

Three new *Aspergillus* species isolated from clinical sources as a causal agent of human aspergillosis

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Three new species of *Aspergillus* isolated from clinical sources in China are described and illustrated: *A. beijingensis*, *A. qizutongii* and *A. wangduanlii*. The first species is characterized by spreading colonies, yellow to grayish green conidial heads, smooth-walled conidiophores with a clavate vesicle, uniseriate aspergilla and nearly globose, micro-verrucose conidia. The second is characterized by spreading colonies, olive-yellow conidial heads, conspicuously roughened conidiophores with a flask-shaped vesicle, uniseriate but often secondarily proliferating aspergilla and globose, smooth conidia. The third is characterized by rapidly growing colonies, dull green conidial heads, smooth to irregularly roughened conidiophores which are often surrounded by coiled hyphae in the basal part and are terminally swollen into a globose or irregular shaped vesicle, uniseriate aspergilla and globose, micro-verrucose conidia.

Key Words—aspergillosis of maxillary sinus; *Aspergillus beijingensis*; *Aspergillus qizutongii*; *Aspergillus wangduanlii*; systematics.

During investigation of the causal agents of several cases of aspergillosis of human maxillary sinus at the Beijing Medical University and the Beijing Tongren Hospital in China, three clinical isolates were found to exhibit unreported features of conidial development. They are described below as new species of the form-genus *Aspergillus* (Raper and Fennell, 1965; Samson, 1979; Samson and Gams, 1985; Pitt and Samson, 1993). Living cultures of the new species as well as the dried specimens are deposited at the Natural History Museum and Institute, Chiba (CBM) and the Research Center for Medical Mycology, Beijing Medical University (CMMB).

Description

Aspergillus beijingensis D.-M. Li, Horie, Y.-X. Wang et R.-Y. Li, sp. nov. Figs. 1, 4–6

Coloniae in agar Czapekii celeriter crescentes, flavae vel olivaceo-flavae, velutinae; conidiogenesis per plurima; reversum flavo-brunneum vel laete brunneum. Coloniae in agar maltoso celeriter crescentes, griseo-virides, floccosae; conidiogenesis per plurima; reversum flavo-album. Capitula conidica flava vel griseo-viridia, radiata vel laxe columnaria, 50–150 × 20–100 µm. Conidiophora ex mycelio basali oriunda; stipites 100–550 × 4–5.5 µm, dilute griseo-virides, leves; vesiculae clavatae vel interdum ampulliformes, dilute griseo-virides, 7–11 (–14) µm diam, in summa 1/2 parte fertiles. Aspergilla uniseriata; phialides dilute griseo-virides, 10–19 × 4–5 µm. Conidia hyalina, globosa vel subglobosa,

3.5–6.5 × 3.5–5.5 µm, micro-verrucosa.

Holotypus: CBM-FD-285, in culture originally from maxillary sinusitis of 38-yr-old man, isolated by Y.-X. Wang, Department of Dermatology, Tongren Hospital, Chong Wen District, Beijing, China, as strain No. CMMB 2456, 21 June 1994, and deposited in the Natural History Museum and Institute, Chiba (CBM).

Etymology: Lat. *beijingensis*, referring to the type locality.

Colonies on Czapek's solution agar (CzA) spreading broadly, attaining a diam of 57–62 mm in 14 d at 25°C, consisting of a thick mycelial felt, velvety; conidial heads very abundantly produced, Virid Yellow (3A8 after Kornerup and Wanscher, 1978) to Olive Yellow (3C7); reverse Yellowish Brown (5E4) to Light Brown (5D4). Colonies on Czapek-yeast extract agar (CYA) spreading broadly, attaining a diam of 56–60 mm in 7 d at 25°C, consisting of a tough mycelium felt, radially furrowed, floccose; conidial heads abundantly produced, Olive (3D5), intermixed with loose white aerial hyphae; reverse Pale Yellow (4A3). Colonies on malt extract agar (MEA) spreading broadly, attaining a diam of 75–80 mm in 14 d at 25°C, consisting of a thin mycelial felt, floccose; conidial heads very abundantly produced, Greyish Green (28C4 to 30C5); reverse Yellowish White (2B2). Colonies on MY20 agar spreading broadly, attaining a diam of 51–52 mm in 7 d at 25°C, consisting of a thin mycelial felt, floccose; conidial heads abundantly produced, Greyish Yellow (3B6); reverse Light Yellow (4A4).

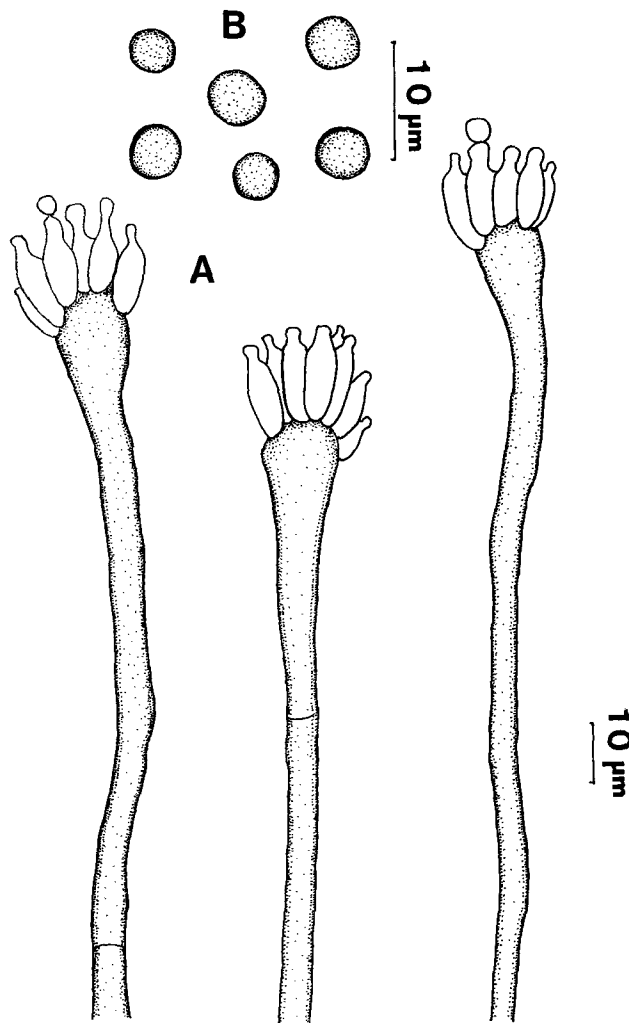


Fig. 1. *Aspergillus beijingsensis*.
A, Aspergilla. B, Conidia.

Conidial heads yellow to greyish green, radiate to loosely short columnar, $50\text{--}150 \times 20\text{--}100\text{ }\mu\text{m}$. Conidiophores arising from basal mycelium; stipes $100\text{--}550\text{ }\mu\text{m}$ long, $4\text{--}5.5\text{ }\mu\text{m}$ in diam at the middle, pale greyish green, septate, thick-walled, smooth; vesicles clavate, sometimes flask-shaped, pale greyish green, $7\text{--}11\text{--}(14)\text{ }\mu\text{m}$ in diam, fertile over the upper half. Aspergilla uniseriate; phialides pale greyish green, $10\text{--}19 \times 4\text{--}5\text{ }\mu\text{m}$. Conidia hyaline, greyish green in mass, globose to subglobose, $3.5\text{--}6.5 \times 3.5\text{--}5.5\text{ }\mu\text{m}$, micro-verrucose (under SEM, Fig. 6). Mycelium composed of hyaline to pale greyish green, septate, smooth, $5\text{--}7\text{ }\mu\text{m}$ wide, thick-walled (up to $1.5\text{ }\mu\text{m}$) hyphae. At 37°C , spreading broadly but growth rate and conidial heads are less than those at 25°C .

Specimen examined: CBM-FD-285 (holotype), isolated from clinical material of 38-yr-old man with maxillary aspergilloma. After the surgical removal of granulomata and draining of cavities of the right maxillary sinus, granulomata and draining of cavities was inoculated in Sabouraud's medium by Y.-X. Wang, Tongren Hospital,

Chongwen District, Beijing in June 21, 1994. The patient was cured by surgical resection of aspergilloma without antifungal agent treatment.

This species is regarded as belonging in the section *Flavi* W. Gams et al. of the subgenus *Circumdati* W. Gams et al. (= *Aspergillus flavus* group sensu Raper et Fennell (Raper and Fennell, 1965)). Within the section, *A. beijingsensis* mostly closely resembles *A. flavus* Link var. *columnaris* Raper et Fennell. Both species have uniseriate aspergilla and globose to subglobose, often delicately roughened conidia that are similar in size (Raper and Fennell, 1965; Christensen, 1981). However, length of conidial heads and width of vesicle of *A. beijingsensis* are significantly smaller than those of the latter species (e.g., up to $400\text{--}500\text{ }\mu\text{m}$ long conidial heads and $15\text{--}25\text{--}(40)\text{ }\mu\text{m}$ diam vesicle in *A. flavus* var. *columnaris*).

Recognition of the species as new is also based on its smooth-walled stipes of conidiophores, and in this character the species somewhat resembles *A. avenaceus* G. Smith, *A. lucknowensis* Rai, Tewari et Agarwal and *A.*

zonatus Kwon et Fennell (Raper and Fennell, 1965; Rai et al., 1968; Samson, 1979; Kozakiewicz, 1989). *Aspergillus beijingensis* is separated from these species by an absence of metulae and conidium size and/or shape (viz., *A. avenaceus*: ellipsoidal, $4\text{--}5\text{--}(6.5) \times 3.2\text{--}4\text{ }\mu\text{m}$ conidia; *A. lucknowensis*: globose, $2\text{--}2.5\text{ }\mu\text{m}$ diam conidia; and *A. zonatus*: oval, $2.8\text{--}3.4\text{--}(4.5) \times 2.2\text{--}2.8\text{--}(3.0)\text{ }\mu\text{m}$ conidia).

Aspergillus qizutongii D.-M. Li, Horie, Y.-X. Wang et R.-Y. Li, sp. nov. Figs. 2, 7, 8

Coloniae in agar Czapekii celeriter crescentes, olivaceo-flavae, velutinae vel floccosae; conidiogenesis perplurima; reversum laete aurantiacum vel griseo-flavum. Coloniae in agar maltoso celeriter crescentes, olivaceo-flavae, floccosae, planae; conidiogenesis perplurima; reversum griseo-flavum. Capitula conidica olivaceo-flava vel olivaceo-brunnea, radiata, $60\text{--}120 \times 30\text{--}70\text{ }\mu\text{m}$, vel laxae columnariae, $120\text{--}300\text{--}(500) \times 25\text{--}50\text{ }\mu\text{m}$. Conidiophora ex mycelio basali vel hyphis aeriis oriunda; stipites $100\text{--}300\text{--}(500) \times 6\text{--}8\text{ }\mu\text{m}$, dilute flavo-brunnei, asperati; vesiculae ampulliformes, dilute

olivaceae, $9\text{--}17\text{ }\mu\text{m}$ diam, in summa $1/2$ vel $3/4$ parte fertiles, saepe secunde prolificantes. Aspergilla uniseriata; phialides dilute olivaceae vel flavo-virides, $7\text{--}12 \times 2\text{--}4\text{ }\mu\text{m}$. Conidia hyalina, globosa vel ovoidea, $5\text{--}6.5\text{--}(7)\text{ }\mu\text{m}$, levia.

Holotypus: CBM-FD-284, in culture originally from maxillary sinusitis of 48-yr-old woman, isolated by Y.-X. Wang, Department of Dermatology, Tongren Hospital, Chong Wen District, Beijing, China, as strain No, CMMB 2516, 20 Jun. 1995, and deposited in the Natural History Museum and Institute, Chiba (CBM).

Etymology: named in memory of Professor Zu-tong Qi, Institute of Microbiology, Academia Sinica, Beijing, China, eminent mycologist.

Colonies on CzA spreading broadly, attaining a diam of $55\text{--}60\text{ mm}$ in 14 d at 25°C , consisting of a thin mycelial felt, velvety to floccose; conidial heads abundantly produced, Olive Yellow (3D8); reverse Greyish Yellow (4B5) to Light Orange (5A4). Colonies on CYA spreading broadly, attaining a diam of $58\text{--}59\text{ mm}$ in 7 d at 25°C , consisting of a tough mycelial felt, radially furrowed, floccose, more or less zonate; conidial heads

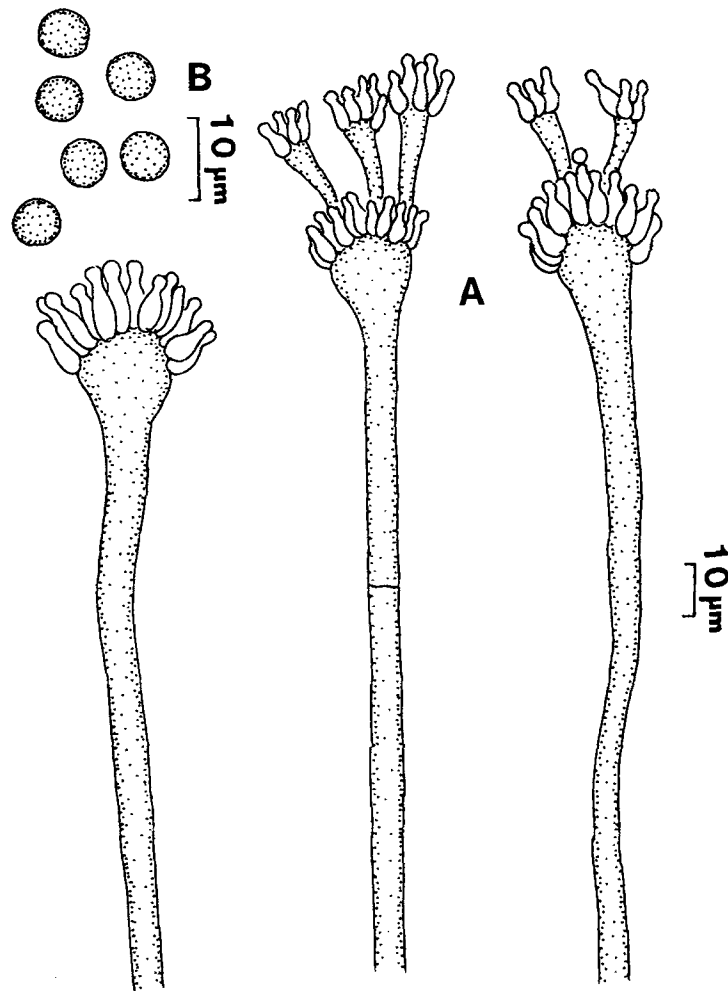


Fig. 2. *Aspergillus qizutongii*. A, Aspergilla. B, Conidia.

abundantly produced, Olive Brown (4D5); reverse Light Yellow (4A4). Colonies on MEA spreading broadly, attaining a diam of 75–76 mm in 14 d at 25°C, consisting of a thin mycelial felt, floccose; conidial heads very abundantly produced, Olive Yellow (2C6); reverse Greyish Yellow (4B4–5). Colonies on MY20 agar growing restrictedly, attaining a diam of 27–29 mm in 7 d at 25°C, floccose, conidiogenesis tardily appeared.

Conidial heads olive-yellow to olive-brown, radiate, 60–120 × 30–70 µm, or loosely long columnar, 120–300(–500) × 25–50 µm. Conidiophores arising from the basal mycelium and aerial hyphae; stipes 100–300(–500) µm long, 6–8 µm in diam at the middle, pale yellowish brown, straight to sinuous, conspicuously roughened, septate, swollen in the upper part; vesicles flask-shaped, pale olive, 9–17 µm in diam, fertile over the upper half or three-fourths; often proliferating (up to 20 µm long) to form a small secondary conidial head with 4–7 µm diam vesicle. *Aspergilla uniseriata*; phialides pale olive to yellowish green, 7–12 × 2–4 µm. Conidia hyalina, globose to ovoid, 5–6.5 (–7) µm in diam, smooth. Mycelium composed of pale greyish green, septate, smooth-walled, 2.5–5 µm wide hyphae. At 37°C, spreading broadly; growth and conidial heads are reduced than at 25°C.

Specimen examined: CBM-FD-284 (holotype), isolated from clinical material of 48-yr-old woman with maxillary aspergilloma. After the surgical removal of granulomata and draining of cavities of the right maxillary sinus, granulomata and draining of cavities was inoculated in Sabouraud's medium by Y.-X. Wang, Tongren Hospital, Chongwen District, Beijing in January 20, 1995. The patient was cured by surgical resection of aspergilloma without antifungal agent treatment.

Besides the frequent occurrence of secondarily proliferating aspergilla, *A. qizutongii* resembles *A. beijinensis* in conidiophore and conidium dimensions and in its production of uniseriate conidiogenous cells (phialides only), but differs from that species in its conspicuously asperate wall of conidiophore stipes and phialide size (7–12 × 2–4 µm in *A. qizutongii* versus 10–19 × 4–5 µm in *A. beijinensis*). The secondary proliferation of aspergilla was often observed on some cultures of other *Aspergillus* species: *A. apicalis* B. S. Mehrotra et Basu, *A. candidus* Link, and anamorphs of *Eurotium* spp. (usually seen in growth on non-osmophilic media) (Raper and Fennell, 1965; Mehrotra and Basu, 1976).

Aspergillus wangduanlii D.-M. Li, Horie, Y.-X. Wang et R.-Y. Li, sp. nov. Figs. 3, 9–11

Coloniae in agar Czapekii celeriter crescentes, dilute flavae vel griseo-flavae, floccosae; conidiogenesis perplurima; reversum dilute aurantiacum vel laete aurantiacum. Coloniae in agar maltoso celeriter crescentes, olivaceo-flavae, floccosae vel lanatae; conidiogenesis perplurima; reversum dilute flavum.

Capitula conidica obscure viridia, radiata vel laxae columnariae, 60–130 × 50–100 µm. Conidiophora ex mycelio basali vel hyphis aeriis oriunda; stipites 90–700(–900) × 6–17.5 µm, interdum ramosi, hyalini vel

dilute flavo-grisei, leves vel tuberculati, septati, basi ex hyphis convolutis cingentes; vesiculae subglobosae, dilute flavo-griseae vel dilute flavo-virides, 13–21 µm diam, in summa 1/2 vel 3/4 parte fertiles. *Aspergilla uniseriata*; phialides brunneo-virides, 8–13 × 4–6 µm. Conidia dilute brunneo-viridia vel brunneo-flava, globosa vel subglobosa, 4–6 µm diam, levia vel scabrella (sub LM), micro-verrucosa (sub SEM).

Holotypus: CBM-FD-283, in culture originally from 35-yr-old woman with maxillary sinusitis, isolated by Y.-X. Wang, Department of Dermatology, Tongren Hospital, Chong Wen District, Beijing, China, as strain No. CMMB 2309, 1 Dec. 1995, and deposited in the Natural History Museum and Institute, Chiba (CBM).

Etymology: named in memory of Professor Duanli Wang, Research Center for Medical Mycology, Beijing Medical University, Beijing, China, eminent medical mycologist.

Colonies on CzA growing rapidly, attaining a diam of 40–45 mm in 14 d at 25°C, consisting of a dense mycelial felt, floccose, furrowed; conidiogenesis abundant, Pale Yellow (4A3) to Greyish Yellow (2B4); reverse Pale Orange (5A3) to Light Orange (5A4). Colonies on CYA spreading broadly, attaining a diam of 61–62 mm in 7 d at 25°C, consisting of a tough basal felt with sparse aerial hyphae, conspicuously radially furrowed, more or less floccose; conidiogenesis limited in number, Yellowish White (4A2); reverse Pale Orange (6A3). Colonies on MEA spreading broadly, attaining a diam of 67–68 mm in 14 d at 25°C, consisting of a thin mycelial felt, floccose to lanose; conidiogenesis very abundant, Olive Yellow (2D7 to 2D8); reverse Pale Yellow (4A3). Colonies on MY20 agar growing rapidly, attaining a diam of 54–56 mm in 7 d at 25°C, floccose, slightly zonate; conidiogenesis abundant at the center, Light Yellow (4A5); reverse mostly uncolored.

Conidial heads dull green, radiate to loosely short columnar, 60–130 × 50–100 µm. Conidiophores arising mostly from the basal mycelium or aerial hyphae; stipes 90–700(–900) µm long, 6–17.5 µm in diam, sometimes irregularly or dichotomously branched, hyaline to pale yellowish grey, smooth to irregularly tuberculate, often constricted below the vesicle, septate surrounded by coiled hyphae in the basal part; vesicles pale yellowish grey to pale yellowish green, subglobose, 13–21 µm in diam, fertile over the upper half or three-fourths of surface. *Aspergilla uniseriata*; phialides brownish green, 8–13 × 4–6 µm. Conidia pale brownish green to brownish yellow, globose to subglobose, 4–6 µm in diam, smooth to minutely roughened (under LM), micro-verrucose (under SEM). Mycelium composed of greyish green to brownish yellow, septate, smooth-walled, 3–7 µm wide hyphae. At 37°C, spreading broadly; growth is slower than at 25°C and conidial heads are less produced.

Specimen examined: CBM-FD-283 (holotype), isolated from clinical material of 35-yr-old woman with maxillary aspergilloma. After the surgical removal of granulomata and draining of cavities of left maxillary sinus, granulomata and draining of cavities was inoculated in

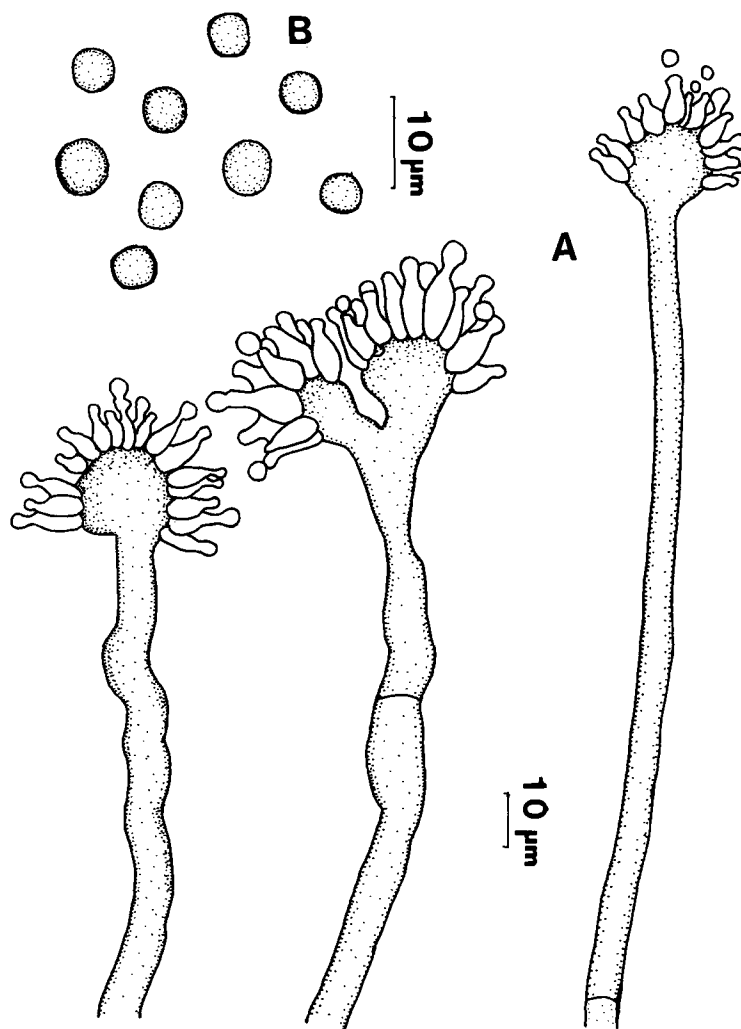


Fig. 3. *Aspergillus wangduanlii*.
A, Aspergilla. B, Conidia.

Sabouraud's medium by Y.-X. Wang, Tongren Hospital, Chongwen District, Beijing in December 1, 1995. The patient was cured by surgical resection of aspergilloma without fungal treatment.

The dull green, radiate to loosely columnar conidial heads, pigmented, smooth to roughened conidiophores that are constricted just below the vesicle and surrounded by coiled hyphae (as a network of distinctive "feeder" hyphae) in the basal part, subglobose vesicles, pale brownish green to brownish yellow conidia, place *A. wangduanlii* in the section *Sparsi* W. Gams et al. (=the *Aspergillus sparsus* group sensu Raper et Fennell) of the subgenus *Circumdati* (Samson and Gams, 1985). In their treatment of the *A. sparsus* group, Raper and Fennell (1965) recognized *A. funiculosus* G. Smith as the only species having uniseriate aspergilla. Conidia of the latter species in large heads borne on long stalks are at first elliptical ($3\text{--}3.5 \times 2\text{--}2.5 \mu\text{m}$) and smooth, subsequently becoming subglobose to globose ($3\text{--}3.5 \mu\text{m}$ in diam), brown and coarsely roughened, as are the conidia

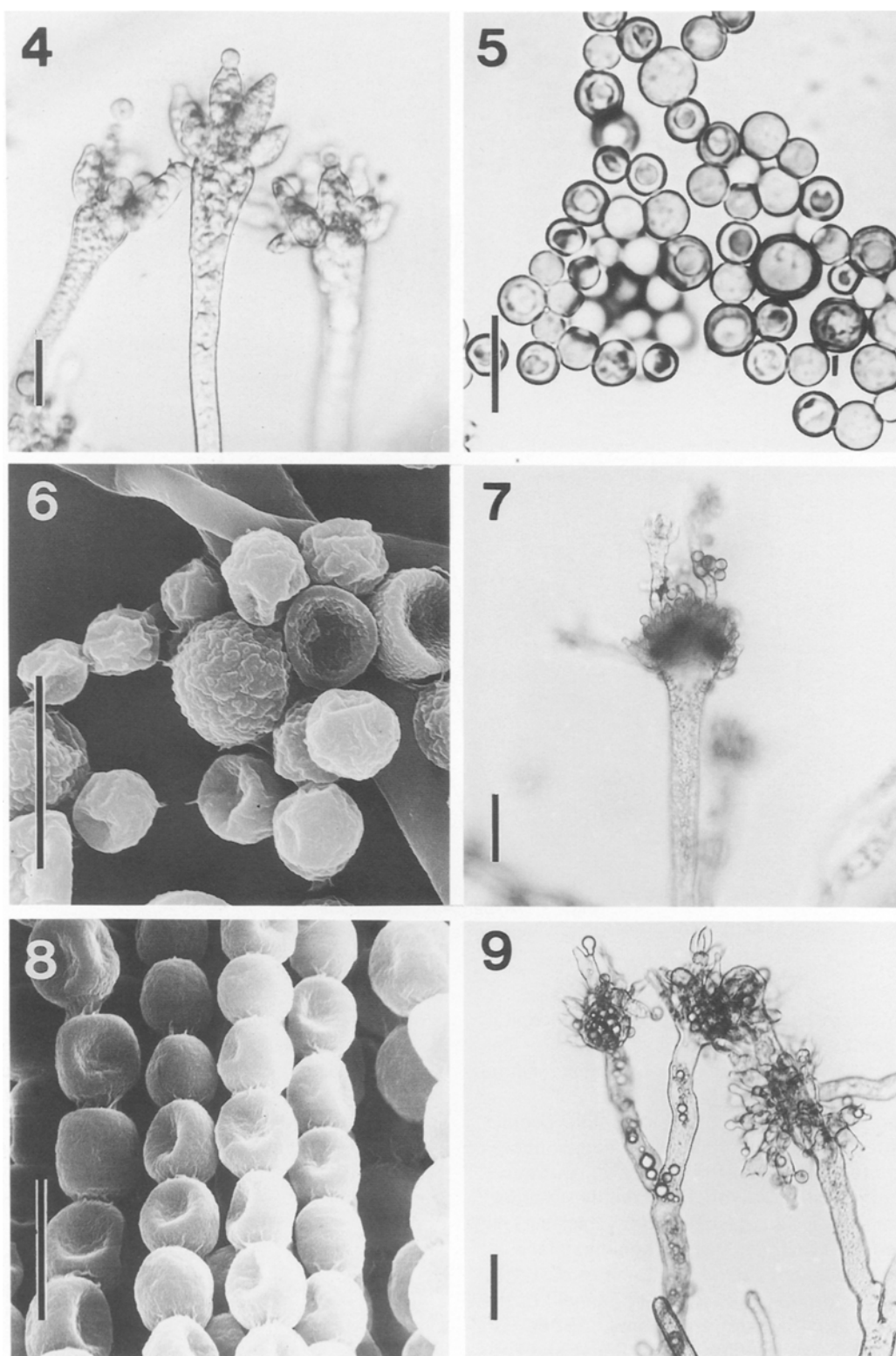
of short-stalked and fragmentary heads.

The conidial heads of *A. wangduanlii* are occasionally irregular due to the occurrence of branched conidiophores (Figs. 3, 9) and superficially resemble those of *A. dimorphicus* Mehrotra et Prasad in the section *Circumdati*. However, *A. dimorphicus* differs in the ochraceous buff or yellow-ochre to dark olive-buff colony color and in having biseriate aspergilla (Mehrotra and Prasad, 1969).

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Figs. 4–6. *Aspergillus beijingsensis*.

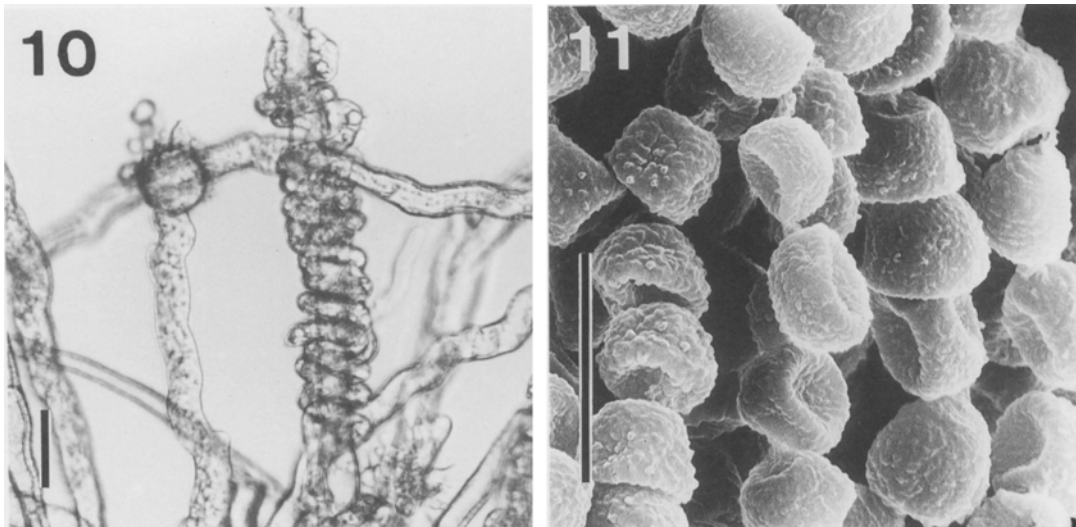
4. Aspergilla. 5. Conidia (LM). 6. Conidia (SEM).

Figs. 7, 8. *Aspergillus qizutongii*.

7. Aspergilla. 8. Conidia (SEM).

Fig. 9. *Aspergillus wangduanlii*. Aspergilla.

Scale bars: Figs. 4–6, 8 = 10 μm ; Figs. 7, 9 = 20 μm .



Figs. 10, 11. *Aspergillus wangduanlii*.
 10. Conidiophore surrounded by coiled hyphae. 11. Conidia (SEM).
 Scale bars: Fig. 10 = 20 μm ; Fig. 11 = 10 μm .

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