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Tortulomyces thailandicus gen. et sp. nov. and Nitschkia siamensis sp. nov. (Coronophorales, Ascomycota) from northern Thailand

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ABSTRACT

Two new species collected in northern Thailand are herein described and discussed. *Tortulomyces thailandicus* is the type of a new genus characterized by a combination of such features as a dense subiculum, non-ostiolate ascomata, two kinds of paraphyses (filiform and bead-like), and 1-septate, brown ascospores. *Nitschkia siamensis* has non-ostiolate, turbinate and stalked ascomata and hyaline, often slightly curved, 1-septate ascospores.

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During an approximately 10-day mycological foray in northern Thailand, specimens representing two species of coronophoraceous fungi were collected. This group has received only limited attention in southeast Asia, and the few data are available. For example, *Bertia tropicalis* Huhndorf, A.B. Mill. & F.A. Fern. occurs primarily in Puerto Rico and Panama but is also occasionally encountered in French Guiana, Costa Rica, and Thailand (Huhndorf et al. 2004). Several species of coronophoraceous fungi, namely *Acanthonitschkea argentinensis* Speg., *A. pulchella* (Sacc.) Nannf., *B. biseptata* Sivan. & W.H. Hsieh, *B. moriformis* (Tode : Fr.) De Not., *Chaetosphaerella phaeostroma* (Durieu & Mont.) E. Müll. & C. Booth, *Nitschkia*

broomeiana (Berk.) Petch, *N. phaeospora* W.H. Hsieh, Chi Y. Chen & Sivan., *Scortechinia chaetomioides* (Penz. & Sacc.) Arx & E. Müll., and *S. conferta* (Schwein.) Subram. & Sekar., were reported from Taiwan by Hsieh et al. (2000).

In the southern provinces of China (Fujian, Hainan, Hunan and Yunnan), which are located at about the same latitudes as Taiwan, such species as *A. argentinensis*, *A. macrobarbata* Fitzp., *B. moriformis*, *Calyculosphaeria tristis* (Fuckel) Fitzp., *Fracchiacea heterogenea* Sacc., *Neofracchiacea callista* (Berk. & Curt.) Teng, *N. hainanensis* Teng & Ou, *N. floridana* Fitzp., *N. macrospora* Teng, and *Tympanopsis euomphala* (Berk. & M.A. Curtis) Starbäck have been reported (Teng 1996).

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The names given above are provided as published in the books by Teng (1996) and Hsieh et al. (2000), although some names (*N. broomeiana* and *F. heterogenea*, *S. conferta* and *T. euomphala*) sometimes have been considered to be synonymous (cf. Vasilyeva 1998). Moreover, the species composition of the assemblage of fungi from the southern provinces of China might need to be reconsidered, since the identifications were made before 1939 (Teng 1939), and information on the coronophoraceous fungi and their distribution in the world was even more limited than is currently the case. One example is *C. tristis*, which was reported to be restricted to Europe (Nannfeldt 1975) and, therefore, would hardly be expected to occur in China. In this paper, two coronophoraceous fungi found in northern Thailand are discussed and illustrated.

The specimens considered herein were collected in Chiang Mai province of northern Thailand (Mae Taeng District near the village of Pha Deng and the Doi Suthep temple in the vicinity of the city of Chiang Mai) in June 2011. These specimens were deposited in the herbarium of Mae Fah Luang University (MFLU). The treatment of material prior to examination followed that described in a previous paper (Vasilyeva et al. 2012). Micrographs of asci and ascospores were obtained with the use of a Leica DM 4500B microscope, whereas a Zeiss EVO 40 scanning electron microscope was used to examine surface details of the ascomata.

Tortulomyces Lar.N. Vassiljeva, S.L. Stephenson, A.V. Chernyshev & K.D. Hyde, gen. nov. (Family Nitschkiaceae)
MB 564276

Similar in appearance to *Spinulosphaeria*, but a quellkörper and setae on the ascomata are absent, whereas two types of paraphyses, the first inflated, bead-like and the second filiform, are present.

Type species: *Tortulomyces thailandicus* Lar.N. Vassiljeva, S.L. Stephenson, A.V. Chernyshev & K.D. Hyde.

Etymology: Refers to the similarity of the ascomata to a small pastry (= tortula, in Latin) or a dumpling in terms of their overall shape.

Ascomata partly imbedded in a dense subiculum, subglobose but pinched at the bluntly-pectinate top, non-ostiolate, quellkörper absent. Asci in fascicles, clavate, stipitate, paraphysate. **Paraphyses** of two kinds, mostly filiform, but some consisting of inflated cells. **Ascospores** oblong-ellipsoid, 1-septate, brown.

Tortulomyces thailandicus Lar.N. Vassiljeva, S.L. Stephenson, A.V. Chernyshev & K.D. Hyde, sp. nov. (Figs. 1a–e and 2 a–e)
Mycobank MB 564277

This species is characterized by subglobose ascomata which are pinched at the bluntly-pectinate top and non-ostiolate.

Holotypus: Thailand, vicinity of Chiang Mai city, near the Doi Suthep temple, on dead branches of an unidentified tree, 21 June 2011, L.N. Vasilyeva (MFLU11-1163).

Etymology: refers to the country where the fungus was collected.

Ascomata partly imbedded in a dense subiculum consisting of dark brown septate and branching hyphae about 8–10 µm

thick, subglobose but pinched at the bluntly-pectinate top, 280–320 × 260–280 µm, non-ostiolate, bluish; surface moderately tuberculate, tubercles 60–80 µm diam.; walls 40–50 µm thick, consisting of 4–5 layers of angular cells 4.5–9 µm diam. Asci elongated-clavate, eight-spored, in the spore-bearing portions 35–55 × (6)8–10 µm, stalks 35–40 µm, oozing from ascomata in fascicles intermingled with numerous filiform paraphyses longer than the asci and sparingly occurring bead-like paraphyses consisting of inflated cells 15–30 × 12–15 µm. **Ascospores** overlapping biserial, oblong-ellipsoid, 1-septate, light brown, 10–12 × 3.5–4 µm.

Tortulomyces thailandicus shares the condition of ascomata embedded in a dense subiculum with members of the genera *Chaetosphaerella* E. Müll. & C. Booth and *Neochaetosphaerella* Lar.N. Vassiljeva, S.L. Stephenson & A.V. Chernyshev (cf. Vasilyeva et al. 2012), as well as *Spinulosphaeria thaxteri* (Pat.) Sivan. Ascomata of the latter species were described as having a metallic iridescence (Sivanesan 1974), whereas ascomata of *C. phaeostroma*, *N. thaxterioides* Lar.N. Vassiljeva, S.L. Stephenson & A.V. Chernyshev, and *T. thailandicus* appear to have a somewhat bluish color. The shape of the ascomata in *T. thailandicus* reminds one of a dumpling or a small pastry, and this peculiar shape does not seem to be the result of collapsing, which is usually the case for many coronophoraceous fungi.

The taxonomic status of *Tortulomyces* as a new genus was reached after comparisons with the few species of coronophoraceous fungi that are also known to possess 2-celled and colored ascospores (Table 1). Two of these species were assigned to *Spinulosphaeria* Sivan. (Sivanesan 1974; Mugambi & Huhndorf 2010), one species is the type of *Gaillardiaella* Pat., which has been discussed repeatedly (Petraik 1950; Müller & Arx 1962; Subramanian & Sekar 1990), and another one was treated as the member of *Nitschkiella* G.H. Oth ex P. Karst. by Hsieh et al. (1998, 2000), which is hardly justified.

The features listed in Table 1 were considered to be important at different taxonomic levels. For example, the presence of a 'quellkörper' (a mucilaginous mass of internal cells just below the apex of an ascoma) was recently acknowledged to be the main characteristic defining the family Scortechiniaceae (Huhndorf et al. 2004). However, the presence or absence of a 'quellkörper' was not assessed as being significant even at the generic level when the new species of *Spinulosphaeria*, *S. nuda* Mugambi & Huhndorf, was described (Mugambi & Huhndorf 2010) as lacking a 'quellkörper' whereas the latter feature is characteristic of the type species of *Spinulosphaeria*, *S. thaxteri* (Sivanesan 1974). The fungus from Thailand is similar to *S. thaxteri* in having ascomata crowded on a dense subiculum, but it lacks a 'quellkörper' similar to that found in *S. nuda*, so the genus *Spinulosphaeria* was first considered as appropriate. Data for other species listed in Table 1 are taken mostly from the original published descriptions. The type species of *Gaillardiaella*, *G. pezizoides* Pat., was once characterized as having a 'quellkörper' (Müller & Arx 1962), but that might have been a wrong interpretation, since the most recent description of this species on the web site "Pyrenomycetes of the World" (<http://www-s.life.illinois.edu>) clearly indicates that this feature is absent. However, this might be worth checking again, since the latter description was not based on the type specimen.

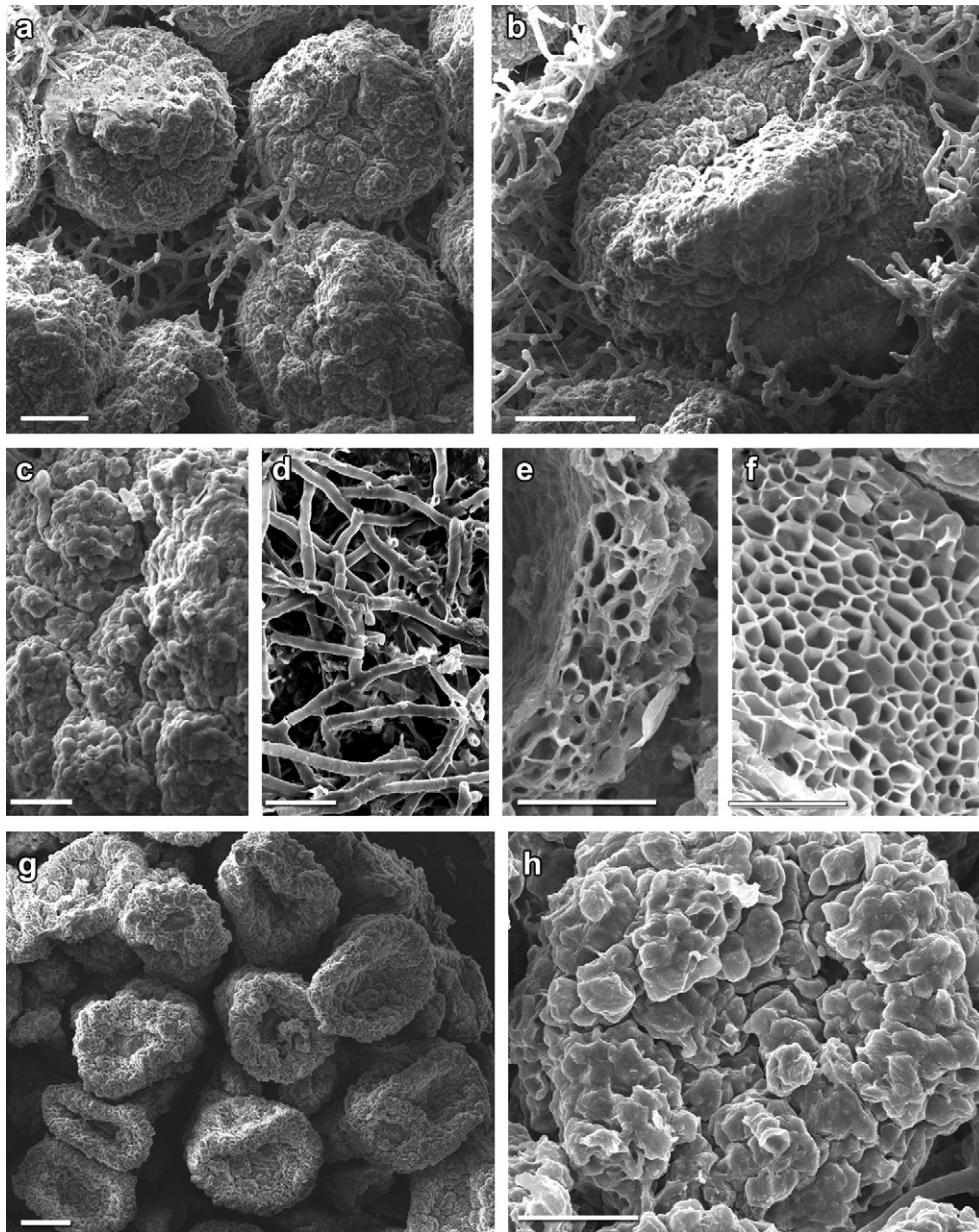


Fig. 1 – a–e, *Tortulomyces thailandicus* (SEM): a, b, ascomata embedded in a subiculum; c, ascomatal surface; d, hyphae of a subiculum; e, cross section through a lateral wall. f–h, *Nitschkia siamensis* (SEM): f, cross section through the base of an ascoma; g, ascomata aggregated on a substrate; h, ascomatal surface. Scale bars: a, b, g, h, 100 μm ; c–f: 0 μm .

The next important feature is the presence or absence of setae on the ascomata. In fact, the genus *Spinulosphaeria* was created as differing from *GaillardIELla* in having an “ascomatal wall furnished with sharply pointed, tooth-like spines”, and this difference is indicated as the only one that distinguishes these genera in a key (Sivanesan 1974). The description of *G. pezizoides* on the web site mentioned above indicates only the presence of a roughened and tuberculate surface on the ascomata, but three earlier mycologists (PetraK 1950; Müller & Arx 1962) spoke of setose ascomata in this species (“Die Oberfläche ist durch konvex vorspringende Zellen rauh und zuweilen mit einzelligen, sich verjüngenden, stumpfen Borsten besetzt”), so the presence or absence of setae might not be

the primary difference between *GaillardIELla* and *Spinulosphaeria*.

The condition of setose or non-setose ascomata is used twice for delimitation of genera (i.e., steps 5 and 13) in the key for some coronophoraceous fungi provided by Mugambi & Huhndorf (2010). Although Arx & Müller (1954) considered this difference to be important only for species delimitation in the group, it has been somewhat traditional to use the presence or absence of setae on ascomata at the generic level (Fitzpatrick 1923; Sivanesan 1974; Nannfeldt 1975; Subramanian & Sekar 1990). In this regard, the fungus from Thailand, which lacks setae on the ascomata, cannot be assigned to *Spinulosphaeria*. However, when taking into

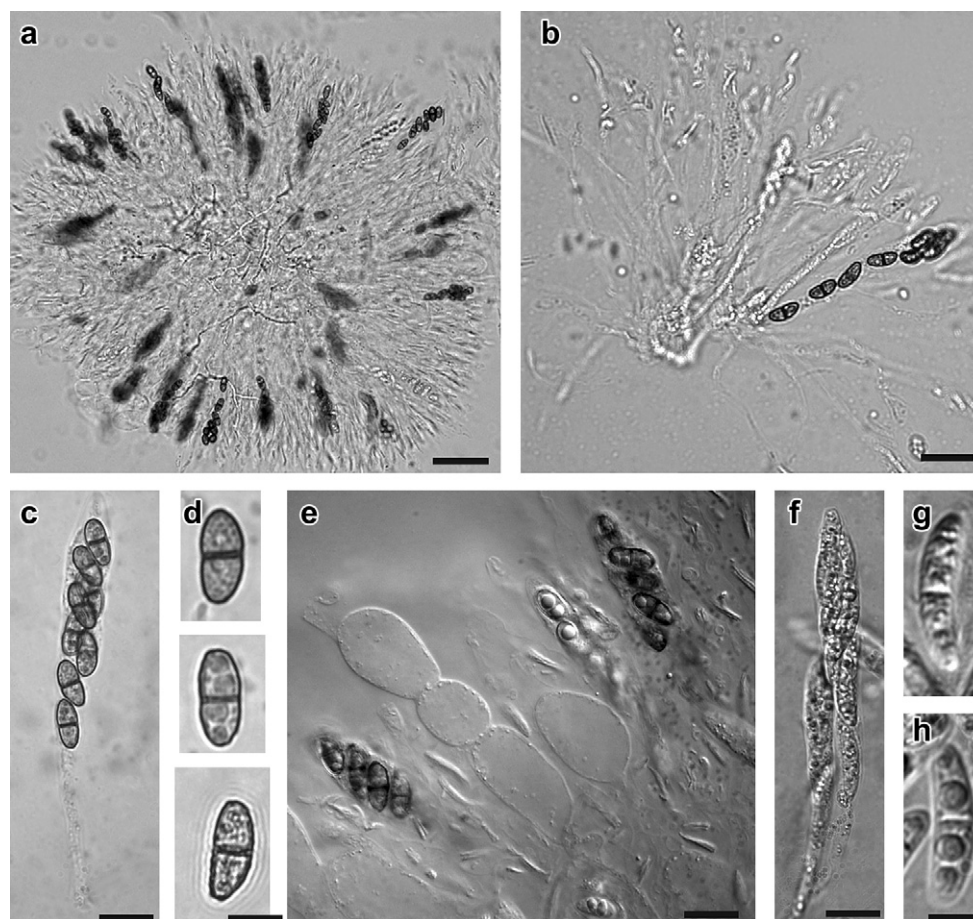


Fig. 2 – a–e, *T. thailandicus*: a, b, asci intermingled with filiform paraphyses; c, ascus; d, ascospores; e, asci and moniliform paraphyses. f–h, *N. siamensis*: f, ascus; g, h, ascospores. Scale bars: a, 45 μm ; b, 17 μm ; c, 11 μm ; d, g, 5.5 μm ; e, 12 μm ; f, 9.5 μm .

account the logic outlined in a previous paper (Vasilyeva et al. 2010), where the presence or absence of setae was not considered to be the generic difference, we could still keep the genus *Spinulosphaeria* in mind. In addition, the ascomata of *S. nuda* without setae did not prevent the placement of the latter species in *Spinulosphaeria* (Mugambi & Huhndorf 2010).

Two newly chosen features have been proposed (Mugambi & Huhndorf 2010) to distinguish the genera *Gaillardiella* and *Spinulosphaeria* (as represented by *S. nuda*). These are the collabent ascomata and ascospores without a mucilagenous sheath in the former genus, whereas the ascomata are not collabent and the ascospores possess a mucilagenous sheath in the latter. However, it was mentioned specifically by these

authors that the ascospores of type species of *Spinulosphaeria* lack a sheath, so this feature cannot be important for delimitation of the two genera under discussion. With respect to collabent or non-collabent ascomata, this difference has not always been considered to warrant generic importance within the group of coronophoraceous fungi. Thus, the genus *Gaillardiella* might be represented by species differing in this respect, since the new description of *G. pezizoides* (see <http://www-s.life.illinois.edu>) indicates that the ascomata flattened at the apex but not additionally collapsing when dry, whereas Petrak (1950) remarked about the strongly collapsing ascomata, which are similar to those of discomycetes (“stark schüsselförmig eingesunken, dick wulstig berandeten

Table 1 – Comparative data relating to some of the most important features often used for delimitation of genera.

Species	Quelkörper	Subiculum	Setae	Paraphyses
<i>Tortulomyces thailandicus</i>	–	+	–	Filiform and inflated
<i>Spinulosphaeria thaxteri</i>	+	+	+	Filiform
<i>S. nuda</i>	–	–	–	Inflated
<i>Gaillardiella pezizoides</i>	?	–	?	Filiform if any are present
<i>Nitschkia phaeospora</i>	+	–	+	Filiform

Apothezien eines Diskomyzeten täuschend ähnlich"). Furthermore, non-collabent ascomata are observed in the type species of *Bertia* De Not. (*B. moriformis*), but several new species of *Bertia* were described with collabent ascomata (Hsieh et al. 1995; Huhndorf et al. 2004; Mugambi & Huhndorf 2010).

It is likely that *S. nuda* should be assigned to a separate genus if one takes into consideration its very characteristic paraphyses, which are broad, septate and constricted at the septa. The type of paraphyses, consisting of inflated cells somewhat similar to beads, is also found in other members of the Coronophorales. Apart from *S. nuda*, they are illustrated for two species of *Chaetosphaerella* (Réblova 1999), *B. tropicalis* (Huhndorf et al. 2004), *B. gigantospora* (Nannf.) Mugambi & Huhndorf, and *B. orbis* Mugambi & Huhndorf (Mugambi & Huhndorf 2010). The three latter species are probably not members of *Bertia*, but the most interesting fact is that the fungus from Thailand possesses both inflated and typically filamentous paraphyses (Fig. 2e). This feature is one of major justifications in support of proposing the separate genus *Tortulomyces*, but it is the unique set of features (brown didymospores, dense subiculum, lack of setae and the absence of a quellkörper), combined with the types of paraphyses that was of primary importance. The filiform paraphyses of *S. thaxteri* were indicated by Sivanesan (1974). The type of paraphyses characteristic of *G. pezizoides* is not quite clear. Petrak (1950) described the condition in this species as scanty and completely dissolving 'paraphyses' (as "Metaphysen spärlich, bald ganz verschleimend"), whereas Müller & Arx (1962) did not mention them at all and illustrated the asci of *G. pezizoides* as without paraphyses.

Nitschkia siamensis Lar.N. Vassiljeva, S.L. Stephenson, A.V. Chernyshev & K.D. Hyde, sp. nov. (Figs. 1f–h, 2f–h)
Mycobank MB 564278

This species is characterized by non-ostiolate ascomata with a thick sterile base and moderately tuberculate surface, as well as 1-septate hyaline ascospores (12.5)14–16 × 3–3.5 μm.

Holotypus: Thailand, Chiang Mai Province, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on dead branches of an unidentified tree, 20 June 2011, L.N. Vasilyeva (MFLU11–1164).

Etymology: refers to the old name of Thailand.

Ascomata superficial, solitary or in large groups, turbinate, collapsing when dry, non-ostiolate, black, with a thick sterile base, 200–300 μm diam., 400–550 μm high including the sterile base; surface moderately tuberculate, tubercles 35–70 μm diam.; walls 45–55 μm, consisting of 5–6 layers of angular cells 7–18 × 2–12 μm. Asci elongated-clavate, eight-spored, sessile, paraphysate, 40–50 × 7–9 μm. Ascospores overlapping biseriate, broadly fusoid, hyaline, often slightly curved, 1-septate, (12.5)14–16 × 3–3.5 μm.

The taxonomic status of *N. siamensis* was not determined easily. The main problem was that the ascomata were characterized by a thick sterile base and were more similar to stalked goblets than to the saucer-like and sessile ascomata of many species of *Nitschkia* occurring in temperate latitudes. The "stalked" ascomata from tropical regions have been observed in species assigned to *Bertia*, for example *B. bisepata*

(Hsieh et al. 2000), *B. orbis*, *B. tropicalis* (Mugambi & Huhndorf 2010), and *N. siamensis* from tropical Thailand appears to be very similar. However, we could not place the newly found species in the family Bertiaceae, which has been separated from three other families (including the Nitschkiaceae) on the basis that ascomata in the former are mostly tuberculate and the ascospores are longer than 15 μm, whereas the members of the Nitschkiaceae should have ascomata that are smooth or have short spines present (Mugambi & Huhndorf 2010).

The ascomata of *N. siamensis* are clearly tuberculate (Fig. 1h), but their tubercles are exactly the same as in *N. parasitans* (Schwein.) Nannf. (cf. Vasilyeva et al. 2010, Fig. 5B). We could also indicate that the ascospores of *N. siamensis* are not longer than 15 μm, although some of them are very slightly longer. In addition, *N. floridana* Fitzp. is known to have ascospores 18–20 μm long, and, on the whole, a feature such as the size of the ascospores is hardly important for the delimitation of families. Within the family Nitschkiaceae, the combination of generic characters—ascomata without a quellkörper and ascospores not spinulose, hyaline and usually 1-septate—corresponds to that described for the genus *Nitschkia*. This largely agrees with the key of Mugambi & Huhndorf (2010) except that they describe *Nitschkia* as not having tuberculate ascomata and characterized by ascospores less than 15 μm long.

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