Hortaea, a New Genus to Accommodate Cladosporium werneckii

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In 1921, Horta1) described the causative agent of tinea nigra, a superficial mycotic infection occurring in South Asia, both American continents, Africa and Australia, as Cladosporium werneckii. Since then, opinions on that binominal have been divided and many synonyms were proposed by mycologists.

In 1970, von Arx2) described the fungus as Exophiala werneckii in his book without any comments. Thereafter, Gustafson et al.3) and Cole4) found annellations on yeast-like cells of the fungus using a transmission and a scanning electron microscope, respectively. McGinnis5) also reported that both the yeast-like and hyphal conidiogenous cells are annellides. On the other hand, Cole6) and Nishimura and Miyaji6) observed annellations on the conidiogenous cells of Exophiala salmonis, the type species of the genus Exophiala, using a scanning electron microscope. Nishimura and Miyaji7) observed E. dermatitidis, E. jeanselmei, and E. spinifera using a scanning electron microscope, and clarified the fact that their conidiogenous cells are annellides.

From these data it seemed reasonable that Cladosporium werneckii was transferred to the genus Exophiala. In fact, mycologists such as Ajello8), Carmichael et al.9), Cole, McGinnis5) and Rippon10) have agreed with von Arx2).

This time, we found a unique sympodial anamorph accompanying an annellidic one in "Exophiala werneckii" during our studies on the phylogenesis of black yeasts. The sympodial anamorph is definitely different from that of other sympodial genera such as Rhinocladiella and Ramichloridium. Therefore, we analyze the process of the conidiogenesis and propose a new genus, Hortaea, to accomodate C. werneckii.

Materials and Methods

A culture, NCMH 75, derived from the neotype of E. werneckii, which had been given to us by Dr. McGinnis (North Carolina Memorial Hospital, North Carolina, U.S.A.), was used in this study. Corn meal agar (Difco) and potato dextrose agar (Difco) were used as media. The culture was incubated on them at 27°C or room temperature which ranged from 5 to 20°C. Colonies were cut into small blocks 14 to 34 days after inoculation and put in 2%
Figs. 1–4. *Hortaea werneckii* (NCMH 75). Scanning electron micrographs. Bar is 1 μm. 1. Rachis with round bud scars. Terminal conidia are acropetally produced. 2. Rachis looking like a pine cone. 3. Bird’s eye view of a rachis. Two round bud scars are observed at the apex. 4. Rachis with a round and lunate scars.
Figs. 5–8. *Hortaea werneckii* (NCMH 75). Scanning electron micrographs. Bar is 1 μm. 5. Lunate bud scars alternate on a rachis. 6. Bud scars are formed in a clockwise direction one after another at higher levels. 7. Bud scars looking like petals. 8. Rachis looking like a rose. A small round bud and falciform scars are observed.
After having been fixed at 3°C for 24 hours and then 1% osmium tetroxide at 3°C for 17 hours, these blocks were dehydrated by a series of gradient alcohols, acetones, and by isoamyl acetate, then dried with a Critical Point Dryer HCP-1 (Hitachi), coated with gold palladium using an Ion Coater IB-3 (Eiko Engineering) Ltd.) and observed by a scanning electron microscope HFS-2 (Hitachi).

**Results**

In its mycelial form, conidiogenous loci occurred on ampullaceous cells formed laterally or terminally on hyphae, lateral branches and projections on hyphae, or directly from lateral walls of hyphae. These conidiogenous loci elongated and swelled in accordance with the production of solitary, terminal conidia, and became thick cylindrical, obclavate or truncate rachises which were 2.0—4.0 (average 3.1)μm in diameter and 1.2—7.3 (average 4.3)μm in length. There were a few zigzag-shaped rachises (Fig. 5). Denticles were not detected on the surface of the rachises. Instead, various shapes of bud scars could be observed. In a few conidiogenous cells, round bud scars were arranged on the rachises in lengthwise rows (Fig. 1). Some rachises were covered with remnants of bud scars which looked like scales. These rachises had the appearance of pine cones or pineapples (Fig. 2).

In most of conidiogenous cells, remnants of bud scars shaped like sickles, half moons or scales were observed on the rachises. Each remnant was formed when a new conidium appeared by breaking through a part of a previously formed bud scar(s). In some cases, both round and lunate scars were observed on a rachis (Fig. 4). At the apices of rachises, one to two round scars were located, which were surrounded by remnants that looked like scales, half moons or sickles (Figs. 3, 6 and 8). In some cases, traces of the torn-off cell walls of the first conidium encircled the first bud scar and in other cases they were inconspicuous. On the other hand, petal-like appendages or short frills attached around the second and successive bud scars (Figs. 6—8). The appendages were parts of the preexisting bud scars, which were broken and lifted when the conidia were formed. The bud scars usually were 0.8—2.4 μm in diameter.

Yeast-like cells produced daughter cells by unipolar or bipolar budding. In most of the yeast-like cells the conidiogenous apices were also covered with round bud scars or variously shaped remnants of bud scars (Fig. 9).

There were a few conidiogenous cells with annellations in both the mycelial and yeast forms (Fig. 10). Their number, in particular, was very low when cultured at room temperature. The annellations, which looked like short frills, were irregular in comparison with those of *E.*
**Discussion**

As reviewed by McGinnis⁵, the taxonomy of this dematiaceous yeast-like fungus has been confusing. Now, many mycologists have regarded this fungus as a member of the genus *Exophiala* and accepted the binomial, *E. werneckii*, even though Hermanides-Nijhof¹² considered it to be a later synonym of *Sarcinomyces crustaceus* and Borelli¹³ described it as *Aureobasidium werneckii*.

Gustafson et al.¹⁴, Cole⁶ and McGinnis⁵ have indicated that *E. werneckii* has an annellidic anamorph. Cole⁶ demonstrated the annellidic anamorph in *E. salmonis*, the type species of the genus *Exophiala*, by scanning electron microscopy. Nishimura and Miyaji⁷,⁸ observed the anamorphs of *E. dermatitidis*, *E. jeaneselmei*, and *E. spinifera* by scanning electron microscopy and found the annellidic anamorph in these fungi, but never did find such anamorphs as the phialidic and sympodial anamorphs which had been reported by some mycologists¹⁴-¹⁶. Furthermore, they demonstrated the annellidic anamorph in the culture derived from the neotype of *C. werneckii* conidium after two revolutions as shown in Fig. 12A. We also refer to it as a “generic spiral” and designate the axis of rotation as the main axis. Additionally, we designate the angle of revolution between a bud scar and the next formed one as a divergence. In botany, a divergence is usually shown by one of the following progressions.

1/2, 1/3, 2/5, 3/8, 5/13, 8/21, 13/34, 21/55, ....

Namely, the denominator or numerator of a term is the sum of the two denominators or numerators just before the term, respectively.

It is well known that the divergence between a scale and the next one formed on a cone of the gymnosperms is shown by a 8/21, 13/34 or 21/55 cycle. If the divergence of the present fungus is a 2/5 cycle, bud scars are formed as shown in Fig. 11B. If the fifth conidium is the newest one, the fourth and fifth bud scars are observed at the conidiogenous cell apex as shown in Fig. 11C. This coincides with the conidiogeneous cell apex shown in Fig. 3. When the sixth conidium is produced just above the first conidium after two revolutions as shown in Fig. 12A, the round bud scars are arranged as shown in Fig. 1. If the pitch of a generic spiral is slightly smaller than the radius of the scar, new conidia occur by breaking through a part of the bud scars formed previously. As shown in Fig. 1, the upper parts of the bud scars on the left side are broken off. When the pitch becomes narrower, the conidiogenous cell is covered with lunate bud scars. When the divergence is a 3/8 cycle and the pitch of a generic spiral is much smaller than the radius of the scar, the scaly bud scars overlap each other and the rachis becomes cone-like (Figs. 2 and 12B).

Next, we demonstrate conidiogeneous cell apices as...
shown in Figs. 5–8, from which a conidium arises by breaking through the bud scar of the next older conidium. As shown in Fig. 6, when the radius of a generic spiral is small, a new conidium emerges by breaking through a part of the next older scar. As a result, lunate or falciform bud scars are spirally arranged around the main axis (Figs. 5 and 13). The rachises shown in Figs. 5–8 seem to be formed by such a process. In fact, the diameters of the rachises shown in Figs. 5–8 are smaller than those shown in Figs. 1–4. When both the radius and pitch of a spiral are small, lunate or falciform bud scars overlap each other, and as a result, the rachis becomes shorter. When bud scars become gradually smaller towards the apices, the rachises become truncate or tapered as shown in Figs. 6 and 8. When the radius of a generic spiral is almost equal to zero, the growing point extends along with the main axis, and as a result, annellations are formed (Fig. 10).

As mentioned above, the process of the conidium ontogenesis of the present fungus is explained quite well by our hypothesis. Even though various forms of rachises are observed, all of them are a homology of the conidiogenous cell shown in Fig. 1 and the mode of conidium formation is fundamentally sympodial. In the present fungus, the annellidic anamorph is also a homology of the sympodial anamorph.

According to Grove et al., the annelloconidia of E. dermatitidis are enteroblastically produced and released by abscission. Gustafson et al., who studied the budding process of the yeast-like cells of C. werneckii with a transmission electron microscope, reported that increased electron opacity at the site of bud initiation, as reported by Grove et al., was not observed. Furthermore, they described that the collars (annellations) are formed not as a result of abscission but rather when successive daughter cells blastically emerge through the existing bud scar at the generative apices. According to them, the conidia of C. werneckii seem to be produced holoblastically and the annellations seem to be formed through processes different from those of E. dermatitidis.

According to Mok, E. werneckii decomposes casein but E. jeanselmei, E. spinifera and E. dermatitidis do not. In our study, the present fungus hydrolyzes skim milk but six species of the genus Exophiala do not (unpublished data). Judging from these results, the present fungus is different from the species of the genus Exophiala.

According to Pechak and Crang, *Aureobasidium pullulans* produces conidia enteroblastically. It also differs...
from the present fungus in conidiogenesis.

In 1977, Hermanides-Nijhof stated that *E. werneckii* is a later synonym of *Sarcinomyces crustaceus* Link. However, her opinion has not been accepted by McGinnis and Carmichael. Furthermore, the reproduction of *S. crustaceus* as indicated by Sigler et al., using a scanning electron microscope, is definitely different from that of the present fungus. Additionally, the conidiogenesis is definitely different from the descriptions and illustrations of Link which were cited by Sigler et al. De Hoog and Rubio found that the *S. crustaceus* strain used by Sigler et al. has both blastic and sarcinic (meristic) conidiogeneses. They did not describe any sympodial conidiogenesis in the strain of *S. crustaceus*. Furthermore, the sympodial conidiogenesis of the present fungus is definitely different from that of any other species of sympodial genera.

The type specimen was prepared from the culture derived from the neotype of *E. werneckii* by McGinnis and has been preserved in New York Botanical Garden. We did not observe the type specimen because the present study was performed using a scanning electron microscope. We prepared slide culture preparations of the present fungus and reconfirmed that many conidiogenous cells of the fungus look like annellides under a light microscope, even though they are sympodulae. We know that the fungus should be compared with the type specimen, when a new taxon is proposed. However, as mentioned above, the new genus *Hortaea* is proposed on the basis of the findings not by a light microscope but by a scanning electron microscope.

In 1982, von Arx et al. observed a sympodial anamorph with an annellidic one in *Taphrina deformans*. However, they did not give detailed explanations of the sympodial anamorph and of the relationship between the two anamorphs.

The conidium ontogenesis of *H. werneckii* may reveal an intermediate form between the annellidic and sympodial conidiogeneses. We suppose that the annellidic conidiogenesis might have been differentiated from the sympodial one in the present fungus. Studies on conidium ontogenesis in pleomorphic fungi will be of importance to clarify the conidium morphogenesis of the Fungi Imperfecti.

Based on this observation, we would like to propose a new genus, *Hortaea*, to accommodate *C. werneckii*.

**Description**

*Hortaea* Nishimura et Miyaji, gen. nov.

Etymology: In memory of Dr. Parreiras Horta, Professor of Dermatology.

Mycelium cylindrical to toruloid, pale to dark brown. Conidiophores semi-macronematous to micronematous, branched or unbranched, septate, pale brown to black. Conidiogenous cells (sympodulae) incorporated or terminal, cylindrical or ampullaceous, pale brown to black; rachises (fertile parts) of sympodulae truncate, cylindrical or obclavate with bud scars in spiral arrangement; bud scars round, lunate, falcate or scalar; denticles absent. Conidiogenous cells (annellides) rarely produced. Conidia solitary, acropleurogenous or acrogenous, one- to two-celled, ellipsoidal to cylindrical, pale brown to black, sometimes aggregated in clusters.

Yeast-like cells one- to two-celled, ellipsoidal to cylindrical, pale brown to black, with bud scars sympodially arranged or with annellations, producing conidia by unipolar or bipolar budding.

Teleomorph unknown.

*Hortaea werneckii* (Horta) Nishimura et Miyaji, comb. nov. (Figs. 1-10)


*Pullularia werneckii* (Horta) de Vries, Contribution to the Knowledge of the genus *Cladosporium* Link ex Fr., Hollandia, Baarn, p. 101, 1952.


*Pullularia fermentans* Wynne et Gott var. *leaoi*

Colonies at first smooth, shiny, moist, becoming dry or moist, thin felt with growth of superficial and immersed hyphae. Colonies on Sabouraud's dextrose agar, potato dextrose agar or corn meal agar at 27°C on the 14th day, attaining 18–20 mm in diameter, greenish buff, brown to greenish black. Conidiogenous cells (sympodulae), incorporated or terminal, cylindrical or ampullaceous, pale brown to black; rachises of sympodulae 1.2–7.3 (av. 4.3) μm in diameter, truncate, cylindrical or obclavate with bud scars 0.8–2.4 μm in diameter, round, lunate, falcate or scaly, encircled with or without frills or petal-like appendages; denticles not produced. Conidiogenous cells (annellides) rarely produced, 0.3–6.2 (av. 2.3) x 1.3–2.6 (av. 1.9) μm, truncate or cylindrical, with 1–9 annellations. Conidia one- to two-celled, ellipsoidal to cylindrical, pale brown to black, 3.8–8.1 (av. 6.0) x 2.3–3.9 (av. 3.1) μm.

Yeast-like cells one- to two-celled, ellipsoidal to cylindrical, pale brown to black, 6.8–13.3 (av. 9.5) x 3.0–4.6 (av. 4.0) μm including conidiogenous apices, producing sympodial or annelloconidia by unipolar or bipolar budding.

Specimen examined: NCMH 75 (=CBS 107.67, ATCC 36317), isolated from a patient with tinea nigra in Portugal, sent by M.R. McGinnis.

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**References**

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