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Neocarpenteles: a new ascomycete genus to accommodate *Hemicarpenteles acanthosporus*

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Abstract A new genus, *Neocarpenteles*, the Trichocomaceae (Eurotiales), and a new combination, *N. acanthosporum*, are proposed to accommodate *Hemicarpenteles acanthosporus* Udagawa et Takada. The fungus is characterized by sclerotioid, nonostiolate, unilocular stromata in which asci gradually produce, outward from the center, lenticular ascospores with two equatorial crests and convex surfaces ornamented by triangular ridges and microtubercles, and an *Aspergillus* anamorph with uniseriate aspergilla. It has the Q-10 system as the major ubiquinone.

Key words *Aspergillus acanthosporus* · Cleistothecial ascomycetes · *Neocarpenteles acanthosporum* · Systematics · Trichocomaceae

Introduction

During the Japanese Mycological Expedition to New Guinea and the Solomon Islands in 1969–1970, Udagawa and Takada repeatedly isolated a cleistothecial ascomycete with an *Aspergillus* anamorph from the soil of Bougainville Island, Papua New Guinea. They described it as *Hemicarpenteles acanthosporus* Udagawa et Takada (Udagawa and Takada 1971), a species resembling *Hemicarpenteles paradoxus* A.K. Sarbhoy et Elphick (Fennell and Raper 1955; Sarbhoy and Elphick 1968). It differs from the latter by the very restricted growth of the colonies on Czapek's agar, the colorless colony reverse, and the shape and size of the spinulose ascospores and the shorter conidiophores. *Hemicarpenteles acanthosporus* might be a form occurring principally in tropical or subtropical soils. Ito and Nakagiri (1991) later reported three isolations from subtropical soil in the Yaeyama Islands, Okinawa Prefecture, southernmost Japan. We also isolated this fungus from forest soil in Iriomote Island, one of the Yaeyama Islands, in 1992.

Because of the production of sclerotioid, uniloculate stromata lacking inner differentiated walls and oblate ascospores, *Hemicarpenteles* A.K. Sarbhoy et Elphick is similar to *Eupenicillium* F. Ludw. but differs by having an *Aspergillus* rather than a *Penicillium* anamorph (Malloch and Cain 1972). Raper and Fennell (1965) placed *Aspergillus paradoxus* Fennell et Raper, the anamorph of *H. paradoxus*, in the *Aspergillus ornatus* group (= subgenus *Ornati*, section *Ornati* W. Gams et al.) on the basis of its somewhat clavate vesicles, absence of metulae, and abundant production of sclerotia (later proved to be ascomata). The outstanding characters of the *A. ornatus* group are (1) aspergilla uniseriate; (2) vesicles fertile over most of the surface; (3) conidiophore stipes smooth to slightly roughened, hyaline or less commonly brown; and (4) conidia gray or yellow-green to olive-brown (Raper and Fennell 1965; Klich and Pitt 1988).

There is, however, some phylogenetic controversy surrounding the teleomorphs of *Ornati*. The teleomorphs of this section are unlike other sections of *Aspergillus* in separating into the three genera with distinct morphological differences: *Hemicarpenteles*, *Sclerocleista* Subram., and *Warcupiella* Subram. (Subramanian 1972; Gams et al. 1985). On the contrary, the other sections, namely sections *Aspergillus* (teleomorph: *Eurotium* Link : Fr.), *Fumigati* W. Gams et al. (*Neosartorya* D. Malloch et Cain), and *Nidulantes* W. Gams et al. (*Emericella* Berk. et Broome), might each form a monophyletic group.

Such heterogeneity (diversity) in the teleomorphs in *Ornati* led to confusion about section delimitation and indicated that chemotaxonomic and molecular studies on *Aspergillus* were essential to reevaluate the classification within this section.

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The early chemotaxonomic treatments of these fungi were presented by Kuraishi et al. (1990) and Sugiyama and Yamatoya (1990). The heterogeneity of the *A. ornatus* group in the Raper and Fennell *Aspergillus* classification was strongly supported by their nonmorphological analysis. They also suggested that *H. acanthosporus* (Q-10) clearly belongs to a different lineage than *H. paradoxus* (Q-9) and *Sclerocleista* species (Q-9) in the teleomorph of *Ornati* (Q-9). Independent studies on the molecular phylogenetics of *Aspergillus* and its teleomorphic genera have provided strong evidence for the separation of the two taxa, *H. paradoxus* and *H. acanthosporus*, at the generic level (Peterson 2000; Tamura et al. 2000). Other than a superficial resemblance in ascomatal morphology and conidial structures, there is little reason to keep *H. acanthosporus* in the genus *Hemicarpenteles*. Thus, we are in a position to reevaluate morphological characters of this unique fungus and to erect a new ascomycete genus to accommodate it.

Taxonomy

Neocarpenteles Udagawa et Uchiyama, gen. nov.

Stromata superficialia, plus minusve globosa, flavo-brunnea vel griseo-brunnea, tomentosa, sclerotioida, non ostiolata, unilocularia, peridio ascomatis interioris non distinguibilia. Asci irregulariter dispositi, ex centrum stromatics maturi, globosi vel subglobosi, octospori, evanescentes. Ascosporae unicellulares, hyalinae, lenticulares, varie sculptae, duabus cristis aequatorialibus praeditae.

Status anamorphus: *Aspergillus*. Aspergilla sine metulis.

Ubiquinonum majus: Q-10.

Species typica: *Neocarpenteles acanthosporum* (Udagawa et Takada) Udagawa et Uchiyama.

Colonies producing coiled branches of hyphae that develop into the ascoma-bearing stromata. Stromata superficial, more or less globose, yellowish-brown to grayish-brown, tomentose, hard and sclerotioid, nonostiolate, uniloculate, containing asci but lacking an inner differentiated ascomatal peridium. Asci irregularly disposed, maturing outward from the center of the stroma, globose to subglobose, 8-spored, evanescent. Ascospores one-celled, hyaline, lenticular, variously sculptured, with two equatorial crests.

Anamorph: *Aspergillus*, characterized by phialides borne directly on the vesicle (uniseriate aspergilla).

Major ubiquinone: Q-10.

Type species: *Neocarpenteles acanthosporum* (Udagawa et Takada) Udagawa et Uchiyama.

Neocarpenteles acanthosporum (Udagawa et Takada) Udagawa et Uchiyama, comb. nov. Figs. 1–7

Hemicarpenteles acanthosporus Udagawa et Takada, Bull. Natl. Sci. Mus. **14**:503, 1971 (basionym).

Holotypus: NHL 22462 (CBM). Living culture ex type: NHL 2462 (= IFO 9490 = ATCC 22931 = IMI 16621 = CBS 558.71).

Colonies on Czapek yeast extract agar (CYA) growing rapidly, attaining a diameter of 45–46 mm in 7 days at 25°C, floccose, plane, thin, with vegetative mycelium submerged, producing loose mycelium and very sparse conidia, white to brownish-orange (M. 6C4, after Kornerup and Wanscher 1978) or slightly cinnamon (Rayner 1970); stromata (ascomata) limited in central area; exudate small, clear; odor earthy; reverse grayish-orange (M. 5B4).

Colonies on malt extract agar (MEA) spreading broadly, attaining a diameter of 85 mm or more in 7 days at 25°C, floccose with dense development of aerial hyphae, plane, consisting of a thin basal felt, producing abundant stromata (ascomata) on the felt, white to pale yellow (M. 4A3) or buff (R); conidiogenesis inconspicuous, not affecting the overall appearance of the culture; reverse pale yellow to greenish-gray (M. 4A3-27E2) or olivaceous-gray (R).

Stromata (ascomata) sclerotioid, subglobose to ovoid or somewhat elongate, 350–1000 × 250–850 μm, fawn, nonostiolate, covered by dense aerial hyphae, maturing gradually from the center outward after 3–4 weeks; stromatal peridium 30–80 μm thick, composed of an outer layer of yellowish-brown textura angularis with thick-walled cells measuring 10–32 × 7.5–15 μm, and an inner layer of hyaline, thin-walled, 20–30 × 12.5–22.5 μm, angular cells. Asci 8-spored, globose to subglobose, 10–12 μm in diameter, evanescent at maturity. Ascospores hyaline, lenticular, 6–7 μm long (including crests), 4–4.5 × 3.5–4 μm, with two equatorial, thin, ruffled crests, about 1 μm wide; convex surface of ascospores ornamented with raised flaps of tissue, in shape resembling triangular projections or long ridge lines, and microtubercles.

Conidial heads small, 100–150 μm in diameter, radiate to loosely columnar. Conidiophores arising from foot cells in the basal mycelium, (50–) 100–400 × 5–12 μm, hyaline, erect or sinuous, smooth-walled, septate; vesicles flask-shaped, 10–26 μm in diameter. Aspergilla uniseriate; phialides 7.5–12.5 × 4–5 μm, fertile over the upper half to two-thirds of the vesicle. Conidia pale greenish-brown, mostly globose to subglobose, 4.5–7 μm in diameter, spinulose.

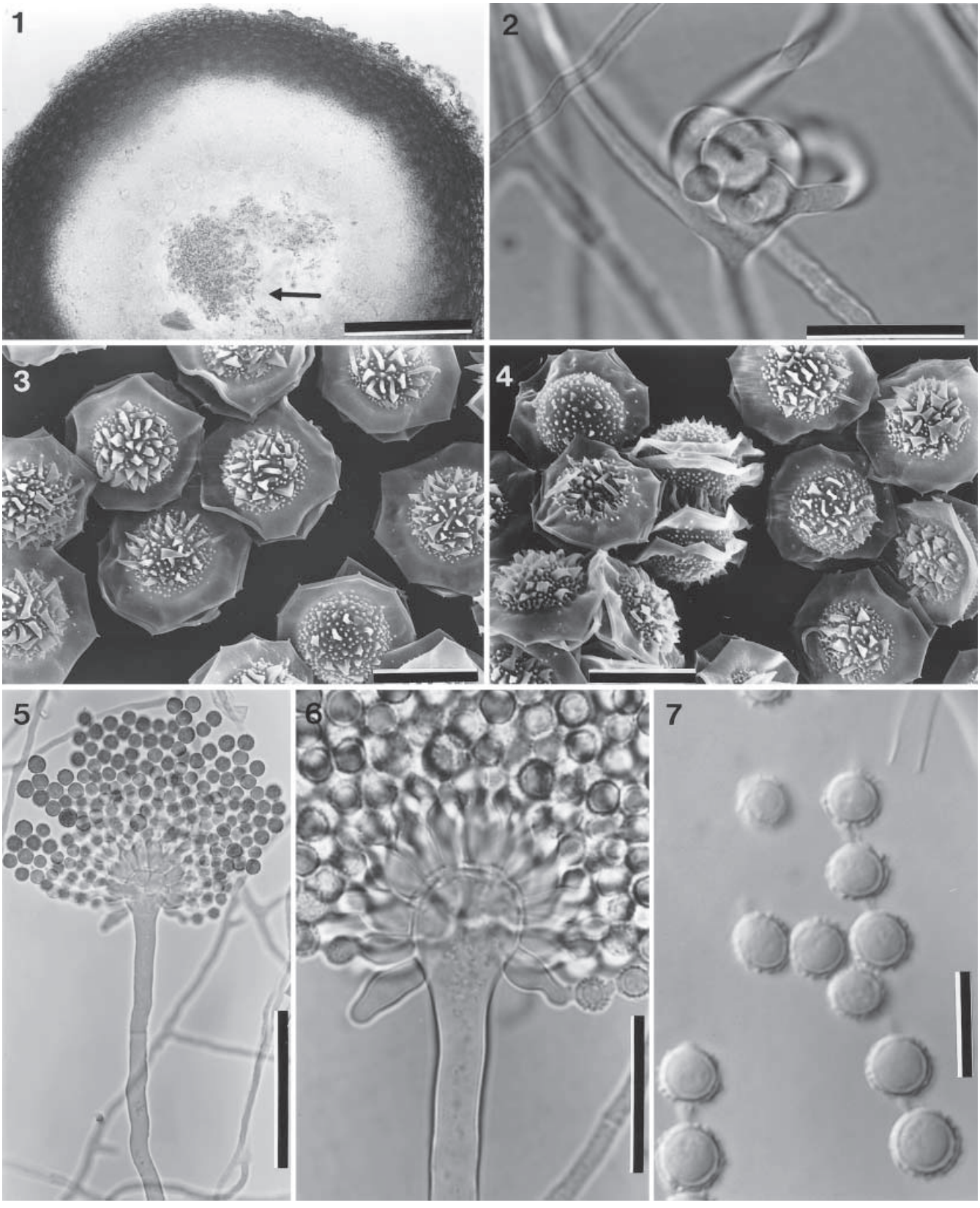
At 37°C, growth is nil.

Major ubiquinone: Q-10.

Distribution: Bougainville Island, Papua New Guinea; Japan.

Specimens examined: NHL 22462, soil, Bougainville Island, Papua New Guinea, 6–8 January 1970, collected by S. Udagawa (holotype); NHL 2656, 2657, 2658, soil, Bougainville Island, Papua New Guinea, 6–8 January 1970, collected by S. Udagawa; SUM 3179, forest soil, Iriomote Island, Taketomi-cho, Okinawa Prefecture, Japan, 11 July 1992, collected by S. Uchiyama.

Note. Tamura et al. (2000) presented an analysis of rDNA sequence data indicating that *Hemicarpenteles paradoxus* is more closely related to *Eupenicillium crustaceum* F. Ludw. and *Penicillium chrysogenum* Thom than to *Aspergillus*-producing teleomorph genera such as *Eurotium* spp., *Fennellia flavipes* B.J. Wiley et E.G. Simmons, and *Petromyces alliaceus* D. Malloch et Cain. On the contrary, their result shows that, with high bootstrap support in neighbor-joining analysis, *H. acanthosporus*



Figs. 1–7. *Neocarpenteles acanthosporum.* **1** Vertical section of stroma (a part), showing ascus development from the center (*arrow*). **2** Stromatal initial. **3,4** Ascospores (Scanning electron photomicrograph). **5,6** Aspergilla. **7** Conidia. *Bars* **1** 100 μm; **2,6** 20 μm; **3,4** 5 μm; **5** 50 μm; **7** 10 μm

(=*N. acanthosporum*) and *Aspergillus clavatus* Desm. are clustered together. The same conclusion was also evidenced in the large subunit rDNA sequence data of Peterson (2000). The *Aspergillus clavatus* group (=subgenus *Clavati*, section *Clavati* W. Gams et al.), a small distinctive group defined by Raper and Fennell (1965) in "the genus *Aspergillus*," is readily recognized by its moderately spread colony, large clavate to splitting bluish gray-green heads, hyaline thick-walled long conidiophores with a clavate vesicle, uniseriate aspergilla, and elliptical gray-green conidia. Unfortunately, there is no species with teleomorphs in *Clavati*, although only one species, *Aspergillus ingratus* Yaguchi et al., produces saffron-colored sclerotia in dark-incubated cultures (Yaguchi et al. 1993). In spite of the sequence similarities noted above, however, we find it difficult to include *A. acanthosporus* (the anamorph of *N. acanthosporum*) in *Clavati* because of its small dull green radiate conidial heads, short conidiophores with a small flask-shaped vesicle, and large globose conidia.

Furthermore, Tamura et al. (2000) suggested that their maximum likelihood tree based on 1614 aligned sites of the 18s rDNA sequence shows a close relationship between *N. acanthosporum* and the *Aspergillus*-producing teleomorph taxon *Neosartorya fischeri* (Wehmer) D. Malloch et Cain (section *Fumigati*). Such a relationship is further suggested by the fact that both taxa have hyaline oblate (bivalved) ascospores with distinct equatorial crests and variously sculptured convex surfaces. The triangular projections from the ascospore wall of *Neocarpenteles acanthosporum* closely resemble the ascospore ornamentation of another *Neosartorya* species, *N. pseudofischeri* S.W. Peterson (Peterson 1992). *Neosartorya* species are characterized by nonstromatic ascomata with a thin pseudoparenchymatous peridium and rapidly matured asci on ascogenous hyphae. Thus, there is no morphological evidence supporting this relationship.

We concluded that *Neocarpenteles acanthosporum* should be excluded from section *Ornati*, where it traditionally has been considered, but further work is required to find a reliable placement of the anamorph of this unique fungus as a monophyletic group within the genus *Aspergillus*.

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