

Three new species and a new combination in the genus *Torulomyces* from soil

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Seven isolates of *Torulomyces* from Asian and Australian soil samples were studied in comparison with known taxa of the genus and with *Monocillium indicum*, the type species of *Monocillium*. Three new species, *Torulomyces parviverucosus*, *T. laevis*, and *T. ovatus*, are described, and *T. brunneus* is described as a new combination. Conidial characteristics, especially their shape and surface structure, are useful taxonomic criteria for distinguishing species of *Torulomyces*. *Monocillium* is considered to be a distinct genus.

Key Words—Hyphomycetes; *Monocillium*; soil fungi; *Torulomyces*.

The genus *Torulomyces* was erected by Delitsch (1943) with *T. lagena* Delitsch as the type species. A second species, *T. viscosus* Delitsch, described at the same time, remains doubtful because type material is not available and the original description was poor (Stolk and Samson, 1983). Barron (1961) described *Monocillium humicola* Barron in that morphologically similar genus without referring to Delitsch (1943). Later, *M. humicola* was recognized by Barron (1967) to be conspecific with *T. lagena*. *Monocillium humicola* var. *humicola* and *M. humicola* var. *brunneum* M. Christensen & Backus were distinguished by Christensen and Backus (1964) on the basis of difference in color and reverse pigmentation colony. These varieties were later reduced to synonymy with *T. lagena* (Stolk and Samson, 1983). Hashmi et al. (1972) compared the conidium ontogeny and karyology of *T. lagena* with *M. indicum* S. B. Saksena, the type species of *Monocillium*, and concluded that they were congeneric with *Monocillium* treated as a synonym of *Torulomyces*. Then *Torulomyces indicus* (S. B. Saksena) Hashmi et al. was illustrated by Kendrick and Carmichael (1973) as a representative species of *Torulomyces*.

On the other hand, *Torulomyces* and *Monocillium* were retained as separate genera by von Arx (1974) and by Domsch et al. (1980), who reported that conidia of *T. lagena* were attached to each other by connectives, as in *Penicillium*, while those of *M. indicum* adhered to each other by truncate ends, as in some *Acremonium* species. The teleomorph of *Monocillium* was reported by Gams (1971) to be in *Niesslia* Auersw., while *T. lagena* is the anamorph of *Eupenicillium limoneum* Gochenaur & Alattner (Stolk and Samson, 1983). However the transfer of *T. lagena* to *Penicillium* by Stolk and Samson (1983) was not

accepted by Pitt and Samson (1993). Based on the protologue described by Matsushima (1987), *Torulomyces macrosporum* Matsushima may belong to *Monocillium*.

During a study of microfungi from soil samples, we obtained seven isolates belonging to *Torulomyces*. From a taxonomic study of these isolates, three new species and a new combination in *Torulomyces* are described below.

Materials and Methods

Fungal cultures Nine fungal strains were used in this study. Two, IMI 92745 and CBS 382.64, originated from the International Mycological Institute, Egham, Surrey, United Kingdom and the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, respectively. The other seven strains were isolated from Asian and Australian soil samples by a soil dilution method. *M. indicum* CBS 313.61 was used for comparing the anamorph characteristics of *Torulomyces* with those of *Monocillium*.

Media A modified malt extract and corn meal agar (MMCA) was used. It was composed of malt extract (Difco), 5 g/L; corn meal agar (Difco), 5 g/L; yeast extract (Difco), 1 g/L; glucose, 2 g/L; and agar, 15 g/L. This medium was useful for studying colony appearance and the production of soluble pigment. Growth rates on MMCA, 2% malt extract agar (MA) and LcA (Miura and Kudo, 1970) were measured after incubation for 14 to 20 d at 25 and 37°C in darkness.

Morphology Microscopic morphology was examined using 20-d-old cultures on LcA mounted in lactophenol. Descriptions are based on cultures grown on LcA. For scanning electron microscope (SEM) observations,

pieces of colonies cultured on LcA for 20 d at 25°C in darkness were fixed with 2% glutaraldehyde, rinsed with two or three changes of distilled water, and dehydrated through a graded ethanol series. They were immersed in 50% ethanol:amyl acetate and 100% amyl acetate, then dried with a Hitachi Critical Point Dryer (Hitachi, Japan). Dried materials were placed on specimen holders with double-sided adhesive tape and coated with gold using an IB-3 Ion Coater (Eiko, Japan) operating under high vacuum. Micrographs were obtained with a S-430 Scanning Electron Microscope (Hitachi, Japan) operating at 20 kV.

Preservation All strains were preserved by freeze-drying in ampules and kept at 5°C. Dried type specimens were deposited in the Herbarium of the National Science Museum, Tsukuba, Ibaraki, Japan (TNS).

Results and Discussion

In the early stages of conidiophore and phialide development in *Torulomyces*, clavate structures were observed (Fig. 1). Similar structures were observed in the early stages of development of phialides in *M. indicum* by Barron (1961). Conidiophores of *Torulomyces* were simple and erected more-or-less at right angles to the vegetative hyphae (Figs. 2–6). A single, terminal, and inflated phialide was formed on the conidiophore. One septum was always observed at the base of phialides (Fig. 7, arrowheads) and another septum was usually observed at the base of conidiophores. Although the phialides of *Torulomyces* are flask-shaped, some of them have very clear long necks (Fig. 8A), while others are short and inconspicuous (Fig. 8B) in a single strain. In *M. indicum*, no septum was observed in the sporogenous cells (Fig. 9), in agreement with the observations of Barron (1961). Hence, the differentiated conidiophores and phialides of *Torulomyces* provide a distinction from the sporogenous cells produced by *Monocillium*. Therefore, *Torulomyces* can be distinguished from *Monocillium*, and the conclusion of Hashmi et al. (1972) reducing *Monocillium* to a synonym of *Torulomyces* is not quite correct.

Conidia were produced enteroblastically from the phialides, forming long conidial chains with small connectives between adjacent conidia, which were hyaline pigmented, and usually roughened. Conidia were globose to subglobose in all isolates except for those of KY12726 in which the shape is obovoid. The surface texture of some isolates was not distinct under light microscopy, but SEM observations showed five types of conidia on the basis of their shape and fine structure as follows:

Type I (Fig. 10): Conidia are globose to subglobose and conspicuously roughened showing large tubercles on their surface. The isolate IMI 192745 produced this type of conidia. Conidial diam averaged 2.0–3.0 µm as measured under the light microscope.

Type II (Fig. 11): Conidia are globose to subglobose and roughened showing moderate tubercles on their surface. This type of conidia is represented by CBS 382.64, KY 12723 and KY 12724. The conidial diam is

slightly smaller than that in Type I, i.e., 1.5–2.5 µm.

Type III (Fig. 12): Conidia are globose to subglobose and roughened, showing small tubercles on their surface. Type III conidia were observed in KY12720. Conidial diam was 1.5–2.6 µm.

Type IV (Fig. 13): Conidia are globose to subglobose and have smooth surfaces. The conidia of three isolates, KY12725, KY12727 and KY15343, are of this type. Conidia are larger, 2.0–3.7 µm in diam.

Type V (Figs. 14, 15): Conidia are obovoid and have smooth surfaces. This type of conidia is observed in KY12726. Conidial dimensions were 2.3–3.6 × 1.8–2.5 µm.

Shape, surface structure and size of conidia are considered to be valuable taxonomic characteristics in *Torulomyces*. Three new species and a new combination in *Torulomyces* are proposed on the basis of the conidial types. A description of *T. lagena*, the only previously known species, is also provided.

Taxonomy

Torulomyces lagena Delitsch, Systematik der Schimmelpilze, Neudamm: 91. 1943. Figs. 1, 2, 10

≡ *Penicillium lagena* (Delitsch) Stolk & Samson, Stud. Mycol. **23**: 100. 1983.

= *Monocillium humicola* Barron, Can. J. Bot. **39**: 1575. 1961.

Conidiophores mononematous, growing out at right angles from hyphae, simple, unbranched, hyaline, smooth, 2.5–9.5 × 2.5–3.5 µm. Phialides solitary, terminal, flask-shaped, smooth, hyaline, 5.0–10.0 µm long, 2.5–3.5 µm wide at the widest part, 0.8–1.6 µm wide at the apex. Conidia globose to subglobose, 2.0–3.0 µm in diam, conspicuously roughened showing large tubercles, in long, simple chains and with small connectives between adjacent conidia.

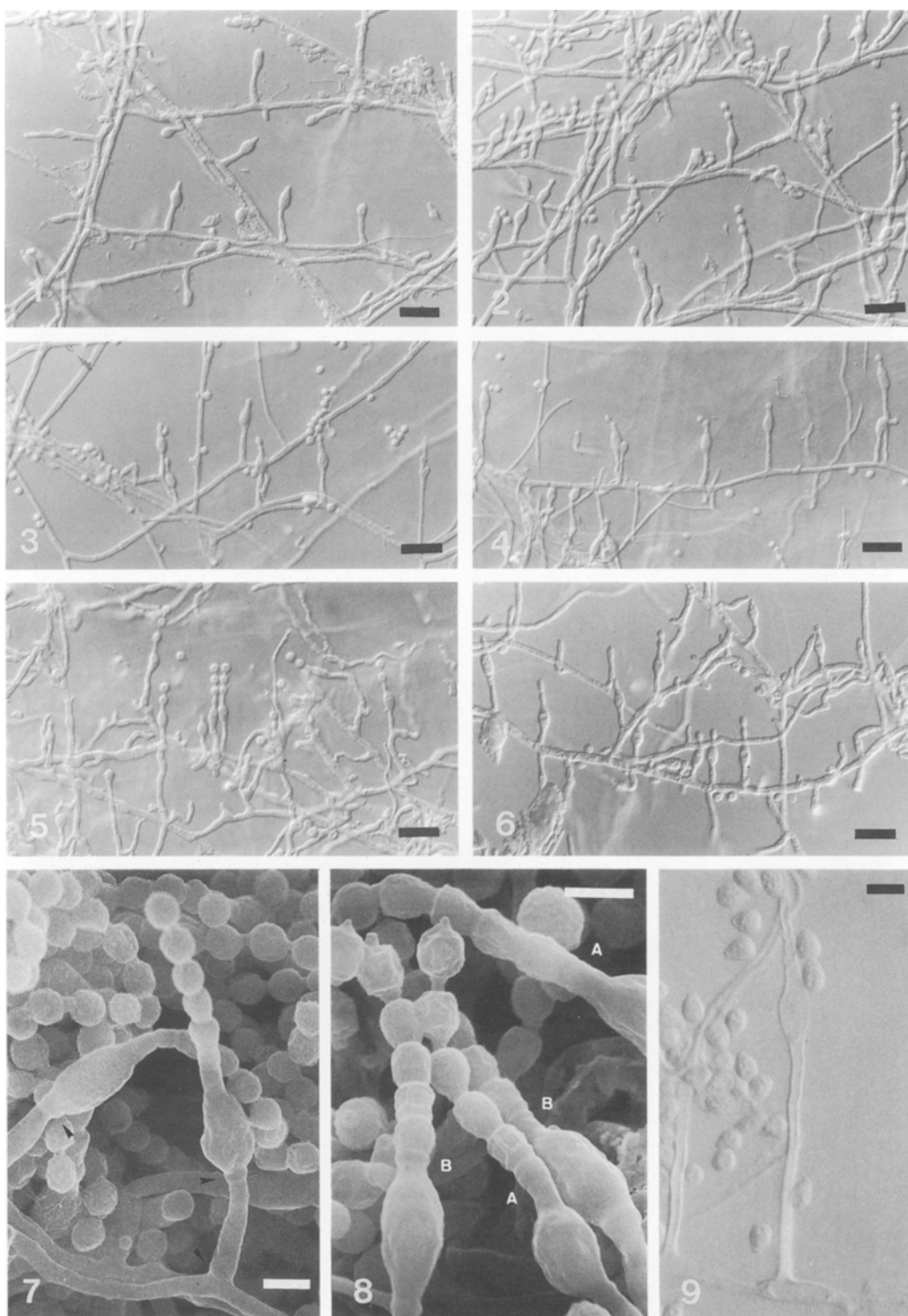
Cultures of IMI 92745 grow slowly, reaching 2.2–2.3 cm in diam on MMCA in 14 d at 25°C. On MA and LcA, colonies 2.9–3.9 cm and 2.5–3.1 cm in diam, respectively, in 20 d at 25°C. The colonies on MMCA are light gray and no soluble pigment is observed. No growth at 37°C.

Material examined: IMI 92745 (= CBS 185.65), identified as *M. humicola*, isolated from soil in cedar bog collected in Guelph, Canada, by G. L. Barron, 1961, designated as neotype of *T. lagena* (Stolk and Samson, 1983).

Torulomyces brunneus (M. Christensen & Backus) Ando, comb. nov. Figs. 3, 7, 11

≡ *Monocillium humicola* Barron var. *brunneum* M. Christensen & Backus, Mycologia **56**: 498. 1964.

Conidiophores mononematous, growing out at right angles from hyphae, simple, unbranched, hyaline, smooth, 4.0–16.5 × 1.0–2.0 µm. Phialides solitary, terminal, flask-shaped, smooth, hyaline, 4.5–8.0 µm long, 2.0–3.2 µm wide at the widest part, 1.0–1.4 µm wide at the apex. Conidia globose to subglobose, 1.5–2.5 (–3.3) µm in diam, conspicuously roughened showing moderate-sized tubercles, in long, simple chains and with



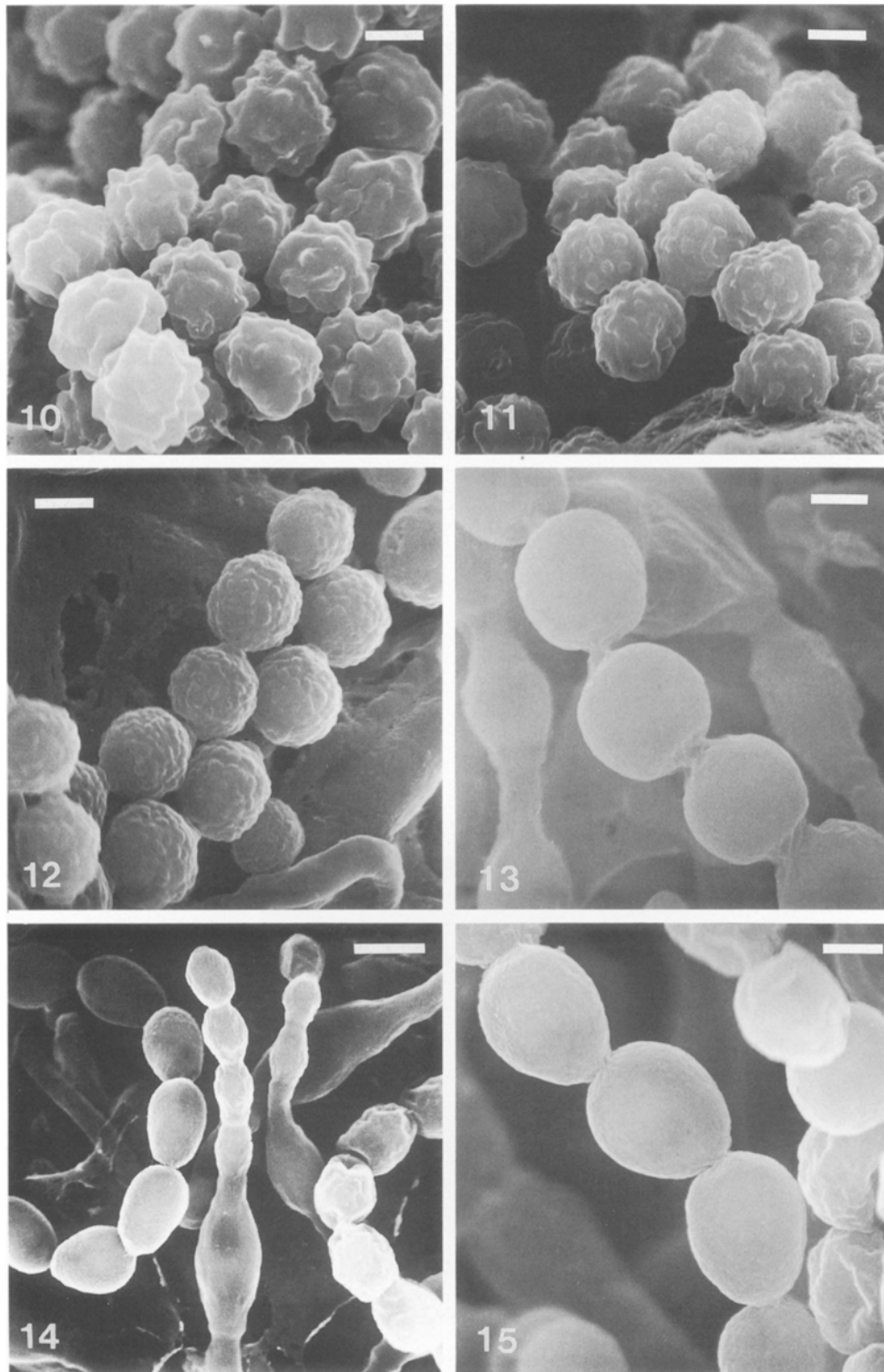
Figs. 1–6. Conidiophores, phialides and conidia of *Torulomyces* spp. grown on LcA.

1, 2. *T. lagena* IMI 92745. 3. *T. brunneus* CBS 382.64. 4. *T. parviverrucosus* KY 12720. 5. *T. laevis* KY 12727. 6. *T. ovatus* KY 12726. Scale bars = 10 μ m.

Fig. 7. A phialide on a conidiophore developed from a hyphal cell of *Torulomyces brunneus* CBS 382.64 on LcA by SEM. Each of the arrow heads shows a septum. Scale bar = 2 μ m.

Fig. 8. Phialides with the clear long neck (A) and without neck (B) of *Torulomyces parviverrucosus* KY 12720 on LcA. Scale bar = 2 μ m.

Fig. 9. A phialide developed directly from a hyphal cell of *Monocillium indicum* CBS 313.61 growing on LcA. Scale bar = 10 μ m.



Figs. 10–15. SEM observations of conidial surfaces of *Torulomyces* spp. grown on LcA.

10. Conidial Type I (globose conidia with large verrucose) of *T. lagena* IMI 92745. 11. Conidial Type II (globose conidia with moderate verrucose) of *T. brunneus* CBS 382.64. 12. Conidial Type III (globose conidia with small verrucose) of *T. parviverrucosus* KY 12720. 13. Conidial Type IV (globose conidia with smooth wall) of *T. laevis* KY 12727. 14. *T. ovatus* KY 12726 showing conidial chain from a phialide. 15. Conidial Type V (obovoid conidia with smooth wall) of *T. ovatus* KY 12726. Scale bars: 10–13, 15 = 1 μm ; 14 = 2 μm .

small connectives between adjacent conidia.

Cultures of CBS 382.64, KY 12723 and KY 12724 grow slowly, reaching 2.6–3.5 cm in diam on MMCA in 14 d at 25°C. On MA and LcA, colonies 3.4–5.2 cm and 2.7–4.1 cm in diam, respectively, in 20 d at 25°C. The colonies on MMCA are light grayish blue to light grayish green. Red brown and brown soluble pigments are observed in CBS 382.64 and KY 12724, respectively. Although Christensen and Backus (1964) reported the production of sclerotia on media soon after isolating, no sclerotia was observed on any media in the three strains used in this study. At 37°C, colonies up to 4.5 mm diam in 14 d on MMCA.

Holotype: A dried plate culture of CBS 382.64 (=WSF 9-c), type of *M. humicola* var. *brunneum*, isolated from soil under *Pinus strobus* collected in a community of eastern white pine located in Vilas County, Wisconsin, U.S.A., by M. Christensen (Christensen and Backus, 1964).

Additional materials examined: KY 12723 isolated from soil collected in Hamilton Island, Qld, Australia, by K. Ando, Oct. 1989; KY 12724 isolated from forest soil collected in Townsville, Qld, Australia, by K. Ando, Oct. 1989.

***Torulomyces parviverrucosus* Ando & Pitt, sp. nov.**

Figs. 4, 8, 12

Conidiophora mononematica, erecta, recta, non ramosa, laevia, non septata, hyalina, 3.5–14.5 × 1.0–2.0 μm. Phialides solitariae, acrogenae, lagenariae, laeves, hyalinae, 4.0–7.0 μm longae, 2.0–3.2 μm crassae, apice 1.0–1.3 μm crassae. Conidia globosa vel subglobosa, 1.5–2.6 μm diam, verrucosa cum granulis parvis numerosis praedita.

Holotypus: TNS-F-238516, colonia exsiccata e cultura ex KY 12720.

Conidiophores mononematous, growing out at right angles from hyphae, simple, unbranched, hyaline, smooth, 3.5–14.5 × 1.0–2.0 μm. Phialides solitary, terminal, flask-shaped, smooth, hyaline, 4.0–7.0 μm long, 2.0–3.2 μm wide at the widest part, 1.0–1.3 μm wide at the apex. Conidia globose to subglobose, 1.5–2.6 μm in diam, roughened, showing small tubercles, in long, simple chains and with small connectives between two adjacent conidia.

Cultures of KY 12720 grow slowly, reaching 2.7–2.8 cm in diam on MMCA in 14 d at 25°C. On MA and LcA, colonies 3.5–4.8 cm and 2.1–3.2 cm in diam, respectively, in 20 d at 25°C. The colonies on MMCA are light grayish blue, grayish green or dark blue green. Red soluble pigment is observed in the medium. At 37°C, colonies 11–12 mm diam in 14 d on MMCA.

Holotype: TNS-F-238516, a dried specimen from the culture of KY 12720 isolated from soil collected in Gap Park, Sydney, N.S.W., Australia, by K. Ando, Oct. 1989.

***Torulomyces laevis* Ando & Manoch, sp. nov.**

Figs. 5, 13

Conidiophora mononematica, erecta, recta, non ramosa, laevia, non septata, hyalina, (1.5–)2.5–10.5 ×

1.0–1.5 μm. Phialides solitariae, acrogenae, lagenariae, laeves, hyalinae, 4.0–8.0 μm longae, 1.5–3.3 μm crassae, apice 1.0–1.5 μm crassae. Conidia globosa vel subglobosa, laevia, 2.0–3.7 μm diam.

Holotypus: TNS-F-238517, colonia exsiccata e cultura ex KY 12727.

Conidiophores mononematous, growing out at right angles from hyphae, simple, not branched, hyaline, smooth, (1.5–)2.5–10.5 × 1.0–1.5 μm. Phialides solitary, terminal, flask-shaped, smooth, hyaline, 4.0–8.0 μm long, 1.5–3.3 μm wide at the widest part, 1.0–1.5 μm wide at the apex. Conidia globose to subglobose, 2.0–3.7 μm in diam, smooth, in long, simple chains and with small connectives between adjacent conidia.

Cultures of KY 12727, KY 12725 and KY 15343 grow slowly, reaching 1.8–2.8 cm in diam on MMCA in 14 d at 25°C. On MA and LcA, colonies 2.7–4.5 cm and 1.8–3.5 cm in diam, respectively, in 20 d at 25°C. The colonies of KY 12727 and KY 15343 on MMCA are light gray, and that of KY 12725 is beige. No soluble pigment was observed. No growth at 37°C.

Holotype: TNS-F-238517, a dried specimen from culture of KY 12727 isolated from forest soil collected in Thailand, by K. Ando, Oct. 1992.

Additional materials examined: KY 12725 isolated from forest soil collected at the Gombak Field Study Center of the Univ. of Malaya, Kuala Lumpur, Malaysia, by K. Ando, Oct. 1989; KY 15343 isolated from forest soil under litter of *Pinus merksii* collected at the Forest Research Institute of Malaysia, Kuala Lumpur, Malaysia, by K. Ando, Nov. 1989.

***Torulomyces ovatus* Ando & Nawawi, sp. nov.**

Figs. 6, 14, 15

Conidiophora mononematica, erecta, recta, non ramosa, laevia, non septata, hyalina, 2.5–8.5 × 1.0–2.3 μm. Phialides solitariae, acrogenae, lagenariae, laeves, hyalinae, 6.0–10.5 μm longae, 2.2–3.7 μm crassae, apice 0.7–1.5 μm crassae. Conidia obovata, laevia, 2.3–3.6 × 1.8–2.5 μm.

Holotypus: TNS-F-238518, colonia exsiccata e cultura ex KY 12726.

Conidiophores mononematous, growing out at right angles from hyphae, simple, unbranched, hyaline, smooth, 2.5–8.5 × 1.0–2.3 μm. Phialides solitary, terminal, flask-shaped, smooth, hyaline, 6.0–10.5 μm long, 2.2–3.7 μm wide at the widest part, 0.7–1.5 μm wide at the apex. Conidia obovoid, smooth, 2.3–3.6 × 1.8–2.5 μm.

Cultures of KY 12726 grow slightly faster than other species, reaching 2.7–3.1 cm in diam on MMCA in 14 d at 25°C. On MA and LcA, colonies 5.7–6.1 cm and 3.4–4.2 cm in diam, respectively, in 20 d at 25°C. The colonies of KY 12726 on MMCA are beige, and no soluble pigment is observed. At 37°C, colonies 4–5 mm diam in 14 d on MMCA.

Holotype: TNS-F-238518, a dried specimen from culture of KY 12726 isolated from forest soil under litter of *Pinus caribaea*, collected at the Forest Research Institute of Malaysia, Kuala Lumpur, Malaysia, by K. Ando, Nov.

Table 1. Sizes of conidiophores, phialides and conidia in *Torulomyces* spp.

Species	Conidiophores ^{a)}	Phialides ^{a)}	Conidia ^{a)}
<i>T. lagena</i>	2.5–9.5 × 2.5–3.5	5.0–10.0 × 2.5–3.5	2.0–3.0 in diam
<i>T. brunneus</i>	4.0–16.5 × 1.0–2.0	4.5–8.0 × 2.0–3.2	1.5–2.5 in diam
<i>T. parviverrucosus</i>	3.5–14.5 × 1.0–2.0	4.0–7.0 × 2.0–3.2	1.5–2.6 in diam
<i>T. laevis</i>	2.5–10.5 × 1.0–1.5	4.0–8.0 × 1.5–3.3	2.0–3.7 in diam
<i>T. ovatus</i>	2.5–8.5 × 1.0–2.3	6.0–10.5 × 2.2–3.7	2.3–3.6 × 1.8–2.5

a) All measurements: μm .

1989.

As taxonomic criteria in the genus *Torulomyces*, the sizes of conidiophores and phialides were of no value, because of the lack of variation among species (Table 1). As two types of the phialides, those with a distinct neck and those without, were observed simultaneously in a culture, this characteristic was also not valuable as a

criterion for species classification in *Torulomyces*. From our study, it was concluded that the shape, surface structure and size of conidia were useful characteristics for distinguishing taxa in the genus *Torulomyces* at the species level.

Torulomyces species are separated as indicated in the following key.

Key to *Torulomyces* species

Conidia globose

Conidium surfaces verrucose

Conidia with large tubercles, 2.0–3.0 μm in diam *T. lagena*

Conidia with moderate-sized tubercles, mostly 1.5–2.5 μm in diam *T. brunneus*

Conidia with small tubercles, mostly 1.5–2.5 μm in diam *T. parviverrucosus*

Conidium surfaces smooth

Conidia smooth, mostly 2.0–3.5 μm in diam *T. laevis*

Conidia obovoid

Conidia smooth, mostly 2.5–3.5 × 2.0–2.5 μm *T. ovatus*

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Literature cited

- Arx, J. A. von. 1974. The genera of fungi sporulating in pure culture, 2nd ed, pp. 192–194. J. Cramer, Vaduz.
- Barron, G. L. 1961. *Monocillium humicola* sp. nov. and *Paecilomyces variabilis* sp. nov. from soil. *Can. J. Bot.* **39**: 1563–1571.
- Barron, G. L. 1967. *Torulomyces* and *Monocillium*. *Mycologia* **59**: 716–718.
- Christensen, M. and Backus, J. W. 1964. Two varieties of *Monocillium humicola* in Wisconsin forest soils. *Mycologia* **56**: 498–504.
- Delitsch, H. 1943. *Systematik der Schimmelpilze*. Druck J. Neumann, Neudamm, Germany.
- Domsch, K. H., Gams, W. and Anderson, T.-H. 1980. *Compendium of soil fungi*, vol. 1, pp. 786–787. Academic Press, London.
- Gams, W. 1971. *Cephalosporium-artige Schimmelpilze*. G. Fischer, Stuttgart.
- Hashmi, M. H., Kendrick, W. B. and Morgan-Jones, G. 1972. Conidium ontogeny in hyphomycetes. The genera *Torulomyces* Delitsch and *Monocillium* Saksena. *Can. J. Bot.* **50**: 1461–1463.
- Kendrick, W. B. and Carmichael, J. W. 1973. Chapter 10. Hyphomycetes. In: *The fungi, an advanced treatise*. vol. IV A, (ed. by Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S.), p. 438. Academic Press, New York.
- Matsuhima, T. 1987. *Matsuhima Mycol. Mem. No. 5*. Published by the author.
- Miura, K. and Kudo, M. Y. 1970. An agar-medium for aquatic Hyphomycetes. *Trans. Mycol. Soc. Japan* **11**: 116–118. (In Japanese.)
- Pitt, J. I. and Samson, R. A. 1993. Species names in current use in the Trichocomaceae (Fungi, Eurotiales). In: *Names in current use in the families Trichocomaceae, Cladoniaceae, Pinaceae, and Lemnaceae*, (ed. by Greuter, W.), *Reg. Veget.* **128**: 13–57.
- Stolk, A. C. and Samson, R. A. 1983. The ascomycete genus *Eupenicillium* and related *Penicillium* anamorphs. *Stud. Mycol.* **23**: 1–149.