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A NEW SPECIES OF HETEROTHALLIC NEOSARTORYA

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SUMMARY

A new heterothallic fungus, *Neosartorya spathulata* (anamorph: *Aspergillus spathulatus*), was isolated from cultivated soil from an *Alocasia* field (*A. macrorrhiza*), Tai-Ping Village, Ilan Prefecture, Formosa. The species is characterized by light yellowish cleistothecia, bivalved ascospores with nearly smooth convex surfaces, and the production of two types of conidiophores. Besides morphological differences, it clearly differs from *N. fennelliae*, the other known heterothallic *Neosartorya*, by the incompatibility with their tester strains.

Among the cleistothecial Ascomycetes, the teleomorphs of *Aspergillus* and *Penicillium* have been considered to be primarily homothallic. There are only two exceptions, *Emericella heterothallica* (Kwon, Fennell et Raper) Malloch et Cain and *Neosartorya fennelliae* Kwon-Chung et Kim; these are described as being heterothallic (Raper and Fennell, 1965; Kwon and Raper, 1967; Malloch and Cain, 1972; Kwon-Chung and Kim, 1974). The anamorphs of these species belong to the *Aspergillus nidulans* group and the *A. fumigatus* group, respectively.

During an investigation of the mycobiota of Formosan soils by one of us (M. T.), a heterothallic fungus with an *Aspergillus* anamorph (assignable to the *A. fumigatus* group)

was isolated and appeared to be sufficiently different from *N. fennelliae* to warrant its description as a second heterothallic new species of *Neosartorya*.

Neosartorya spathulata Takada et Udagawa, sp.nov.
(Figs. 1-6)

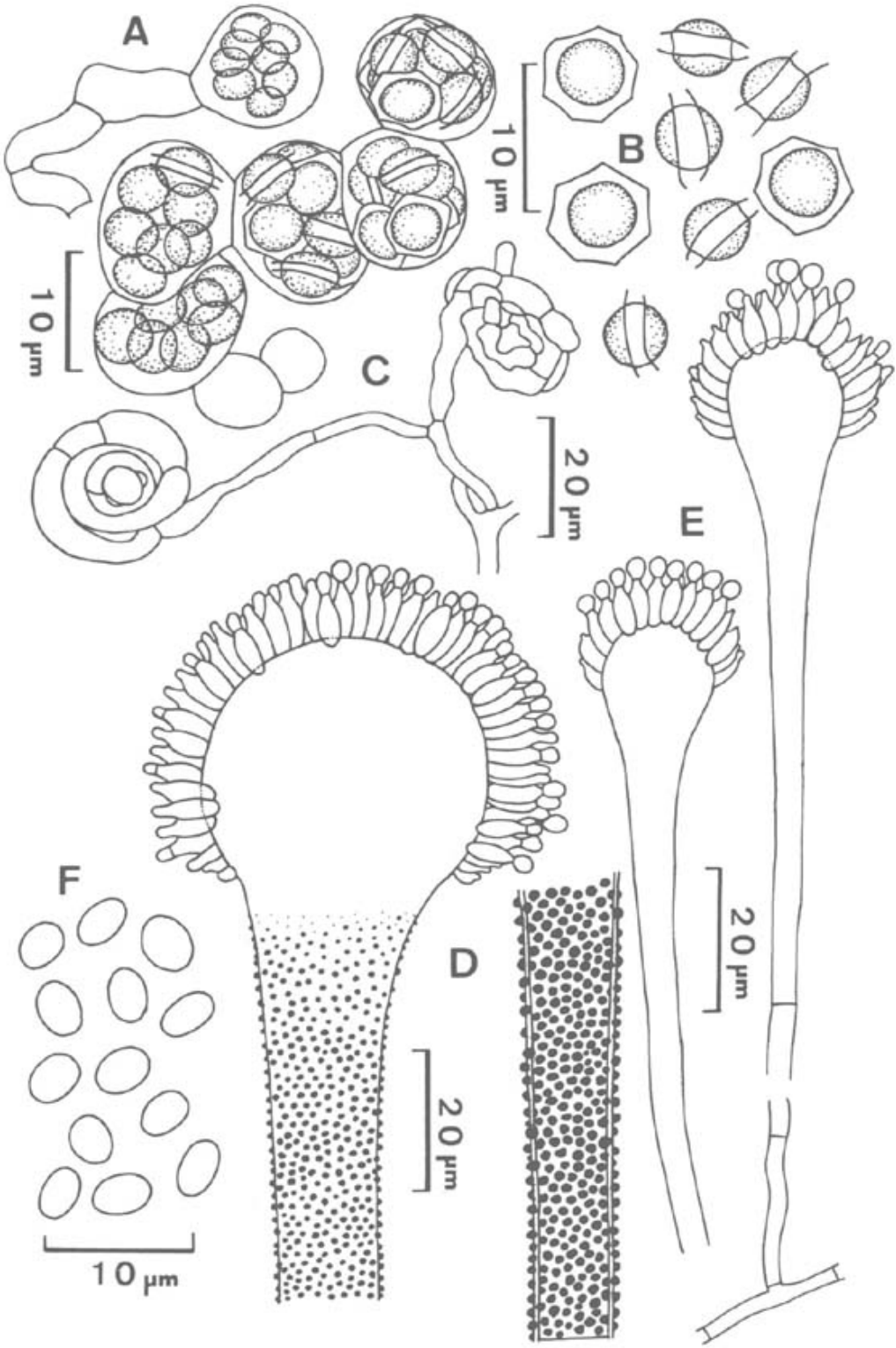
Stat. Anam. *Aspergillus spathulatus* Takada et Udagawa, anam. sp.nov.

Fungus heterothallicus. Cleistothecia superficialia, dispersa vel confluentia, primo alba, deinde flavescentia vel primulina vel luteola, globosa vel subglobosa, 100-260 µm diam, e filis hypharum hyalinis vel flavescentibus vestita; peridium tenue, membranaceum, pseudoparenchymatum, 'textura epidermoidea' vel 'textura angularis', e stratis duobus compositum; stratum externum e cellulis complanatis, hyalinis, irregularibus, incrassatis, 3.5-10 µm latis compositum; stratum internum e cellulis hyalinis, tenuibus, angularibus compositum. Asci octospori, irregulatim dispositi, catenati in ascogonicis hyphis, globosi vel subglobosi, 12-15.5 x 10-12 µm, postremo evanescentes. Ascosporae hyalinae, unicellulares, lenticulares, 5-7 x 3-4.5 µm, duabus cristis aequatorialibus usque 1-1.5(-2.5) µm latis praeditae, superficies convexae leves.

Mycelium e hyphis hyalinis vel flavescentibus, ramosis, septatis, granularibus, 1.5-3.5 µm latis compositum. Capitula conidica primo hemisphaerica, deinde laxe columnaria, 120-300 x 40-80 µm. Conidiophora vulgo e coacto basali vel interdum e hyphis aeriis exorientia, biformia: (a) principia magniora, plus minusve dispersa, 500-1500 x 11-18(-25) µm, supra striam secundariam capitulum protrudentia, valde verrucosa, incrassata, hyalina vel dilute flavo-brunnea, superne gradatim ampliata et (22-)25-52 µm diam vesicularia; (b) secundaria conidiophora minora, 60-250 x 4-10 µm, levia, incrassata, hyalina, superne ampliata et 8-15(-24) µm diam vesicularia. Metulae nullae. Phialides confertae, in summa 1/2-3/4 vesicula sitae, ampulliformes, 6-10 x 2-4 µm. Conidia dilute flavo-viridia, vulgo ellipsoidea, interdum subglobosa vel ovoidea, 3-5.5 x 2-4.5 µm, levia.

Coloniae in agaro Czapekii paulo lente crescentes, tenues, e mycelio vegetativo submerso constantes, sparsis conidialibus capitulis formantes, griseo-viridiae vel

Figure 1. *Neosartorya spathulata*.
A. Asci. B. Ascospores. C. Cleistothecial initials. D. Large type of conidial head. E. Small type of conidial heads. F. Conidia.


A
10 µm
B
10 µm
C
20 µm
E
20 µm
D
20 µm
F
10 µm

griseo-venetae; reversum incolor.

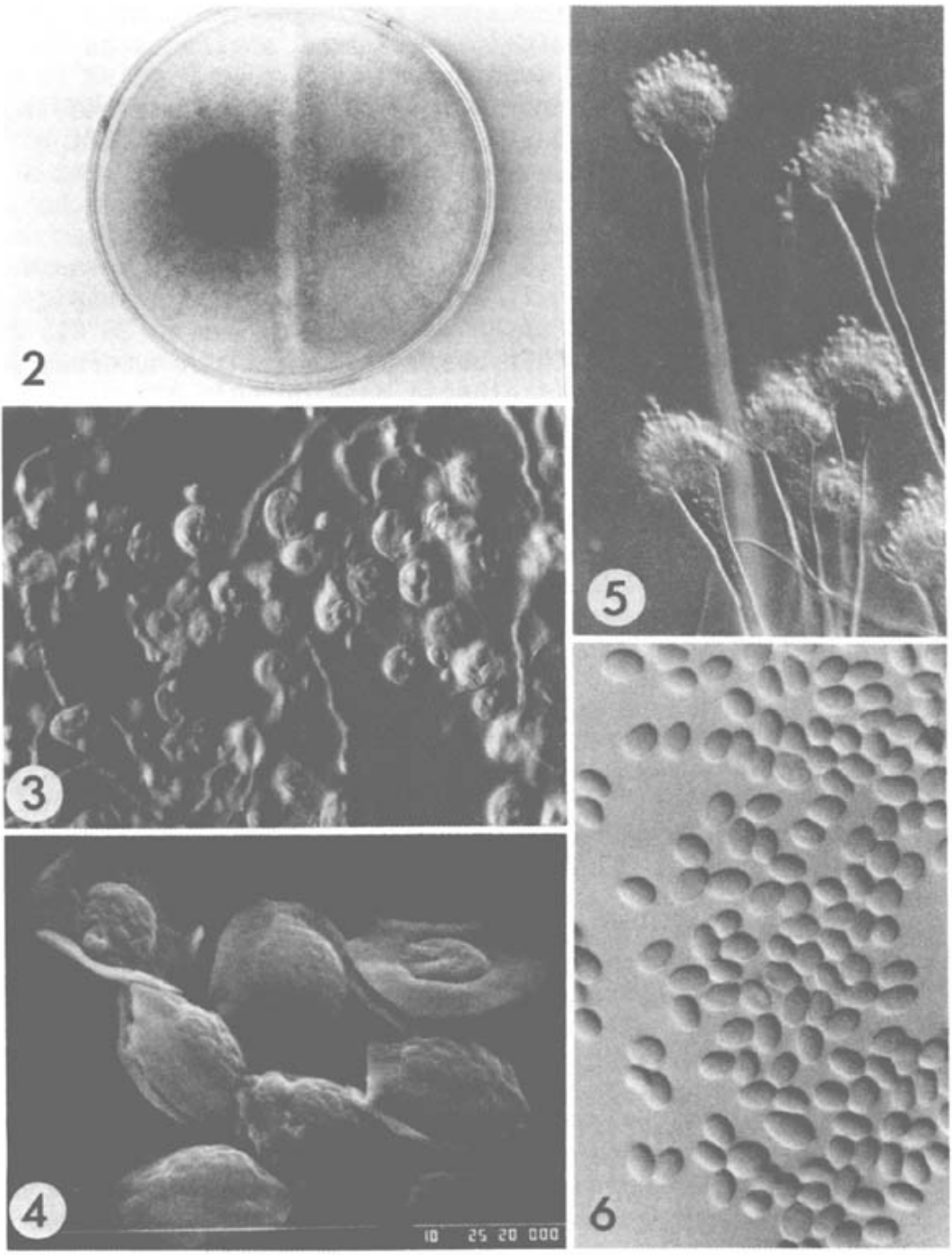
Coloniae in agaro maltoso effusae, floccosae, e mycelio tenui coacto constantes, abundantibus conidialibus capitulis formantes, obscure virides vel griseo-virides vel glauco-venetae vel pistaceae; reversum incolor vel aliquantum griseo-brunneum vel in centro coloniae hinnuleum.

Holotypus NHL 2947, colonia exsiccata ex crusi NHL 2948 x NHL 2949, uterque ex solo sativo in Tai-Ping, Ilan, Formosa, 13.x.1982, a M. Takada, isolata. In herb. NHL.

Etymology: lat. *spathulatus*, referring to spathulate shape of vesicle in large type of conidiophores.

Heterothallic. Cleistothecia superficial, scattered or aggregated in small clusters, at first white, then becoming pale yellow to light yellow (M.4A3-4 after Kornerup and Wanscher, 1978) or 'Primrose' to 'Pale luteous' (Rayner, 1970), globose to subglobose, 100-260 µm in diam, covered by hyaline to pale yellow hyphal strands; peridium thin, membranaceous, pseudoparenchymatous, composed of several layers of flattened cells; outer layer composed of hyaline, thick-walled, elongate, irregular cells measuring 3.5-10 µm wide, forming a 'textura epidermoidea'; inner layer of hyaline, thin-walled, angular cells, forming a 'textura angularis'. Asci 8-spored, irregularly disposed, borne in short, often twisted chains, arising as branches from ascogenous hyphae, globose to subglobose, 12-15.5 x 10-12 µm, evanescent at maturity. Ascospores maturing rapidly (8 days at 37°C), hyaline, one-celled, lenticular with smooth convex walls and two equatorial crests, 5-7 x 3-4.5 µm including crests, spore body 3.5-4 µm long; crests up to 1-1.5(-2.5) µm wide, angular in face view.

Mycelium composed of hyaline to pale yellow, branched, septate, roughened by pigmented granules, 1.5-3.5 µm wide hyphae. Cleistothecial initials beginning as upright branches of hyphae with loose curled tips (ascogonium), becoming surrounded by thin hyphae arising from the opposite mating strain. Conidial heads hemispherical when young, then loosely columnar, 120-300 x 40-80 µm. Conidiophores usually arising from the basal felt, sometimes from aerial hyphae, of two kinds: (a) large type, more or less scattered, 500-1500 x 11-18(-25) µm, projecting above the layer of smaller conidiophores, distinctly roughened by numerous warts, thick-walled, hyaline to pale yellow-brown, gradually broadening to dome- or flask-shaped vesicles measuring (22-)25-52 µm in diam; (b) small type, 60-250 x 4-10 µm, smooth, thick-walled, hyaline, broadening to small vesicles measuring 8-15(-24) µm in diam. Metulae lacking. Phialides borne on the upper 1/2 to 3/4 surface of vesicle,



Figures 2-6. *Neosartorya spathulata*.
2. Paired culture of 'a' x 'A' on malt extract agar at 23° C, 10 days. x 1/2. 3. Asci. x 480. 4. Ascospores (SEM photograph). x 4600. 5. Conidial structures. x 200. 6. Conidia. x 1000.

crowded, flask-shaped, 6-10 x 2-4 μm. Conidia faintly yellow-green, mostly ellipsoidal, sometimes subglobose to ovoid, 3-5.5 x 2-4.5 μm, smooth.

Colonies on Czapek agar growing somewhat restrictedly, attaining a diameter of 33-38 mm within 7 days at 23°C, vegetative mycelium thin, almost submerged, bearing scattered grayish green (M.25B-C4, after Kornerup and Wanscher, 1978) or 'Greyish blue green' (Rayner, 1970) conidial heads; cleistothecia not appearing within 7 days; reverse uncolored. Colonies on malt extract agar spreading rapidly, attaining a diameter of 80 mm within 7 days at 23°C, floccose, composed of a thin basal felt in which numerous white to pale yellow cleistothecia develop only after appropriate pairings, producing abundant dull green to grayish green (M. 25D4-25C5 after Kornerup and Wanscher) or 'Glaucous blue-green' to 'Pistachio green' (Rayner) conidial heads, with an irregular lobate margin; reverse uncolored to somewhat grayish brown (M.8D3 after Kornerup and Wanscher) or 'Fawn' (Rayner) at center. Colonies on oatmeal agar growing moderately, attaining a diameter of 40-46 mm within 7 days at 23°C, floccose, grayish green, conidial heads and cleistothecia as described on malt; reverse uncolored. Colonies on both malt extract and oatmeal agars at 37°C growing more rapidly than at 23°C, thin, producing abundant conidia and cleistothecia after pairings.

Thermotolerant: The optimal temperature for growth and conidiogenesis is 28-43.5°C, reduced growth occurs within the range of 22.5-25.5°C, and the upper limit is near 47°C. No growth was observed at temperatures either below 20°C or above 50°C. In the response to temperatures, no difference was observed between the two mating strains.

Isolation: cultivated soil of *Alocasia macrorrhiza* fields in Tai-Ping Village, Ilan Prefecture, Formosa, 13. x.1982, coll. M. Takada.

Holotype: The type of *N. spathulata* is a dried culture of NHL 2948 x 2949 preserved in NHL 2947. Subcultures of the 'A' (NHL 2948) and 'a' (NHL 2949) mating types are deposited in the American Type Culture Collection.

Neosartorya spathulata is clearly distinguished from *N. fennelliae* by its yellow cleistothecia, catenated asci, and ascospores with nearly smooth convex surfaces in contrast with reticulate surfaces of ascospores of the latter species. Additionally, the conidial structures of *N. spathulata* are of two kinds and the conidia are large ellipsoidal, whereas *N. fennelliae* has one type of conidial structure and small globose-subglobose or ellipsoidal conidia.

Results of mating tests: All mating tests were carried out on malt extract and oatmeal agars at 25°C, 14 days or at 37°C, 8-14 days. The two tester lines of mating type 'A' and 'a' were established in the monoconidial strains from the Formosan isolates of this fungus. Matings between the two 'A' and 'a' tester lines, all of which were inoculated onto the agar plates, resulted in the appearance of two bands of fertile cleistothecia near the junction of the two colonies between the compatible strains (Fig. 2, Table 1). None of the crosses between *N. spathulata* and each strain of *N. fennelliae* (NRRL 5534 'A', 5535 'a'; NHL 2951 'A', 2952 'a'), however, was fertile (Table 1).

Table 1. Results of crosses between *N. spathulata* and two *N. fennelliae*

	N.spathulata NHL		*N.fennelliae* NRRL		*N.fennelliae* NHL	
	2948(A)	2949(a)	5534(A)	5535(a)	2951(A)	2952(a)
N.spathulata						
NHL 2948(A)	-	+	-	-	-	-
NHL 2949(a)		-	-	-	-	-
N.fennelliae						
NRRL 5534(A)			-	+	-	+
NRRL 5535(a)				-	+	-
NHL 2951(A)					-	+
NHL 2952(a)						-

+: Fertile cleistothecia produced.
-: Fertile cleistothecia not produced.

Interspecific pairings between *N. spathulata* and *N. fischeri*: Kwon-Chung and Kim (1974) tested interspecific pairings between strains of *N. fennelliae* and other members of *Neosartorya* and reported that the strains of *N. fennelliae* stimulate hyphal growth of *N. fischeri* var. *glabra* along the line of contact. As summarized in Table 2, similar reaction was observed on the interspecific pairings between mating type strains of *N. spathulata* and *N. fischeri*. Stimulation of cleistothecial formation in *N. fischeri* var. *glabra* and var. *verrucosa* occurred along the line of contact when paired with *N. spathulata*.

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Table 2. Results of interspecific pairings of *N. spathulata* and other *Neosartorya* spp. on oatmeal agar at 26°C

Pairings of strains		Reactions	
		Clear zone formation	Cleistothecial band produced*
NHL2948(A)	xM3061 *N.fischeri* var.*fischeri*	+	-
	XM4690 *N.fischeri* var.*fischeri*	-	-
	xM2522 *N.fischeri* var. *glabra*	+	-
	xM3159 *N.fischeri* var. *glabra*	+	+
	xM4693 *N.fischeri* var. *glabra*	+	+
	xM4691 *N.fischeri* var.*verrucosa*	-	+
	xM2311 *N.fischeri* var. *spinosa*	-	-
	xM4165 *N.fischeri* var. *spinosa*	+	-
NHL2949(a)	xM3061 *N.fischeri* var.*fischeri*	±	+
	xM4690 *N.fischeri* var.*fischeri*	-	-
	xM2522 *N.fischeri* var. *glabra*	+	±
	xM3159 *N.fischeri* var. *glabra*	+	+
	xM4693 *N.fischeri* var. *glabra*	+	+
	xM4691 *N.fischeri* var.*verrucosa*	±	+
	xM2311 *N.fischeri* var. *spinosa*	-	-
	xM4165 *N.fischeri* var. *spinosa*	+	-

* Stimulation of cleistothecial production in *Neosartorya fischeri* strains, which was shown by dense band of cleistothecia, occurred along the line of contact.

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