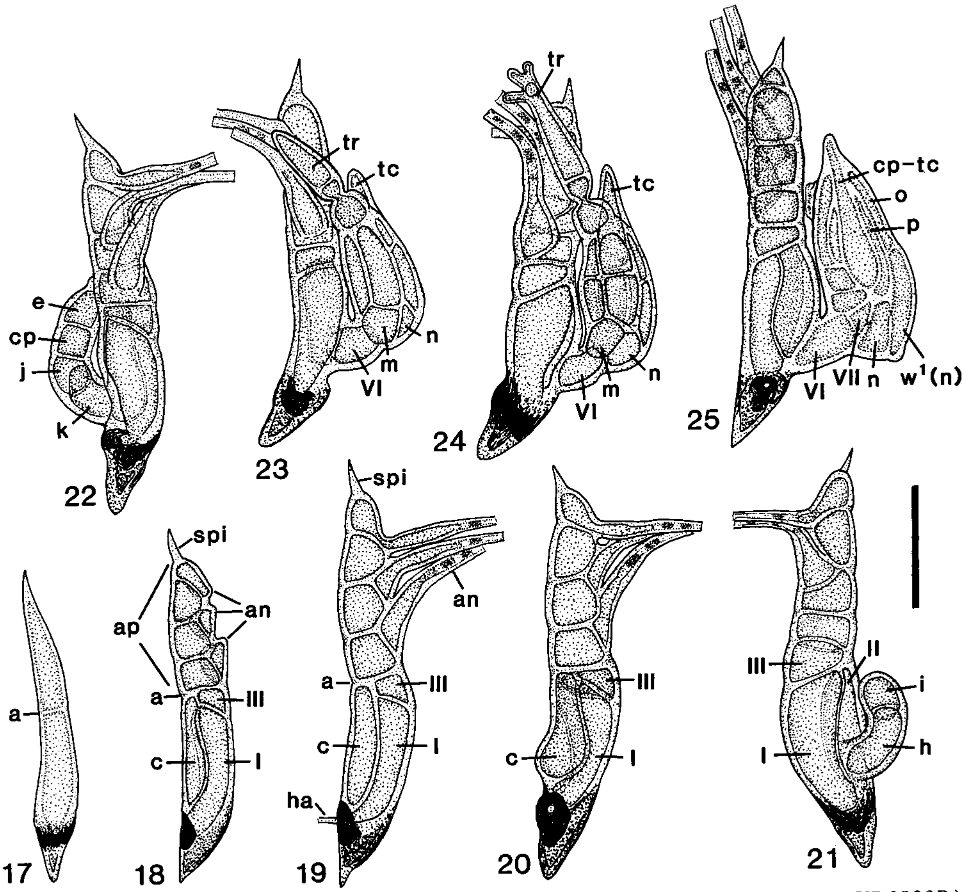

Fig. 17–25. *Phalacrichomyces anomalus.* (Fig. 17–21, 24, 25: *RKB 2818A*; Fig. 22, 23: *RKB 2823B.*) Stages of development of the receptacle, appendage, and perithecium. Details and terminology are given in the text. (All figures, bar = 20 μm.)

elongate perithecial rostrum; the outermost tier-two (w^2) outer wall cells with proximally thickened outer walls, ending in elongate, attenuate, ±curved free appendages, 30–50 μm long, 7–11 μm wide near the base, 1.5–2 μm wide at the tip; the convex upper surface of the body enclosed by the relatively unmodified lower three tiers (w^1, w^2, w^3) of outer wall cells derived from basal cells *m* and *n*; the perithecial rostrum, comprised of tier-four (w^4) and tier-five (w^5) outer wall cells, 45–55 μm long, 10–12 μm wide at the base, 4–6 μm wide at the tip, equaling ca. one third the total length of the body above the base of the ascigerous cavity; the w^4 wall cells 14–20 μm long, the w^5 wall cells 29–37 μm long; the tip proper undistinguished. Ascospores hyaline, 50–65 × 3.5–4 μm.

Etymology.—From *anomalus* (L.) in reference to the irregular developmental pattern of the ascoma.

Type.—VENEZUELA: Guárico; 12 km S of Calabozo, Est. Bioilogia los Llanos, 6–12 Feb 1969, P. & P. Spangler coll. (blacklight); on the pronotum and elytra of *Phalacrichus diligens* (holotype: *RKB 2818A*; RSA [designated slide]; isotypes: RSA).

Other specimens examined.—VENEZUELA: Guárico; Calabozo, 7 Feb 1969, P. & P. Spangler coll. (blacklight); on the left elytron of *P. diligens* (*RKB 2823B*; RSA).

Approximately 200 mature or nearly mature and 40 immature individuals of *P. anomalus* were prepared for study from among the several hundred infected insects available. The perithecial primary stalk cell of this species is fragile and easily broken in the process of removing specimens from the host; as a result, many of the receptacles and perithecia are separated from one another in the slide mounts.

Despite the bizarre modifications of the perithecium of *P. anomalus,* the close relationship of this species and *P. normalis,* is evidenced by marked similarities in the structure of their ascomata, i.e., receptacle, appendage, and perithecial rostrum, which consists of an elongate terminal tier of cells and a relatively short subterminal tier.

MORPHOLOGY AND DEVELOPMENT

Ascospore

The relatively large, acicular, hyaline, two-celled ascospore of *Phalacrichomyces* spp. has a submedian cross wall (*a*) (Fig. 11). The longer cell, which is directed upward in the perithecium prior to spore discharge (Fig. 9, 10, 29, 30), comprises ca. 60% of the total length of the body of the spore. The hyaline sheath surrounding the spore is progressively thicker toward the end of the longer segment where, just beyond the tip of the body proper, it narrows abruptly to a point (Fig. 11).

Receptacle

Development of the ascoma begins with the formation of the foot at the base of the longer of the two segments of the ascospore (Fig. 17). The foot darkens and becomes completely opaque where it surrounds the point of egress of the haustorium (*ha*) (Fig. 19). The nature of the haustorium inside the host was not observed.

Juvenile individuals showing the earliest divisions of the basal cell of a germinated spore were not found. Receptacles of the youngest germlings encountered were at the three-cell stage (Fig. 1, 18–20), i.e., with the basal cell (I) lying parallel to cell *c* and subtending the terminal cell (III). Division of cell *c* gives rise to a perithecial initial and the subbasal cell of the receptacle (II) (Fig. 21 [the perithecial initial has divided once in this example]). Receptacular cells (I, II, III) enlarge somewhat as the ascoma matures, but they do not divide.

Appendage

As in other Stigmatomycetinae, with the possible exception of *Dipodomyces* Thaxter (Tavares 1985:205), the appendage of *Phalacrichomyces* develops from the upper cell of the ascospore, and it is delimited from the receptacle by the enlarged original septum of the spore (*a*) (Fig. 11, 17). There are only two divisions of the primordial appendage, i.e., the upper spore segment. The result is an appendage (*ap*) (Fig. 1, 18) consisting of three superposed cells, each of which cuts off a single cell that is converted into a simple antheridium (*an*), with the indurate upper end of the original spore persisting as a spine (*spi*) near the base of the terminal antheridium (Fig. 1, 2, 18–20). In rare instances, one cell of an appendage may fail to give rise to an antheridium (Fig. 21, 27).

In both *Phalacrichomyces normalis* and *P. anomalus,* immature appendages are similar to one another (Fig. 1, 18). In the former species, mature proximal and median antheridia are directed upward, have somewhat sigmoid discharge tubes, and are more or less parallel to the long axis of the appendage; the distal antheridium is continuous with the long axis and the spine diverges laterally from the posterior surface of its venter (Fig. 2). In the latter species, however, the antheridial discharge tubes are nearly straight or slightly arcuate and project nearly at right angles or diverge upward only slightly relative to the long axis of the appendage; the spine borne on the venter of the distal antheridium terminates the appendage (Fig. 19, 20, 31). Antheridia are mature and may be discharging spermatia by the time perithecial development begins (Fig. 19–21).

Perithecium

The earliest observed stage of formation of a perithecial initial in *Phalacrichomyces* is shown in Figures 20 and 31 where cell *c* of a germling of *P. anomalus* has begun to grow outward at the base. In a slightly more advanced stage shown in Figure 21 the initial has separated from cell *c,* which now constitutes the subbasal cell of the receptacle (II), and has divided into two cells, the primordial cell of the perithecium (*h*) and the primordial cell of the procarp (*i*). As shown in Figures 22 and 32, cells *h* and *i* have divided; *h* has formed an upper cell *j* and a lower cell *k*; *i* has formed cell *cp* (the carpogenic cell) below and cell *e* above. Early stages of perithecial development in *P. normalis* like those shown in Figures 20–22 for *P. anomalus* were not found; however, the position of the primary stalk cell (VI) of immature perithecia of *P. normalis* at the upper end of cell II of the receptacle (Fig. 2–8) indicates that the perithecial primordium arises distally from cell *c* in this species rather than proximally as in *P. anomalus.* Continued development of the perithecium from the cells shown in Figure 22 involves formation of the primary stalk cell of the perithecium (VI) and one of the three perithecial basal cells (*m*) from cell *k,* and formation of the secondary stalk cell of the perithecium (VII) and the other two basal cells (*n* and *n*′) from cell *j.* Further early stages of perithecial development are depicted in Figures 2–5, 12–14, and 23–24. The five tiers of inner and outer wall cells that eventually surround the centrum arise from the three basal cells (Fig. 3–10, 13–14, 23–25, 26–30).

Figures 2 and 12 show an early stage of development of the trichogyne (*tr*) as a lateral bulblike outgrowth separated from the trichophoric cell (*tc*), which is subtended by the carpogenic cell (*cp*). An intermediate stage of development of the two-celled trichogyne is shown in Figures 3 and 23 where a cross wall has formed, separating an elongate upper cell from the distally strongly constricted lower cell. By the time the perithecium has reached the two-outer-wall-cell stage of development the apex of the upper cell of the mature two-celled trichogyne has formed several short or elongate outgrowths (Fig. 4, 5, 24). If fertilization of the carpogenic cell indeed occurs, it must take place at about this time, for the trichogyne soon degenerates, except for a remnant consisting of part of its bulbous basal cell (Fig. 6, 7, 14), which is conspicuous even on mature perithecia (Fig. 9, 10, 29). In the absence of well-fixed and stained material a precise study of the development of the centrum from the carpogenic cell (*cp*) and trichophoric cell

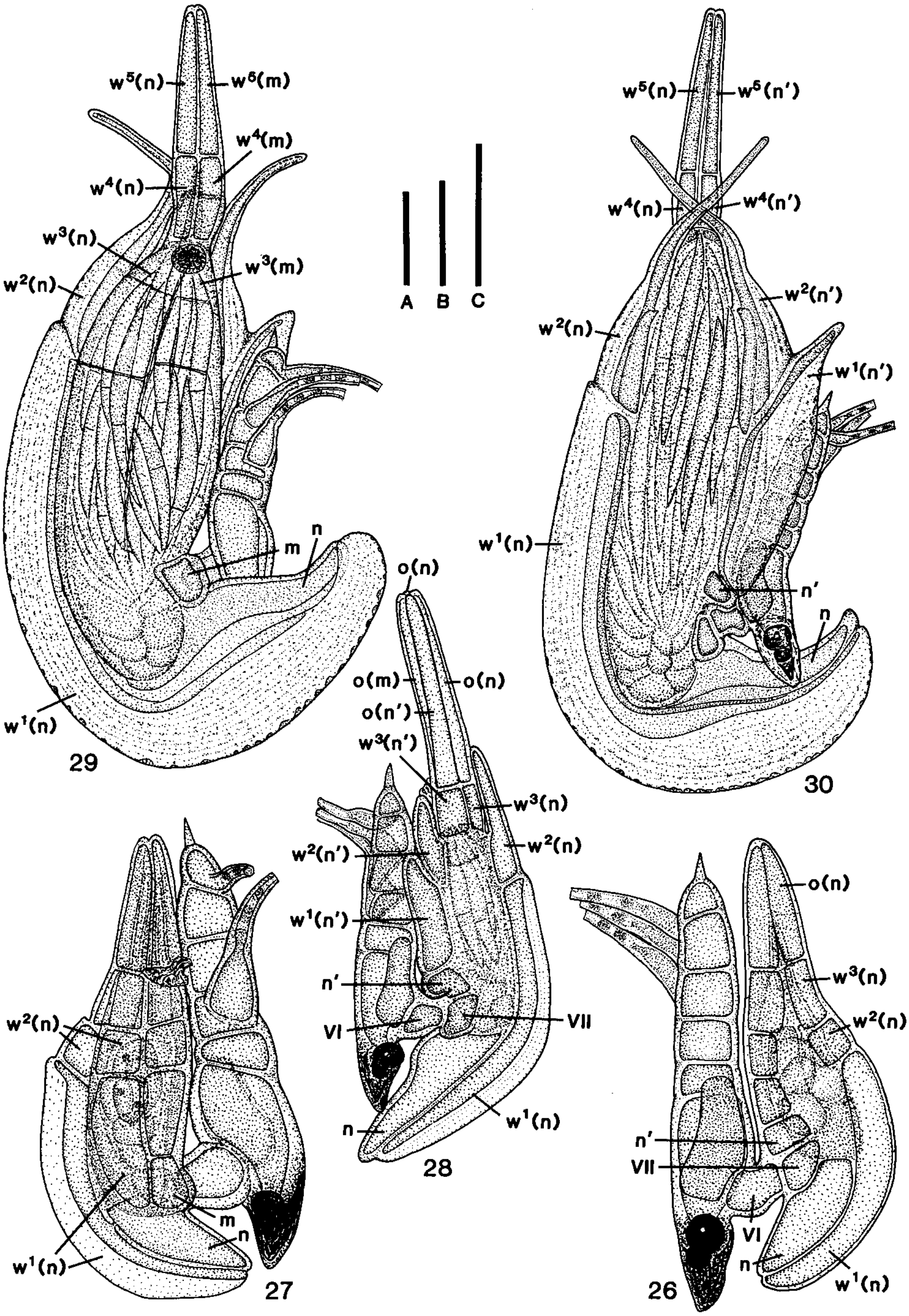

Fig. 26–30. *Phalacrichomyces anomalus (RKB 2818A).*—Fig. 26–28. Intermediate stages of development of the perithecium.—Fig. 29, 30. Two mature individuals shown from above (Fig. 29) and below (Fig. 30) (see Fig. 35 and 36 for photographs). Details and terminology are given in the text. (Bars = 20 μm: Fig. 26, 27, bar C; Fig. 28, bar B; Fig. 29, 30, bar A.)

(*tc*) was not feasible, but several early stages of centrum development should be noted. Before the carpogenic and trichophoric cells fuse, the latter grows upward and forms an acute termination around which the wall cells grow as they bypass the base of the trichogyne (Fig. 3, 4, 13, 23, 24). Fusion of the carpogenic and trichophoric cells (*cp-tc*) has occurred at the stage of perithecial development shown for *P. anomalus* in Figures 25 and 33. Figure 15 shows a mature five-celled centrum (enclosed by the developing inner and outer wall cells) derived from a *cp-tc* cell in *P. normalis* before the formation of ascal initials by the ascogenous cell (*ac*) (see legend for details).

Except for the location of the primary stalk cell (VII) on cell II of the receptacle, perithecial development is similar in both *Phalacrichomyces normalis* and *P. anomalus* through stages shown in Figures 6 and 14 for the former and Figures 25 and 33 for the latter. Subsequent development of the perithecium in *P. anomalus* differs greatly in the degree of modification of several of the outer-wall cells compared to that of the perithecium of *P. normalis.*

As the perithecium of *P. normalis* continues to develop, the four vertical rows of inner and outer wall cells extend upward around the centrum. In Figures 7 and 8, two stages of enlargement of the perithecium are shown in the four-outer-wall-cell stage where there are three tiers of permanent outer wall cells (w^1, w^2, w^3) and a fourth tier of elongating wall-cell primordia (*o*). Cells *o* divide again prior to maturation of the perithecia and form the short fourth and elongate fifth tiers of permanent outer wall cells (w^4 and w^5), which constitute the perithecial rostrum (Fig. 9, 10). The conspicuous trichogynic remnant straddles two of the tier-three outer wall cells, the one derived from basal cell *m* and one of those derived from basal cell *n* (Fig. 10). When in situ, immature specimens like those shown in Figures 2–7 are somewhat appressed to the host integument; however, when mature, the perithecium is more or less erect or ascending and the rostrum is nearly in line with the body (Fig. 9, 16).

Like *P. normalis,* the ascoma of young individuals of *P. anomalus* also is appressed to the surface of the host, but in this species the developing ascoma remains in this position as it matures. Immature and mature ascomata are regularly associated in pairs, and one member of each pair is a mirror image of the other (Fig. 37–40). This noncongruence is reflected in all aspects of the ascomata of opposite members of each pair (Fig. 29–30).

The first visual evidence of the anomalous development of the perithecium of *P. anomalus* compared to that of *P. normalis* takes place at about the two-outer-wall-cell stage as seen in Figures 25 and 33. Primordia of the outer wall cells (*o*) and inner wall cells (*p*) have grown upward around the now-fused carpogenic and trichophoric cells (*cp-tc*). Here, also, basal cell *n* and one of the two lowermost outer wall cells derived from cell *n* have begun to grow downward. By the time the perithecium has reached the four-outer-wall-cell stage (Fig. 26, 34), these two cells have enlarged greatly and during their downward growth have recurved toward the foot at the base of the receptacle. Figure 26 depicts the lower surface of an individual, which originally was closely appressed to the surface of the host, and shows basal cell *n′* and the vertical row of outer wall cells derived therefrom. Figure 27 illustrates an individual as seen from above that is slightly more advanced in development than the one shown in Figure 26. This figure shows the vertical row of outer wall cells derived from basal cell *m* and both of the vertical rows derived from basal cell *n,* where the cells in one row remain essentially

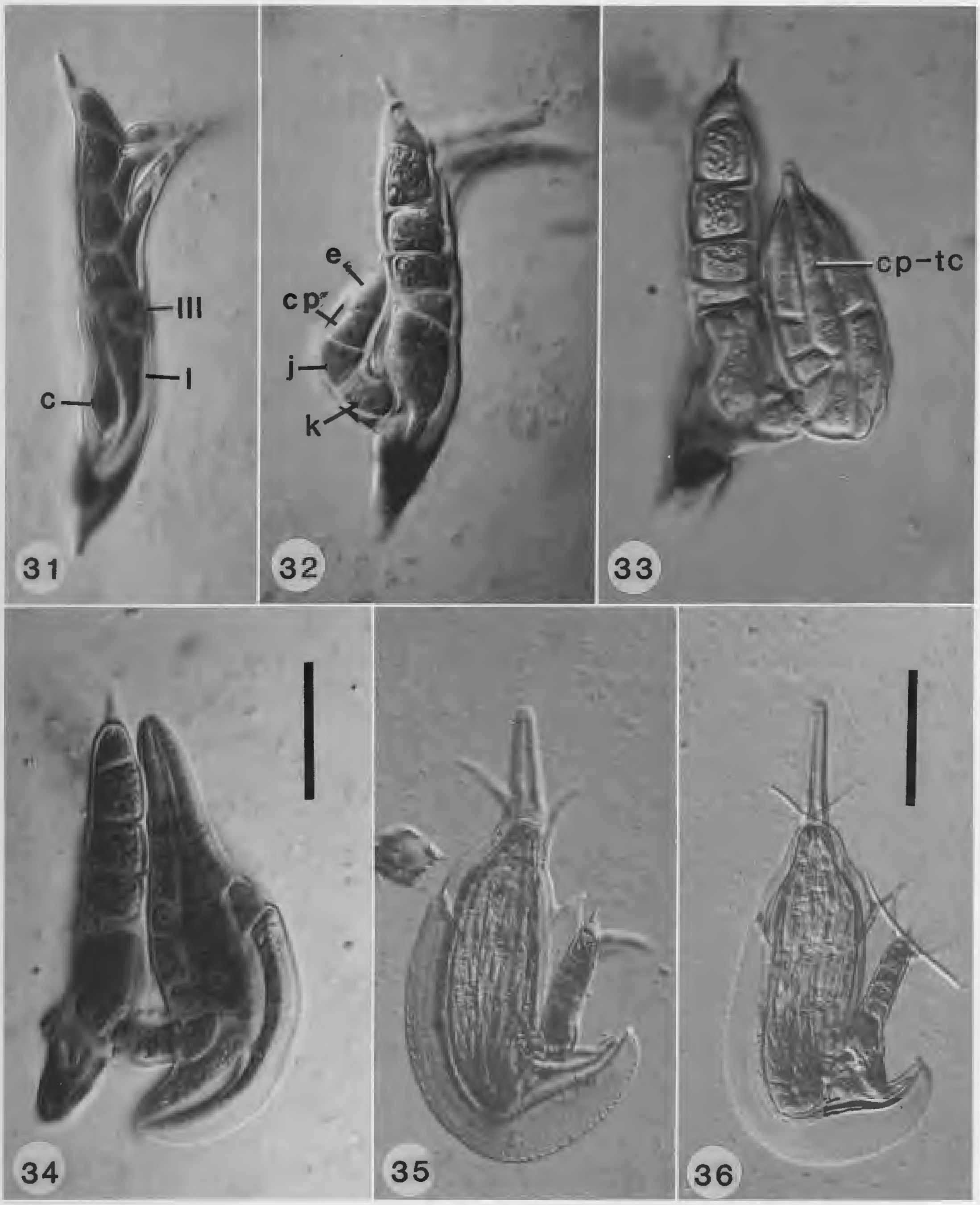

Fig. 31–36. *Phalacrichomyces anomalus (RKB 2818A).*—Fig. 31–34. Photographs of the specimens depicted in Figures 20, 22, 25, and 26, respectively. Note the fused carpogenic and trichophoric cells (*cp-tc*) constituting the young centrum as shown in Figure 33.—Fig. 35–36. Two mature individuals as seen from above (Fig. 35) and below (Fig. 36) (see Fig. 29 and 30 for drawings). (Fig. 31–34, bar with Fig. 34 = 20 μm; Fig. 35, 36, bar with Fig. 36 = 50 μm.)

unmodified compared to the lower two cells in the other row. Also shown are the early stage of thickening of the wall of the lowermost cell derived from basal cell n [($w^1(n)$] and the first evidence of the formation of an upgrowth from the outer wall cell immediately above [($w^2(n)$].

In the intermediate stage of perithecial development shown in Figure 28—as seen from the lower surface—the centrum has begun to form asci, but the perithecium still is in the four-outer-wall-cell stage. The subbasal outer wall cell [$w^2(n)$] in the same row as the highly modified basal cell [$w^1(n)$] is forming a distal prolongation; at the same time the basal and subbasal outer wall cells derived from basal cell n', i.e., $w^1(n')$ and $w^2(n')$, respectively, also are growing upward and forming free terminations. Finally, in mature ascomata (Fig. 29, 30, 35, 36) the highly modified basal cell n and basal wall cell $w^1(n)$ have reached the full extent of their curvature around the base of the receptacle; basal wall cell $w^1(n')$ has, like cell $w^1(n)$, developed a greatly thickened exterior wall and, in addition, has formed an acute apical termination; and cells $w^2(n)$ and $w^2(n')$ have formed free, elongate, slender extensions along with moderately thickened exterior walls below these appendages. The elongate primordial perithecial outer wall cells (o) (Fig. 28) have divided and given rise to a fourth tier of short outer wall cells (w^4) and a fifth tier of elongate outer wall cells (w^5). The resulting perithecial rostrum projects upward at an angle of about 45° relative to the rest of the dorsiventrally flattened, appressed ascoma (Fig. 37–40).

DISCUSSION

Genera Possibly Allied with Phalacrichomyces

In regard to the strongly diagonal to nearly parallel, i.e., side-by-side, positioning of cells I and II of the receptacle, *Phalacrichomyces* resembles only six of the other 39 genera of Stigmatomycetinae (Tavares 1985; Tavares and Balazuc 1989; Benjamin 1992). In three of these genera, i.e., *Acrogynomyces* Thaxter (1931:356), *Cupulomyces* Benjamin (1992:356), and *Prolixandromyces* Benjamin (1970:174, 1981; Santamaria 1988), cell I is *anterior* in relation to cell II, which subtends not only the primary stalk cell (VI) of the perithecium but also cell III, the uppermost cell of the receptacle. In the other three genera, i.e., *Ilyomyces* Picard (1912:445), *Stemmatomyces* Thaxter (1931:107), and *Synandromyces* Thaxter (1912:174; Benjamin 1984), cell I, as in *Phalacrichomyces,* is *posterior* with reference to cell II, and cell III is subtended by cell I.

Acrogynomyces includes six species parasitic on undetermined species of *Eumicrus* Laporte (Coleoptera: Scydmaenidae) from the then German colony of Kamerun, Africa. Depending on the species, the appendage consists of one to several superposed cells and bears sterile branchlets and antheridia laterally and/or distally. Cell I is in contact above with cell VI and in some species extends upward along the outside of this cell and abuts the base of cell VII as well. Cell III is united on the inside throughout most of its length with cell VI, which in all

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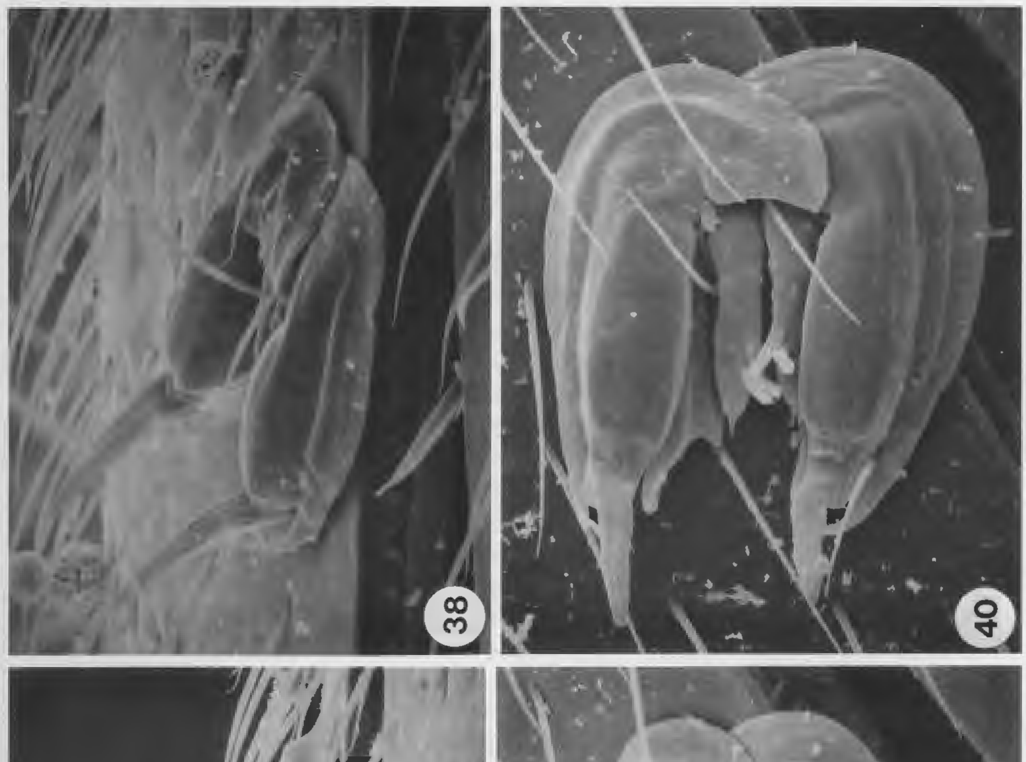
Fig. 37–40. *Phalacrichomyces anomalus (RKB 2818A).*—Scanning electron micrographs of an associated, in situ pair of mature individuals showing mirror-image asymmetry. Note the divergent perithecial rostra. Angle of view: 90°, 60°, 30°, and 0°, respectively. (All figures, bar with Fig. 37 = 50 μm.)


38
40
37
39

but one species, *A. ellipsoideus* Thaxter, is nearly enclosed by cells VII, I, II, and III. The number of tiers of perithecial outer wall cells appears to be four; however, Thaxter (1931) stated that in a few instances he counted five cells in one of the rows. The abruptly narrowed perithecial apex bears a more or less prominent appendage or a conical extension that in some cases appears to derive from the modified base of the trichogyne (Thaxter 1931).

Cupulomyces with one species, *C. lasiochili* (Thaxter) Benjamin (1992), occurs on a member of the Anthocoridae (Heteroptera) from Grenada, West Indies. The appendage lacks sterile branchlets, and consists of but one elongate, sterile cell surmounted by two antheridia-bearing cells. Cell I subtends cell VII, and cell II subtends not only cell III but also cell VI, which lies between cells VII and III, forming a transverse series of three cells. The outer wall cells of the perithecium are disposed in five tiers. Cells of the terminal tier form a distinctive minaret-shaped perithecial apex with each cell bearing a short, divergent upgrowth near its base.

Prolixandromyces includes six species, all occurring on Veliidae (Heteroptera) from both the Old World and New World (at least 15 additional species are in my collection awaiting description). The appendage is without sterile branchlets and consists of three to several superposed cells; the lowermost two cells invariably are sterile, whereas the distal one to several cells give rise to one or two antheridia, which may have greatly elongate necks. Cell VI is free except on the inside at the base and often forms an elongate perithecial stalk. There are only four tiers of perithecial outer wall cells, and one or more cells of the terminal tier may develop a distinctive short or elongate appendage.

On the basis of the anterior vs. posterior placement of cell I in *Acrogynomyces, Cupulomyces,* and *Prolixandromyces,* these genera are readily separated from *Phalacrichomyces,* but, as summarized above, other features of the ascoma, especially characteristics of the perithecium and appendage, serve also to distinguish them from the latter.

There are but two species of *Ilyomyces,* both collected on insects of the genus *Stenus* Latreille in France and still known only from their original descriptions (Picard 1917)[1]; they are the only Laboulbeniales yet found on members of the subfamily Steninae (Staphylinidae: Coleoptera). Although cells I and II have the same relationship to one another in *Ilyomyces* as in *Phalacrichomyces,* the two genera differ greatly in the structure of their appendages and in the conformation of their perithecial apices. On the basis of Picard's illustrations, the appendage of *Ilyomyces* apparently consists of at least two, perhaps three, superposed sterile cells that subtend one or two smallish cells bearing single or paired antheridial phialides. One of the antheridia is subtended by a spine, undoubtedly representing the indurated apex of the original ascospore. The perithecium of *Ilyomyces,* like *Phalacrichomyces,* probably has five tiers of outer wall cells, but the relatively broad perithecial tip is distinguished by four mammillate or somewhat slender terminal lobes.

Stemmatomyces and *Synandromyces* are the two genera of the six being discussed here that have receptacles and appendages showing the greatest correspondence to those of *Phalacrichomyces. Stemmatomyces* includes two species on Elateridae (Coleoptera) from Central and South America (Thaxter 1931); *Synandromyces* has nine species occurring on representatives of four families of Coleoptera, i.e., Cryptophagidae, Cucujidae, Nitidulidae, and Tenebrionidae, from several areas in the New World and Old World (Thaxter 1912, 1931; Benjamin 1984). Both species of *Stemmatomyces* and the seven species of *Synandromyces* parasitizing Cucujidae and Tenebrionidae have receptacles in which cells II and III extend downward alongside cell I, front and rear respectively, with their lower ends often reaching nearly to the base of this cell. There is a slight tendency for cell III to grow down along the outside of cell I in *Phalacrichomyces normalis,* but not in *P. anomalus.* In the two species of *Synandromyces* found on Cryptophagidae and Nitidulidae, there is little or no downward extension of cells II and III beside cell I (Thaxter 1931), and these species are allied with other members of the genus by the structure of the appendage.

Perithecia of *Stemmatomyces, Synandromyces,* and *Phalacrichomyces* are similar in having five tiers of outer wall cells and an essentially free stalk cell (VI), which, except in the anomalous perithecium of *P. anomalus,* forms a well-defined, more or less elongate perithecial stipe. The basal tier of outer wall cells usually is somewhat longer than the subbasal, and together these tiers comprise 60% or more of the total length of the perithecial body. Cells of the next two tiers usually are relatively much shorter than cells of the lower tiers, although in some species of *Synandromyces* cells of tier three may be nearly equal in length to those of tier two. Unlike *Phalacrichomyces,* the terminal tier of outer wall cells in *Stemmatomyces* and *Synandromyces* are relatively short compared to cells of the preceding one or two tiers. The perithecial apex of *Stemmatomyces* differs from that of both *Phalacrichomyces* and *Synandromyces* in having erect, free, terminal lobes, which extend well beyond the ostiole. In *Phalacrichomyces* and *Synandromyces* the apex is unmodified except for *S. floriformis* Thaxter, where the abruptly narrowed, short-cylindrical tip forms horizontally flattened, petallike extensions (Thaxter 1931). The trichogyne of members of *Stemmatomyces* and *Synandromyces,* where known, is comparable to that of *Phalacrichomyces* in consisting of two superposed cells, often adorned distally with few or many globoid or lobate prominences (Thaxter 1931; Benjamin 1984).

The appendages of *Stemmatomyces* and *Synandromyces* are very similar to one another in basic structure (Thaxter 1931; Benjamin 1984), and though they resemble the appendage of *Phalacrichomyces* they are distinctly different when examined closely. In *Stemmatomyces* and *Synandromyces,* the body of the appendage consists of a small number of superposed cells in which the basal cell of the young appendage invariably divides and forms a sterile cell and what Thaxter termed an "antheridiiferous supernumerary cell," which gives rise to the lowermost antheridium developed on the appendage. The antheridium-bearing supernumerary cell of *Synandromyces* is positioned towards the inside of the appendage, i.e., facing the perithecium, whereas that of *Stemmatomyces* is positioned towards the outside of the appendage. In *Synandromyces,* each fertile cell of the appendage above the supernumerary cell forms a single antheridial phialide, which, like the lowermost antheridium, is directed inward. Antheridia formed by successive cells of the appendage of *Stemmatomyces* are directed outward; however, in this genus the subterminal cell usually forms additional antheridia laterally. The terminal antheridium may be spinose in both *Stemmatomyces* and *Synandromyces.*

In conclusion, among all of the Stigmatomycetinae, *Stemmatomyces* and *Synandromyces* appear to be the genera most nearly related to *Phalacrichomyces* on the basis of the morphology of the receptacle, appendage, and perithecium. *Phalacrichomyces* is separated from these genera primarily by the structure of the appendage, which lacks a basal supernumerary antheridiiferous cell accompanied by a sterile cell, and secondarily by the greatly elongate tier-five outer wall cells, which form a distinctive beaklike perithecial rostrum.

Two such morphologically disparate species as *P. normalis* and *P. anomalus* illustrate how certain perithecial characteristics such as modifications of outer wall cells and the presence of absence of appendages can vary among species of a given genus. This points to the need for one to take care not to overly stress such features when describing a new generic entity in the Laboulbeniales. In the absence of *P. normalis,* a characterization of *Phalacrichomyces* based only on *P. anomalus* doubtless would have been very different from the one given in this paper.

Mirror-image Asymmetry

As alluded to earlier in this paper, ascomata of *Phalacrichomyces anomalus* typically are found in pairs. Juxtaposition of two individuals occurs frequently in the Laboulbeniales, a result of the fact that two ascospores appear often to exit the perithecium simultaneously and develop side-by-side on the host (Thaxter 1896:203). The phenomenon is especially conspicuous in the male and female ascomata of dioecious taxa, where the two sexes not only may vary greatly in size and morphology but also in some genera the ascospores may exhibit more or less extreme dimorphism, e.g., *Aporomyces* Thaxter (Benjamin 1989) and *Dioicomyces* Thaxter (1901, 1908; Benjamin 1970). It is, of course, common in many genera to find ascomata growing singly or in clusters of various sizes on any given host, for there undoubtedly are internal as well as external factors, physiological or mechanical, that can affect the discharge of spores from the perithecium and their attachment to and subsequent growth on the host's integument.

The paired, dorsiventrally flattened ascomata of *Phalacrichomyces anomalus* illustrate a phenomenon, which can be termed mirror-image asymmetry, that probably is characteristic of many Laboulbeniaceae. I have not studied this condition in detail, but I have noticed it over the years in many taxa of this family, especially in members of the Laboulbenieae where, owing to a relatively simple receptacle and usually a single perithecium, it is easily observed. Detection of such asymmetry in these fungi owes to the development of four vertical rows of outer perithecial wall cells from just three basal cells, one row each from basal cells *m* and *n'* and two rows from basal cell *n*. Thus, with reference to cell *m,* cells *n* and *n'* are positioned more or less on opposite sides of the perithecial base. In my study of *Rhizopodomyces* Thaxter, I noted (Benjamin 1979:385) that in the female of *R. californicus* Benjamin the position of cells *n* and *n'* varied, *n* being dextral and *n'* sinistral in one individual and vice versa in another individual. As an aside to the present study, I mounted two juxtaposed individuals of each of several species of *Laboulbenia,* i.e., *L. acupalpi* Spegazzini (*RKB 3744*), *L. flagellata* Peyritsch (*RKB 2640, 3746, 3747*), and *L. parvula* Thaxter (*RKB 2624B*). Each pair presumably represented ascomata developed from a pair of ascospores formed in an ascus. In each instance, as seen under the microscope, cell *n* was dextral to cell *m* in one member of a pair and sinistral in the other member. In another observation, 81 pairs of male and female individuals of the dioecious *L. formicarum* Thaxter (*RKB 353*) (see Benjamin and Shanor 1950) were examined, and the relative position of the basal cells of the perithecium of the female member of each pair was similarly scored; with reference to cell *m*, cell *n* was dextral in 43 females and sinistral in the other 38, a close fit to a 1:1 ratio.

The early cytological study by Faull (1912) and the more recent work by Tavares (1985:54–57) on *Laboulbenia chaetophora* Thaxter and *L. gyrinidarum* Thaxter have demonstrated that only four of eight nuclei formed in the young ascus are incorporated into four ascospores disposed in two pairs, the four nonfunctional nuclei degenerating. A similar mode of development of four-spored asci appears to be the rule in Laboulbeniales—with a few exceptions, i.e., species of *Herpomyces* Thaxter and some species of *Compsomyces* Thaxter (Tavares 1985) where there are eight—and the emergence of paired ascospores from perithecia so often observed apparently reflects the manner in which they are delimited within the ascus. Just as the paired ascospores that give rise to juxtaposed male and female individuals in dioecious species must reflect a genetic process, so too must the phenomenon of mirror-image asymmetry as described above for the several taxa named.

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FOOTNOTE

[1] *Note added in press*: Sergio Santamaria [New and interesting Laboulbeniales (Fungi, Ascomycotina) from Spain. Nova Hedwigia 54:479–492. 1992] recently recorded *Ilyomyces mairei* Picard from Spain (see pp. 484–486, fig. 16–19).