# Pyrenomycetes of the Russian Far East 4: family *Nitschkiaceae* (Coronophorales, Ascomycota)

Larissa Vasilyeva

Institute of Biology and Soil Sciences, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia

Aleksey Chernyshev

A.V. Zhirmunsky Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia

Steven L. Stephenson<sup>1</sup>

Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas

Abstract: Ascomata of 10 species assigned to family Nitschkiaceae were examined with a scanning electron microscope to reveal their distinctive features. These observations, along with biogeographical considerations of the 10 species, form the basis for a revised interpretation of the concepts used for those nitschkiaceous taxa in the Russian Far East. Four genera instead of five are recognized as a result of the unification of Nitschkia and Calyculosphaeria, whereas Calyculosphaeria grevillei is replaced by Nitschkia grevillei. The name Fracchiaea subcongregata is now applied to the entity formerly recognized as Fracchiaea broomeiana, and the species recorded originally as Nitschkia cupularis is now identified as Nitschkia parasitans. Two new species, Fracchiaea seticoronata and Nitschkia modesta, are described and discussed.

Key words: Fracchiaea, Loranitschkia, Nitschkia, nitschkiaceous fungi, Tympanopsis

#### INTRODUCTION

This paper continues a series of publications devoted to additions and corrections of the species of pyrenomycetous fungi reported from the Russian Far East (Vasilyeva 2001, 2007; Vasilyeva and Stadler 2008). It is based on the results obtained from a detailed study of all specimens currently available in the Herbarium of the Institute of Biology and Soil Science (Vladivostok) for a small group of pyrenomycetous fungi belonging to family *Nitschkiaceae*.

Members of the *Nitschkiaceae* (*Coronophorales*, *Sordariomycetes*) usually inhabit dead branches of deciduous trees but also sometimes parasitize the stromata of other pyrenomycetous fungi. The nitschkiaceous fungi produce ascomata that often become collapsed to cupulate on a typically hyphoid subiculum or stroma. Such ascomata usually lack the true (perforated) ostiolum and are believed to become ruptured as a result of the presence of a quellkörper that consists of a mucilaginous mass of thick-walled cells. This mass hangs from the apex of the locule of an ascoma and enlarges before the latter ruptures. The quellkörper is thought to be characteristic of all nitschkiaceous fungi (Kirk et al 2008), but in some members of the group it is replaced by a low cushion that is interpreted as being either an incipient or reduced quellkörper (Nannfledt 1975).

In one of the recent taxonomic treatments of the nitschkiaceous fungi, those species with cushions were left in the nitschkeaceous complex, whereas three species possessing a well developed quellkörper were segregated into family Scortechiniaceae (Huhndorf et al 2004). Scortechinia culcitella (Berk. et Ravenel) Speg., the type species of the type genus in the Scortechiniaceae, differs from other nitschkiaceous fungi in having a thick and peculiar subiculum that consists of dichotomously branching hyphae (cf. von Arx and Müller 1954, FIG. 116) as well as in the soft consistency of the ascomata. These differences would seem sufficiently appreciable to consider S. culcitella as representing a separate family, but the small assemblage of nitschkiaceous fungi in the Russian Far East, including Neofracchiaea callista (Berk. et M.A. Curtis) Teng, which is placed in the Scortechiniaceae (Huhndorf et al 2004), does seem to comprise a group of closely related species that warrant inclusion in the same family. The degree of quellkörper development-even the absence of that structure-might resemble the variation observed in the same genera with respect to the presence or absence of a subiculum and the extent to which it develops. In this context Nitschkia parasitans (Schwein.) Nannf. var. mijuskovicii Vujanovic, which possesses a good quellkörper in its ascomata (Vujanovic 2002), could be mentioned, whereas the typical variety of N. parasitans is characterized only by a cushion (Nannfeldt 1975).

Family *Nitschkiaceae* is represented in the Russian Far East by a small number of species that were distributed among five genera in the previous taxonomic treatment (Vasilyeva 1998). In this paper some of the taxonomic concepts are reconsidered and some specimens are now reassigned to other taxa.

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The new information presented herein includes micrographs of ascomata obtained with a scanning electron microscope; such micrographs are provided for nitschkiaceous fungi for the first time. These micrographs reveal details of the surface of ascomata that are different for particular species and can serve as additional characteristics for their delineation.

#### MATERIALS AND METHODS

Most of the specimens considered in this study are deposited in the Herbarium (VLA) of the Institute of Biology and Soil Science of the Russian Academy of Sciences. A Zeiss EVO 40 scanning electron microscope was used to examine surface details of the ascomata. Ascomata were washed several times in distilled water, placed in 96% ethanol for a few seconds, mounted on stubs and sputter-coated with platinum. The micrographs of asci and ascospores were taken with a Leica DM 4500B microscope and Leica DFC300FX video camera. Because most specimens were not fresh some problems were encountered with respect to obtaining asci and ascospores of all species in good condition.

#### TAXONOMY

# KEY TO GENERA OF THE *NITSCHKIACEAE* FOUND IN THE RUSSIAN FAR EAST

- I. Asci 8-spored, ascospores of different shapes.

  - - B. Ascospores clavate, 1-septate, each with a long appendage ..... Loranitschkia
- II. Asci polysporous, ascospores allantoid . . . . Fracchiaea

Fracchiaea Sacc., Atti Soc. Ven. Trent. Sci. Nat., 2:163, 1873.

Neotrotteria Sacc., Bull. Orto Bot. Univ. Napol., 6:45, 1921. Petelotia Pat., Bull. Soc. Mycol. France, 40:35, 1924. Neofracchiaea Teng, Sinensia, 9:255, 1938.

Genera *Neotrotteria* and *Neofracchiaea* currently are recognized as separate from *Fracchiaea* (www.mykoweb.com), but all three are characterized by polysporous asci and small allantoid ascospores. Both the type species of *Neotrotteria* and that of *Neofracchiaea* have ascomata seated on a hyphoid subiculum in contrast to the members of *Fracchiaea*, which have cespitose ascomata on a basal stroma. However the arrangement of ascomata on a subiculum, a stroma or the bark surface on which the fungus occurs is of importance at the species level within the closely related genus *Nitschkia*. The logic of taxonomic delineation requires that a consistent approach be applied toward the same differences within a family, and this entails the unification of *Neotrotteria*, *Neofracchiaea* and *Fracchiaea* under the latter name.

Neofracchiaea callista was placed in genus Fracchiaea in early monographs (Saccardo 1882, Berlese 1900), but Neotrotteria pulchella Sacc. did not receive a proper comparison with the members of that genus and even escaped the attention of the mycologists (cf. Berlese 1900, Fitzpatrick 1924). Nannfeldt (1975) placed N. pulchella in genus Acanthonitschkea, which was recognized as possessing strong bristles on the ascomata. In contrast Fracchiaea and Neofracchiaea callista appeared to be members of genus Nitschkia, which seem to lack bristles. However the ascomata of Fracchiaea heterogenea Sacc. (the type species) are described as prominently spiny (Fitzpatrick 1924) and Neotrotteria pulchella does not differ from other members of genus Fracchiaea in this respect.

- Fracchiaea callista (Berk. et M.A. Curtis) Sacc., Syll. Fung., 1:94, 1882. FIGS. 1A–D, 3A–D, 7A, 8A Cucurbitaria callista Berk. et M.A. Curtis in Berk., Grevillea, 4:47, 1875.
  - Cryptosphaerella callista (Berk. et M.A. Curtis) Fitzp., Mycologia, 16:108, 1924.
  - Neofracchiaea callista (Berk. et M.A. Curtis) Teng, Sinensia, 9:255, 1938.
  - *Nitschkia callista* (Berk. et M.A. Curtis) Nannf., Svensk Bot. Tidskr., 69:309, 1975.

Ascomata superficial on a hyphoid subiculum, early collapsing to become cupulate, nonostiolate, with elongated and crest-like tubercles 10–20  $\mu$ m long at the surface covered in the early stages with thin hyaline hairs up to 50  $\mu$ m long, becoming almost glabrous with age when the hairs break off. Walls 25–30  $\mu$ m thick, consisting of 4–5 layers of rounded, ovoid or angular cells, 6–9  $\mu$ m diam. Asci clavate, polysporous, aparaphysate, p. sp. 45–65 × 8–12  $\mu$ m, stalks up to 35–40  $\mu$ m. Ascospores allantoid, hyaline, 8–10 × 1.5–2  $\mu$ m long.

Specimens examined. RUSSIA. Primorsky Territory, Kedrovaya Pad Biosphere Reserve, on dead twigs of Carpinus cordata Blume, 27 Aug 1956, Koval, E.Z. (VLA P-295); District Shkotovo, Anisimovka vicinity, on dead branches of Carpinus cordata, 1 Sep 1993, Vasilyeva, L.N. (VLA P-296); Vladivostok vicinity, on dead branches of Carpinus cordata, 5 Oct 2006, Vasilyeva, L.N. (VLA P-1968). KOREA. Jeonnam Province, Hongo Island, on dead branches of Carpinus sp., 30 Aug 2006, Lee, C.-J. (NIAST). USA. Tennessee, Great Smoky Mountains National Park, Twin Creeks Trail, on the bark of dead branches of Carpinus caroliniana Walter, 14 Sep 2005, Vasilyeva, L.N. (VLA P-1697).

*Commentary.* This species occurs exclusively on dead branches of *Carpinus* (on *C. cordata* in eastern Russia), and this is the same for *Fracchiaea callista* (on



FIG. 1. Ascomata (SEM). A–D. *Fracchiaea callista*. A–C. Almost glabrous old ascomata covered by scanty soft hairs. D. Young hairy ascomata. E, F. *Fracchiaea subcongregata*. Bars: A, C, D = 200  $\mu$ m, B, E, F = 100  $\mu$ m.

*C. caroliniana*) in North America. Nannfeldt (1975), indicating that the latter (as *Nitschkia callista*) seemed to be confined to eastern North America (CANADA: Ontario; USA: Alabama, Connecticut, Maryland, Pennsylvania, South Carolina, Virginia) and that a record from China (Teng 1936) was not convincing. He suggested that the Chinese collection might belong to an undescribed species, but the occurrence of *Fracchiaea callista* in eastern Asia is now confirmed. It also has been found on the Korean Peninsula (the *Carpinus* sp. specimen is kept in the National Institute of Agricultural Science and Technology, Suwon). As



FIG. 2. Ascomata (SEM). A, B. Fracchiaea seticoronata. C–E. Loranitschkia viticola. C. Young ascomata. D. Collapsed ascomata. E. Top of an ascoma. Bars: A, C = 200  $\mu$ m, B, D, F = 100  $\mu$ m, E = 50  $\mu$ m.

such *Fracchiaea callista* displays a so-called Grayan disjunction in its distribution (Petersen and Hughes 2007), as also seems to be the case for *Fracchiaea subcongregata* (as described below).

*Fracchiaea callista* lack setae on the ascomata, said Nannfeldt (1975), but the micrographs taken with the use of a scanning electron microscope clearly show the presence of fine, soft hairs (FIG. 1B). Moreover Berlese (1900, Tab. XXXVI) illustrated in the same figure that contained *Fracchiaea callista* the hairy, young ascomata (as is the case for the specimen VLA P-296 from eastern Russia; FIG. 1D) and the almost glabrous old ascomata

with only a few hairs (as is the case in specimens P-1968 from eastern Russia and P-1697 from eastern USA).

**Fracchiaea seticoronata** Lar.N. Vassiljeva, S.L. Stephenson et A.V. Chernyshev, sp. nov.

FIGS. 2A, B; 3J–O; 7B; 8B, C

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*Etymology.* seticoronata = crowned with setae, referring to the bristles on ascomata.

Ascomata 600–700  $\mu$ m diam, in subiculo late effuso superficialia, valde cupulato collapsa, non-ostiolata, nigra,



FIG. 3. Details of ascomata surface (SEM). A–D. *Fracchiaea callista*. A. Hyphae of the subiculum. B, C. Hairs on young ascomata. D. Broken hair on an older ascoma. E–I. *Fracchiaea subcongregata*. E. Conical bristles and spiny tubercles at the surface of an ascoma. F. Separate conical bristles. G. Spiny tubercles. H, I. Truncated papillae on small and wart-like tubercles. J–O. *Fracchiaea seticoronata*. J. Hairs on the lower surface of an ascoma. K. Polygonal cracks at the surface of an ascoma. L–N. Bristle with an impression at the top. O. Pores in the lateral wall of an ascoma. Bars: A, J, K = 50 µm, B, D–F, L = 20 µm, C, M, N = 2 µm, G, O = 10 µm, H = 5 µm, I = 1 µm.



FIG. 4. Ascomata (SEM). A, B. Nitschkia floridana. C, D. Nitschkia grevillei. E, F. Nitschkia macrospora. Bars: A, C = 200  $\mu$ m, B, F = 100  $\mu$ m, D = 50  $\mu$ m, E = 300  $\mu$ m.



FIG. 5. Ascomata (SEM). A, B. Nitschkia parasitans. C, D. N. modesta. E, F. Tympanopsis confertula. Bars:  $A = 100 \ \mu m$ , B, D,  $F = 50 \ \mu m$ , C,  $E = 200 \ \mu m$ .



FIG. 6. Details of ascomata surface (SEM). A. Loranitschkia viticola. B. Nitschkia floridana. C. Nitschkia grevillei. D. Nitschkia macrospora. E, F. Nitschkia parasitans. G, H. Nitschkia modesta. I. Tympanopsis confertula. A, C, E–G, I. Surface of lateral walls. B, D, H. Surface of apical walls. Bars: A–C, E, H = 20  $\mu$ m, D, G, I = 50  $\mu$ m, F = 2  $\mu$ m.

facies in polygona fisso, setosa, setae rigidae ad 200 µm longa. Paries ascomatis sectione longitudinali 20–30 µm crassus, 4–5 stratis e cellulis polygonalis 3–7 µm diam compositus. Asci clavati, longe stipitati, polyspori, aparaphysati, p. sp. 60–70 × 16–21 µm, stipitis ad 100 µm longis. Ascosporae allantoideae, hyalinae, interdum pseudoseptatae,  $6-8 \times 1$  µm.

Ascomata 600–700  $\mu$ m diam, superficial on a hyphoid subiculum, strongly collapsing to become cupulate, nonostiolate, black, surface with low tubercles, numerous holes and polygonal cracks, outlining tiles 50–80  $\mu$ m, covered by thick bristles up to 200  $\mu$ m long and slightly pressed inward at the apices. Walls 20–30  $\mu$ m thick, consisting of 4–5 layers of polygonal cells, 3–7  $\mu$ m diam, becoming smaller (< 1.5–2  $\mu$ m diam) toward outside. Asci clavate, polysporous, aparaphysate, p. sp.  $60-70 \times 16-21 \mu m$ , with stalks up to 100  $\mu m$  long. Ascospores allantoid, hyaline, sometimes pseudoseptate,  $6-8 \times 1 \mu m$ .

Specimens examined. RUSSIA. Primorsky Territory, Vladivostok vicinity, on the bark of dead branches of *Carpinus cordata*, 11 Oct 1991, *Vasilyeva, L.N.* (VLA P-158, HOLO-TYPE).

*Commentary.* The specimen of this new species was identified originally as *Neotrotteria pulchella* Sacc. and placed in genus *Fracchiaea* (Vasilyeva 1998). Nannfeldt (1975) treated *Neotrotteria pulchella* as the member of *Acanthonitschkea* because of its spiny ascomata, and this was the only species with polysporous asci in this genus. Several known localities of *Acanthonitschkea* 



FIG. 7. Walls of ascomata as viewed in cross section (SEM). A. Fracchiaea callista. B. Fracchiaea seticoronata. C, K. Fracchiaea subcongregata. C. Cell layers in the wall. K. Munk pores. D, L. Loranitschkia viticola. D. Cross section through lateral wall. L. Cross section through the base of an ascoma. E. Nitschkia floridana. F, J, M. Nitschkia macrospora. F. Cross section through lateral wall. J. Munk pores. M. Cross section through the base of an ascoma. G. Nitschkia parasitans. H. Nitschkia grevillei. I. Tympanopsis confertula. N. Nitschkia modesta. Bars: A–C, F–I, M, N = 20 μm, D, E, J, L = 10 μm, K = 5 μm.

*pulchella* (Sacc.) Nannf. are distributed along the Pacific Coast of Asia. Saccardo (1921) described *Neotrotteria pulchella* from the Singapore region, whereas the entity under the synonymous name *Petelotia tonkinensis* Pat. was found in northern Vietnam near Tonkin Bay (Patouillard 1924). The record from Taiwan (Hsieh et al 2000) extended the range along the Pacific Coast to the north, and the specimen collected near Vladivostok would conform to this peculiar longitudinal distribution because another example of a pyrenomycetous fungus has a similar distribution. We refer to species *Spirodecospora melnikii* (Lar.N. Vassiljeva) K.D. Hyde & Melnik, described originally from Kunashir Island (Vasilyeva 1990) and later recorded from Hong Kong (cf. Melnik and Hyde 2003) and the Korean Peninsula (Melnik et al 2005).

However, despite the possible occurrence of some members of the eastern Asian population of *Acanthonitschkea pulchella* near Vladivostok, we suspect the existence of a species complex under that name, especially if the records from other parts of the world, such as Ghana, Ceylon (Nannfeldt 1975) and India (Subramanian and Sekar 1990), are taken into consideration. The ascomata in our specimen are larger than those described for *A. pulchella* by Nannfeldt (1975) and Hsieh et al (2000), and they often are arranged on a subiculum in a characteristic concentric manner (cf. Vasilyeva 1998,



FIG. 8. Asci and ascospores. A. *Fracchiaea callista*: Ascus with ascospores. B, C. *Fracchia seticoronata*. B. Ascus with ascospores. C. Ascospores (two spores in the lower right corner as viewed with a fluorescence microscope). D. *Fracchiaea subcongregata*: Ascus with ascospores. E, K, L. *Loranitschkia viticola*. E. Asci with ascospores. K. Ascospore stained with iodine, with an arrow indicating the septum separating an appendage. L. Ascospore without staining. F. *Tympanopsis confertula*: Ascus with ascospores. G–I. *Nitschkia macrospore*. G, H. Fascicles of asci and paraphyses. I. Ascospore. J. *Nitschkia modesta*: Ascospore. Bars: A, G = 50 µm, B, D, E, I = 20 µm, C, F, K, L = 10 µm, H = 100 µm, J = 5 µm.

FIG. 2). Furthermore the subiculum lacks the type of branched spines that were illustrated for *Neotrotteria pulchella* from India (Subramanian and Sekar 1990, FIG. 36). The appearance of 32–64-spored asci from the Indian specimen (Subramanian and Sekar 1990, FIG. 38) and the 64-spored asci of *Acanthonitschkea pulchella* from the Taiwanese specimen (Hsieh et al 2000, p 20), as well as the overall appearance of ascospores in illustrations of the two specimens, are

more similar to asci and ascospores of *Fracchiaea callista* in our material (FIG. 8A). However they are clearly different from those in *F. pulchella* from the Russian Far East (FIG. 8B. This photograph corresponds to the drawing of asci and ascospores in Vasilyeva [1998, FIG. 3], but unfortunately the ascospore sizes in the descriptions of *Fracchiaea callista* and *F. pulchella* were noted incorrectly, with those of the latter indicated as the larger of the two

species when they were actually smaller.) All these differences let us describe our specimen as the new species *Fracchiaea seticoronata*.

Fracchiaea subcongregata (Berk. et M.A. Curtis) Ellis et Everh., North Amer. Fungi, p 244, 1892.

FIGS. 1E, F; 3E–I; 7C, K; 8D Sphaeria subcongregata Berk. et M.A. Curtis, Fungi Carol. exs., fasc. 4, N 57, 1855.

Ascomata 330–500 µm diam, gregarious on stromatic base and erumpent in clusters from the bark, turbinate, slightly collapsing to become cupulate, nonostiolate, covered with a diverse ornamentation: conical bristles up to 15–25 µm long (FIG. 3F), wart-like and spiny tubercles up to 10–16 µm long with triangular spines (FIG. 3G), simple tubercles, 8–11 µm diam, and small wart-like tubercles, 5–14 µm diam, studded with truncated papillae, 03–0.4 µm long (FIG. 3H, I). Walls 65– 75 µm thick, consisting of 7–8 layers of rounded or elongate-ovoid cells 10–20 × 7.5–12.5 µm diam. Asci clavate, polysporous, aparaphysate, nonstipitate, 120– 140 × 20–22 µm. Ascospores allantoid, hyaline, sometimes pseudoseptate, 6–8 × 1.3–1.6 µm.

Specimen examined. RUSSIA. Khabarovsk Territory, Big Khekhtsir Nature Reserve, on dead branches of *Acer mono* Maxim., 27 Aug 1983, *Vasilyeva L.N.* (VLA P-1397).

*Commentary.* This species was identified originally as *Fracchiaea broomeiana* (Berk.) Petch (Vasilyeva 1998), whereas the name *F. subcongregata* was considered to be a synonym in accordance with Nannfeldt (1975). At present we have doubts about the presence of *F. broomeiana* in the Russian Far East because this species was described from Ceylon (now Sri Lanka) and no biogeographical relationships are evident in the pyrenomycete mycobiotas of eastern Russia and that part of southern Asia. The patterns of distribution associated with the so-called Pacific baseline and Indian Ocean baseline (Cox and Moore 2000) might be taken into consideration when trying to explain the different species composition in these regions.

In contrast *Fracchiaea subcongregata* was described and known from eastern USA (Ellis and Everhart 1892) and many other species from that region later were found to occur in eastern Russia or eastern China. Moreover Nannfeldt (1975) indicated that *Nitschkia broomeiana* (Berk.) Nannf. has spores 8– 11  $\mu$ m long, whereas spores only 6–7  $\mu$ m long were observed in the Russian specimen (Vasilyeva 1998). Ellis and Everhart (1892) also emphasized that spores in American specimens of *Fracchiaea subcongregata* were mostly 6–8  $\mu$ m long.

Fitzpatrick (1924) considered the name S. subcongregata a synonym of Fracchiaea heterogenea Sacc. described from Europe. However the original diagnosis of the latter species indicated that it was characterized by ascospores  $12 \times 2 \,\mu\text{m}$  (Saccardo 1873), which means that there is no overlap in length with those of *F. subcongregata*. Berlese's (1900) measurements of ascospores of *F. heterogenea* from the type specimen were 9–11 × 2.5–3 µm. The specimen of *Nitschkia broomeiana* from Taiwan, with ascospores 6–9 µm (Hsieh et al 2000, www.bcrc.firdi.org.tw), could be the same species as the one in the Russian Far East.

Loranitschkia Lar.N. Vassiljeva, Mikologiya i Fitopatiologiya, 24:207, 1990

The name of this genus refers first to genus *Loramyces* W. Weston because of the shape of ascospores and then to genus *Nitschkia* because of the nitschkiaceous type of ascomata, which lack the typical ostiole and collapse to become cupulate. In contrast to other species in family *Nitschkiaceae* considered herein the ascomata of the type species of this genus are more pear-shaped than globose before collapsing, and the walls of ascomata are characteristically porous.

Loranitschkia viticola Lar.N. Vassiljeva, Mikologiya i Fitopatologiya, 24:207, 1990.

FIGS. 2C–E; 6A; 7D, L; 8E, K, L Ascomata 260–320  $\mu$ m diam, superficial on scanty subiculum or the twig periderm of host plants, gregarious or scattered, pear-shaped, collapsing to become cupulate, nonostiolate, black, without hairs or bristles. Walls with a characteristic porous texture, 20–25  $\mu$ m thick. Asci arranged on the branching ascogenous system, clavate, aparaphysate, on short stalks, 90–110 × 20–24  $\mu$ m. Ascospores biseriate, hyaline, clavate, with a septum below the middle, 25–33 × 10–12  $\mu$ m, rounded at the apex, attenuated below in a tail-like appendage, 24–26  $\mu$ m long, that also is separated by a septum and directed toward the base of the ascus.

Specimens examined. RUSSIA. Kuril Islands, Kunashir Island, on dead twigs of Vitis cognetiae Pulliat ex Planch., 23 Aug 1987, Vasilyeva L.N. (VLA P-1123, HOLOTYPE); Primorsky Territory, Ussuriysk Nature Reserve, on dead twigs of Vitis amurensis Rupr., 27 Aug 1989, Vasilyeva L.N. (VLA P-235); District Shkotovo, Anisimovka vicinity, on dead twigs of Vitis amurensis, 2 Sep 1993, Vasilyeva L.N. (VLA P-2317); Jewish Autonomous Region, Bastak Nature Reserve, on dead twigs of Vitis amurensis, 19 Aug 2004, Vasilyeva L.N. (VLA P-334). CHINA. Jilin Province, Changbai Mountain: Dayangcha, on dead twigs of Vitis amurensis, 4 Aug 2008, Vasilyeva L.N. (VLA P-2172).

Nitschkia G.H. Otth in Fuckel, Jahrb. Nass. Ver. Naturk., 23–24:165, 1869 (1870). *Coelosphaeria* Sacc., Atti Soc. Ven. Trent. Sci. Nat., 2:163, 1873.

## Calyculosphaeria Fitzp., Mycologia, 15:45, 1923.

Genus Calyculosphaeria was accepted (Vasilyeva 1998) in accordance with Fitzpatrick's (1923) treatment. This genus was segregated from Nitschkia on the basis of the presence of a septum in the ascospores. However Nannfeldt (1975) and Subramanian and Sekar (1990) indicated that a distinct septum also occurs in ascospores of typical Nitschkia, although they sometimes refer to this structure as a pseudoseptum (Teng 1934). The term pseudoseptum has been applied to a protoplasmic or vacuolar membrane looking like a septum (Kirk et al 2008) but differing from a true septum in that it disappears after treatment by substances such as KOH and Melzer's reagent. Young ascospores of Nitschkia can lack a septum, but the latter is evident in mature ascospores of those species that are included herein.

# Nitschkia floridana Fitzp., Mycologia, 15:31, 1923.

FIGS. 4A, B; 6B; 7E

Ascomata 400–500  $\mu$ m diam, superficial on the substrate, scattered or gregarious, rounded, collapsing to become cupulate, nonostiolate, black, without hairs or bristles, the surface roughened by aggregations of small and numerous rounded tubercles 2– 5  $\mu$ m diam. Walls 15–20  $\mu$ m thick, consisting of 4–5 layers of rounded or ellipsoid cells 6–10 × 3.5  $\mu$ m becoming smaller (< 1.5–2.5  $\mu$ m diam) toward outside. Asci clavate, sp. p. 50–60 × 8–9  $\mu$ m, stalks up to 25  $\mu$ m. Ascospores biseriate, hyaline, fusiform, slightly curved, with a septum in the middle, 18–20 × 3.5–4  $\mu$ m.

Specimens examined. RUSSIA. Primorsky Territory, Kedrovaya Pad Biosphere Reserve, on wood, 26 Oct 1987, Vasilyeva L.N. (VLA P-356); Ussuriysk Nature Reserve, 21 Aug 1989, Vasilyeva L.N. (VLA P-357). KOREA. Gangwon Province, Chiaksan National Park, Mount Chiak, on wood, 22 Sep 2006, Vasilyeva L.N. (NIAST).

Commentary. This species was described from eastern USA (Fitzpatrick 1923) and later was found in China (Teng 1934). Such distribution, together with records from eastern Russian and Korea, would fit the Grayan disjunction, but the species also has been reported from southern India (Subramanian and Sekar 1990) and French Guiana (Courtecuisse et al 1996). Nannfeldt (1975) indicated that Nitschkia floridana occurs in Brazil, whereas the specimen in question was identified by the collector as Bertia submorifornis (Plowr.) Sacc. The ascospores of the latter species are larger and somewhat more narrow (Saccardo 1882: 20–23  $\times$  3 µm) than is the case in Nitschkia floridana. If Nannfeldt's (1975) observations that some species of Nitschkia thought to be widely distributed actually have a more limited distribution

are taken into consideration, it might be anticipated that different species would occur in southern India, French Guiana and Brazil.

# Nitschkia grevillei (Rehm) Nannf., Svensk Bot. Tidskr., 69:53, 1975. FIGS. 4C, D; 6C; 7H

- Melanopsamma grevillei Rehm in Starbäck, Bih. K. Svensk. Vet. Akad. Handl., 16, 3(3):5, 1890.
  - Calyculosphaeria grevillei (Rehm) Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloaskomitsety (Sankt-Petersburg): 17, 1998.
  - Winteria tuberculifera Ellis et Everh., Proc. Acad, Nat. Sci. Phil., 1890:240, 1891.
  - *Winterella tuberculifera* (Ellis et Everh.) Berl., Icon. Fung., 1:94, 1894.
  - Winterina tuberculifera (Ellis et Everh.) Sacc., Syll. Fung., 14:589, 1899.

Ascomata 300–400  $\mu$ m diam, gregarious on a thick subiculum within cracks in the bark, rounded, collapsing to become cupulate, nonostiolate, black, without hairs or bristles, surface consisting of densely arranged and characteristically elongated tubercles, 10–25 × 7–15  $\mu$ m. Walls 30–35  $\mu$ m, consisting of 4–5 layers of rounded or slightly angular cells 6–16 × 5– 9  $\mu$ m. Asci clavate, p. sp. 25–35 × 5–6  $\mu$ m, with stalks 10–16  $\mu$ m long. Ascospores biseriate, hyaline, fusiform, with a septum in the middle, 6–8 × 1.5–2  $\mu$ m.

Specimen examined. RUSSIA. Amur Region, Khingansky Nature Reserve, in dead branches of *Padus maackii* (Rupr.) Kom., 31 Aug 1992, *Vasilyeva L.N.* (VLA P-300).

*Commentary.* This species was reported previously to occur on several different kinds of deciduous trees in Primorsky Territory and the Amur Region (Vasilyeva 1998), but only a single specimen from Amur that was collected on *Padus maackii* (= *Prunus maackii* Rupr.) is now assigned to *Nitschkia grevillei*. It should be emphasized that the types of both *Melanopsamma grevillei* (Europe) and *Winteria tuberculifera* (eastern North America), whose names were synonymized by Nannfeldt (1975), were collected from *Prunus* spp. (cf. Saccardo 1891).

Because some species in the *Nitschkiaceae* appear to be restricted to a certain kind of host plant (e.g. *Fracchiaea callista* on *Carpinus* spp., *Nitschkia parasitans* on *Nectria cinnabarina*, *Tympanopsis euomphala* on *Fraxinus* spp.) it is possible that *Nitschkia grevillei* prefers *Prunus* spp. As such the specimens from other genera of host trees should be checked. In the case of collections from the Russian Far East that were identified previously as *Nitschkia grevillei*, all are characterized by the small uniseptate ascospores but clearly differ in also having larger ascomata with a different type of surface. These specimens are identified as *N. modesta* (see below).

## Nitschkia macrospora Teng, Sinensia, 4:368, 1934. FIGS. 4E, F; 6D; 7F, J, M; 8G–I

Ascomata 400–550  $\mu$ m diam, superficial on substrate, scattered or gregarious, rounded, collapsing to become cupulate, nonostiolate, black, without hairs or bristles, with strongly tuberculate and cracked surface. Walls 50–55  $\mu$ m thick, consisting of 7–8 layers of rounded-angular or irregularly elongated cells 10–14 × 8–10  $\mu$ m. Asci paraphysate, clavate, p. sp. 90–110 × 12–14  $\mu$ m, with stalks up to 70  $\mu$ m long. Ascospores biseriate or crowded, hyaline, fusiform, slightly curved, with a septum in the middle, 30–45 × 6–7  $\mu$ m.

Specimens examined. RUSSIA. Primorsky Territory, Kedrovaya Pad Biosphere Reserve, on dead branches of *Philadelphus tenuifolius* Rupr. et Maxim., 17 Oct 1987, *Vasilyeva L.N.* (VLA P-2318); Vladivostok vicinity, on the bark of a deciduous tree, 10 Sep 1996, *Vasilyeva L.N.* (VLA P-2319).

*Commentary.* These specimens are unusual in having paraphysis-like elements (FIG. 8G, H), which are not observed in other nitschkiaceous fungi found in the Russian Far East. However the occurrence of coarsely tuberculate and cupulate ascomata without typical ostioles does not allow us to remove this species from the *Nitschkiaceae.* In addition paraphyses were described for *Nitschkia phaeospora* W.H. Hsieh et al (1998), and the diversity of structural elements associated with asci in the *Coronophorales* might appear to be much greater than is currently known. In this context *Bertia tropicalis* Huhndorf, A.N. Mill. et F.A. Fern. (*Bertiaceae, Coronophorales*), described as possessing paraphysoids (Huhndorf et al 2004), could be mentioned.

Nitschkia modesta Lar.N. Vassiljeva, A.V. Chernyshev et S.L. Stephenson, sp. nov.

FIGS. 5C, D; 6G, H; 7N; 8J

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*Etymology*. modesta = modesty, referring to the lack of bright features.

Ascomata 300–400 µm diam, superficialia in substrato, sparsa vel aggregata, globosa, cupulato collapsa, nonostiolata, nigra, glabra sed tuberculis 9–17 µm diam papillae minutae 0.5–1.5 µm conspersis exasperata. Paries ascomatis sectione longitudinali 30–35 µm crassus, 3–4 stratis e cellulis polygonalis  $3-7 \times 2.5-5$  µm compositus. Asci clavati, stipitati, octospori, aparaphysati, p. sp.  $25-35 \times 5-6$  µm, stipitis ad 16 µm longis. Ascosporae biseriatae, hyalinae, fusoideae, 1-septatae,  $6-8 \times 2-2.5$  µm.

Ascomata 300–400  $\mu$ m diam, superficial on the substrate, scattered or gregarious, rounded, collapsing to become cupulate, nonostiolate, black, without hairs or bristles, surface of rounded or irregular tubercles 9–17  $\mu$ m diam; tubercles studded by small papillae 0.5–1.5  $\mu$ m. Walls 30–35  $\mu$ m thick, consisting of 3–4 layers of rounded or elongated cells,  $3-7 \times 2.5-5 \,\mu\text{m}$ , becoming smaller toward outside. Asci aparaphysate, clavate, p. sp.  $25-35 \times 5-6 \,\mu\text{m}$ , with stalks 10–16  $\mu\text{m}$  long. Ascospores biseriate, hyaline, fusiform, with a septum in the middle,  $6-8 \times 2-2.5 \,\mu\text{m}$ .

Specimens examined. RUSSIA. Primorsky Territory, Kedrovaya Pad Biosphere Reserve, on the dead branch of a deciduous tree, 16 Oct 1987, Vasilyeva L. N. (VLA P-2340, HOLOTYPE).

*Commentary.* Only a few species of *Nitschkia* have ascospores as small as those found in this species. These include *Nitschkia grevillei*, *N. tristis* (Pers.) G. Winter and *N. calyculus* (Mont.) Kuntze. As was noted above *Nitschkia grevillei* differs in the presence of a thick subiculum and, as the scanning electron micrographs show, has ascomata with a peculiar surface (FIGS. 5D, 6C).

*Nitschkia tristis* was reported to be restricted to Europe and characterized by the presence of bristles on a subiculum, especially around the bases of ascomata (Nannfeldt 1975). These bristles are absent in our specimens, and the subiculum is almost absent or barely visible.

Nitschkia calyculus would not be expected to occur in the Russian Far East. The type specimen of N. calyculus was collected in tropical South America (French Guiana), and this species seems to have a distribution limited to the tropical zone that extends from the equator to 10°N. For example French Guiana is at 0–10°N, whereas the record from Ghana (Nannfeldt 1975) is from a locality at  $5-10^{\circ}$ N. The later Indian collections (Subramanaian and Sekar 1990) were found in the Tirunelveli District (Tamil Nadu State), which is within  $10^{\circ}$ N, and the Palghat (= Palakkad) District (Kerala State), which occurs at a latitude only slightly greater than 10°N. The only disharmonious record of Nitschkia calyculus is from Florida (Nannfeld 1975), and this specimen probably belongs to a different species. The name Nitschkia modesta might be suitable for the specimen in question if one takes into account the occurrence of Nitschkia floridana (described from Florida) in eastern Asia (China, Korean Peninsula and the Russian Far East).

Nitschkia parasitans (Schwein.) Nannf., Svensk Bot. Tidskr., 69:52, 1975. FIGS. 5A, B; 6E, F; 7G Sphaeria parasitans Schwein., Trans. Amer. Phil. Soc., 4:206, 1832.

Ascomata 210–240  $\mu$ m diam, superficial and gregarious on stromata of *Nectria cinnabarina*, globose or slightly collapsing, nonostiolate, black, without hairs or bristles, surface with densely gregarious, rounded tubercles 10–15 µm diam and uneven under strong magnification (FIG. 6F). Walls 10–20 µm thick, consisting of 2–3 layers of angular cells  $3.5-9 \times 7-12.5$  µm. Asci clavate, p. sp.  $30-40 \times 8-12$  µm, with short stalks up to 10–12 µm. Ascospores biseriate or crowded, hyaline, fusiform, slightly curved, with a septum in the middle,  $10-16 \times 2$  µm.

Specimens examined. RUSSIA. Khabarovsk Territory, Komsomolsk vicinity, on stromata of *Nectria cinnabarina* (Tode:Fr.) Fr. on *Malus domestica* Borkh., 25 Sep 1959, *Nelen E.S.* (VLA P-1407); Kamchatka Peninsula, Milkovo vicinity, on stromata of *Nectria cinnabarina* on *Padus avium* Mill., 20 Aug 1982, *Vasilyeva L.N.* (VLA P-1409).

*Commentary.* This species was reported earlier as *Nitschkia cupularis* (Pers.:Fr.) P. Karst. by Vasilyeva (1998) because its ascomata disguised the host stromata and only the presence of *Nectria cinnabarina* on some parts of the same twigs suggested the true state of affairs. As is the case for *Nitschkia tristis, N. cupularis* is considered a European species (Nannfeldt 1975), and all records from Asia need to be checked.

Tympanopsis confertula (Schwein.) Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloaskomitsety (Sankt-Petersburg): 22 (1998).

FIGS. 5E, F; 6I; 7I; 8F

- Sphaeria confertula Schwein., Trans. Amer. Phil. Soc., 4:211, 1832.
- Nitschkia confertula (Schwein.) Nannf., Svensk Bot. Tidskr., 69:59, 1975.
- Sphaeria euomphala Berk. et M.A. Curtis in Ravenel, Fungi Carol. exs., fasc. 4, N 54, 1855.
- Botryosphaeria euomphala (Berk. et M.A. Curtis) Sacc., Syll. Fung., 1:462, 1882.
- *Byssosphaeria euomphala* (Berk. et M.A. Curtis) Cooke, Grevillea, 15:122, 1887.
- Nitschkia euomphala (Berk. et M.A. Curtis) Ellis et Everh., North Amer. Pyren., p. 246, 1892.
- *Tympanopsis euomphala* (Berk. et M.A. Curtis) Starbäck, Bih. K. Svensk Vet. Akad. Handl., 19, 3(2):24, 1894.
- Scortechinia euomphala (Berk. et M.A. Curtis) Arx et E.Müll., Beitr. Krypt. Fl. Schweiz., 11(1):380, 1954.

Ascomata 370–500  $\mu$ m diam, scattered or gregarious, superficial on a barely visible subiculum, rounded, collapsing to become cupulate, nonostiolate, black, without hairs or bristles, with faint polygonal cracks on the rough surface. Walls 24–28  $\mu$ m thick, consisting of 4–5 layers of slightly angular cells 8–14 × 4–7  $\mu$ m. Asci clavate, p. sp. 40–50 × 10–12  $\mu$ m, with thin stalks up to 50  $\mu$ m that are visible in Melzer's reagent. Ascospores biseriate or irregularly crowded, ellipsoid or kidney-shaped, smoky, 9–12 × 3.5–4  $\mu$ m.

Specimen examined. RUSSIA. Khabarovsk Territory, Big Khekhtsir Nature Reserve, on dead branches of *Fraxinus* mandshurica Rupr. (around stromata of *Hypoxylon perfor*- atum (Schwein.: Fr.) Fr., 28 Aug 1983, Vasilyeva L.N. (VLA P-1373).

Commentary. We consider genus Tympanopsis to be valid, and because the names Sphaeria confertula Schwein. and S. euomphala Berk. et M.A. Curtis were synonymized by Nannfeldt (1975) the combination Tympanopsis confertula was necessary (Vasilyeva 1998). However, if the concept of this species appears to be too broad, as in many other cases within the Nitschkeaceae, the name Tympanopsis euomphala (Berk. et M.A. Curtis) Starbäck seems to be more appropriate for the specimen from eastern Russia because that species was collected from the same host plant (Fraxinus) as the type of T. euomphala (Ravenel's Fungi Caroliniani Exsiccati, fasc, 4, N 54). It might be relevant to indicate the possible association of its ascomata with those of Hypoxylon perforatum because some nitschkiaceous fungi display a tendency to parasitize stromata of other pyrenomycetes. Nannfeldt (1975) also indicated that this species was associated mostly with H. rubiginosum, but he almost certainly treated the latter species as also including *H. perforatum*, which would have been in accordance with Miller's monograph (1961), the best available on species of Hypoxylon at that time.

#### DISCUSSION

Scanning electron microscope studies revealed numerous features, but some of these were not included in the descriptions of species, especially those that were not highly specific to particular taxa. All species examined in this study have Munk pores (FIG. 7J, K) and characteristic pores of irregular shape in the walls of ascomata, mostly in their lateral surfaces (FIGS. 3O, 6A). These pores are sometimes numerous (especially in species of Fracchiaea), but they are lacking in the inner surface of ascomatal walls. Some other features are characteristic of only a few species. For example the ascomata of Fracchiaea seticoronata, Nitschkia macrospora and N. modesta all have cracks in the surface that delimit more or less isolated tiles of irregular shape (FIGS. 2B, 3K, 4F, 5D, 6D). These tiles are less expressed in other species or could not be distinguished at all.

All species of *Nitschkia* in this study differ in the shape of the tubercles found on the surface of the fruit body. The most diverse ornamentation was observed on the ascomata of *Fracchiaea subcongregata* (see description with the reference to figures). Sections through the walls of ascomata also showed some peculiarities that are characteristic for certain species. *Nitschkia parasitans* has ascomata with the thinnest walls (FIG. 7G), whereas those of *Fracchiaea seticor* 

onata consist of small cells (FIG. 7B). The cell walls in the ascoma wall in *Loranitschkia viticola* (FIG. 7D) are thicker than in other nitschkiaceous fungi, and this fact probably explains why many ascomata of this species do not collapse after drying. In addition the characteristic porous texture of ascoma walls also distinguishes *L. viticola* from other species. Two micrographs (7L, M) show sections through the basal walls of *L. viticola* and *Nitschkia macrospora* respectively; the differences here correspond to those shown in sections of lateral walls (FIG. 7D, F).

In summary our studies seem to indicate that there are a number of structural features other than those traditionally included in taxonomic descriptions of nitschkiaceous fungi that can be used to distinguish closely related species. Moreover it is apparent that some reports of particular taxa that appear to represent major range extensions need to be examined if we are to develop a more complete understanding of biogeographical relationships and patterns of host associations in this group of fungi.

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