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NOTES ON ZYGORHYNCHUS SPECIES

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An account is given of species of *Zygorhynchus* maintained in the CBS collection, with emphasis on zygospore formation. A key to species is added.

Two difficult to identify isolates of *Zygorhynchus* spp., prompted a study of all CBS strains in the genus for comparison with the original diagnoses and with the descriptions given in the monograph of the genus by Hesseltine & al. (1959). This study, with emphasis on zygospore formation, led to a re-evaluation of some key characteristics used by Hesseltine & al. (1959).

All strains maintained in the CBS collection sub *Zygorhynchus californiensis, Z. exponens* var. *exponens, Z. exponens* var. *smithii, Z. heterogamus, Z. japonicus, Z. macrocarpus, Z. moelleri, Z. psychrophilus,* and *Z.* sp. were examined.

METHODS

Cultures were grown on beerwort agar at temperatures from 15 to 36°C, at 3°C increments; *Z. psychrophilus*, which does not grow at or above 20°C, was grown from 0 to 15°C. For similar reasons, *Z. japonicus* was grown at 5–20°C. Following the methodology of Hesseltine & al. (1959), cultures were also grown on D-glucose and D-xylose medium, and furthermore tested for amylolytic and lipolytic activity (after Hankin & Anagnostakis, 1975).

Media: beerwort agar containing 4% resp. 2% sugar; D-glucose and D-xylose medium, containing D-glucose or D-xylose 20 g, $MgSO_4$ $7H_20$ 0.25 g, L-asparagin 2.0 g, KH2PO4 0.5 g, thiamine-HCl 0.25 g, trace elements solution 1 ml, agar 15 g in 1 l de-ionized water; malt yeast agar: malt extract 10 g, yeast extract 4 g, glucose 4 g per litre; V8 juice agar (200 ml V8 per litre); oatmeal agar: extract of 30 g flakes per litre.

Zygorhynchus Vuillemin (1903)

Type species: *Zygorhynchus heterogamus* (Vuill.) Vuill. (1903) = *Mucor heterogamus* Vuill. (1886).

Vuillemin (1903) distinguished *Zygorhynchus* from *Mucor* on differences of the zygospore-apparatus.

In *Zygorhynchus* the suspensors are unequal, both in size and shape, the smaller one straight and short, the larger long, curving upwards and widening towards a pyriform shape. The most conspicuous feature in *Zygorhynchus* is the occurrence of the com-

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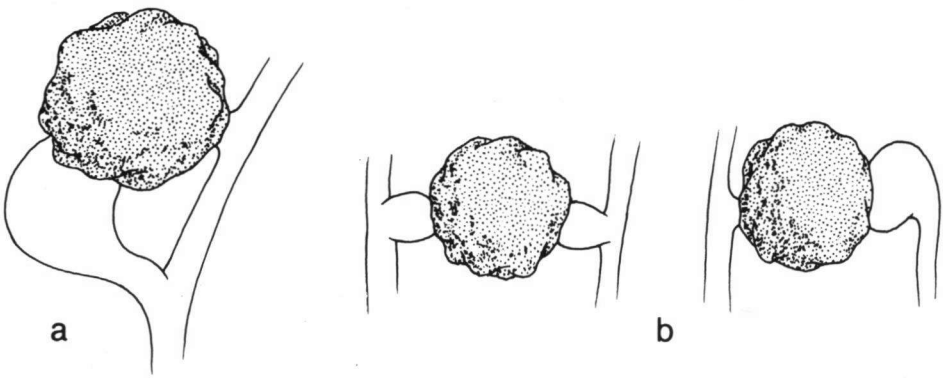

Fig. 1. Zygosporic stages. — a. *Zygorhynchus*. — b. *Mucor*. (Schematic.)

plete zygospore apparatus, with extremely unequal suspensors, on the same aerial hypha.

In the closely related genus *Mucor*, suspensors, varying from equal to rather unequal, always originate from different hyphae, both in heterothallic and homothallic species (Fig. 1). In some species of *Zygorhynchus*, however, some zygospores may be formed following the *Mucor* pattern. Green (1927) found that in *Z. moelleri* Vuill. 'sometimes the gametangia were produced on different zygophores and that one branch might produce two gametangia which might fuse with others on independent zygophores'. Hesseltine & al. (1959) described and discussed the mixture of *Zygorhynchus* and *Mucor* patterns in *Z. exponens* Burgeff, where both patterns are quite common. The same situation was observed in the present study in CBS 154.69, *Z. japonicus* Kominami.

Zygorhynchus exponens Burgeff — Figs. 2, 4e

Zygorhynchus exponens Burgeff in Bot. Abh. 4: 34. 1924.

The three strains of *Z. exponens* maintained in the CBS collection were all derived from the type strain. The strain denoted *'niger'* by Burgeff, a single-spore isolate of the type strain, is (now) not different from the others.

On beerwort agar at 24°C colonies of all three showed grey and white patches, when grown at 27–30°C they were yellow and grey. Zygospores were formed in both the *Zygorhynchus* and *Mucor* fashion, the latter prevailing.

Material examined. — CBS 141.20, type strain of *Zygorhynchus exponens*, ex forest soil, Geisenheim, Germany, *H. Burgeff* = CBS 403.58 = NRRL 1492 = Blakeslee C–972, subculture of 141.20. — CBS 508.48, strain *'niger'*, single spore isolate from CBS 141.20, *H. Burgeff*.

Hesseltine & al. (1959) considered zygospore formation in this species intermediate between *Z. moelleri* and homothallic *Mucor* species.

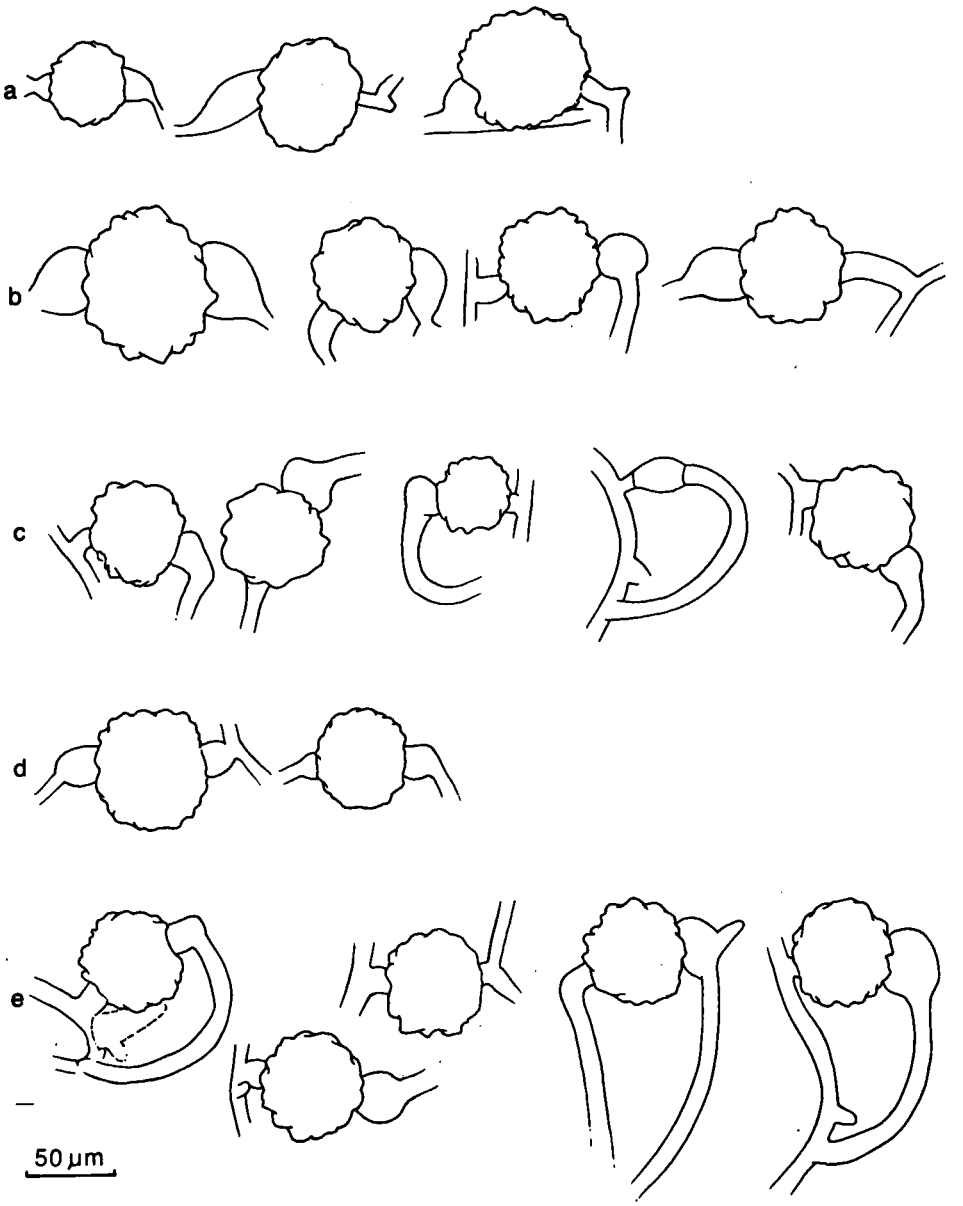

Fig. 2. Zygosporic stages. — a. *Mucor plumbeus*, CBS 213.75 (+) × 848.73 B (–). — b. *Zygorhynchus exponens*, CBS 141.20. — c. *Z. exponens*, CBS 508.48. — d. *Z. exponens*, CBS 403.58 — e. *Z. exponens* var. *smithii*, CBS 404.58.

The patchy colouring of *Z. exponens* colonies is reminiscent of strains with an unbalanced sexual state e.g. azygosporic descendants of normally heterothallic species. Morphologically, *Z. exponens* is most closely related to *Mucor plumbeus*: like *M. plumbeus*, *Zygorhynchus exponens* has short racemose branches with small sporangia; breaking sporangial wall; (some) conical columellae with a short apical projection; globose, verrucose sporangiospores; brown zygospores which are (often) borne between equal or inequal suspensors originating from different hyphae and zygospore ornamentation of similar patterns (Schipper & al., 1975).

Occasionally sporangia and zygospores are formed on the same hypha. Sporangia, sporangiospores and zygospores of *Mucor plumbeus* and *Zygorhynchus exponens* are of about the same size. The differences between the two species are the shape of the majority of columellae and the occurrence of zygospores borne between suspensors originating from one hypha.

Zygorhynchus exponens in the *Zygorhynchus* stage showed only moderately enlarged suspensors, rather variable in shape (Fig. 2). Unusual though the characters may seem, the species has proved to be stable. After sixty years of repeated culturing, the type strain still fits the author's drawings, and so does the 1948-single spore isolate.

Dr. W. Gauger, University of Nebraska, kindly germinated zygospores of CBS 141.20. Germination was unusually fast: after only 8 days the first germination was observed. Thirteen viable germinations were obtained: two germ sporangia and eleven mycelial germinations. Both single germ spore isolates (five from one germ sporangium) and hyphal tip isolates (nine, taken at random) produced colonies identical to the parental one.

CBS 404.58, the type strain of *Z. exponens* var. *smithii*, shows similar features and is regarded as synonymous.

Material examined. — CBS 404.58, type strain of *Zygorhynchus exponens* var. *smithii* Hesseltine & al. = NRRL 2572 = IMI 79624 = IFO 6665, ex soil, U.K.

Zygorhynchus japonicus Kominami — Fig. 3

Zygorhynchus japonicus Kominami in Mykol. Zentbl. 5: 3. ('1914') 1915.

Kominami's strain is no longer known to exist. In 1968 a similar fungus was isolated by A. A. Milko (Pidoplichko & Milko, 1971) which was available for study: CBS 154.69 (= VKM F-1382, ex forest soil, Chesnovizkaja Region, USSR). In this strain, a *Mucor* zygosporic condition was found to occur alongside the *Zygorhynchus* condition.

CBS 154.69 did not grow at 25°C, however good growth and sporulation occurred between 5 and 20°C. It was grown on 4% beerwort agar, 2% beerwort agar, maltyeast agar, oatmeal agar and V8 juice agar, at 20°C. In each colony the *Mucor* pattern of zygospore formation seemed to prevail. The typical '*japonicus*' pattern, as described by Kominami, was rare, and occurred mixed with the '*Mucor*' pattern on the same hyphae; indisputable azygospores were observed (Fig. 3). Pairs of zygospore-bearing aerial hyphae arising separately from the substratum were distinct in young cultures. Colonies derived from isolated zygospores were similar.

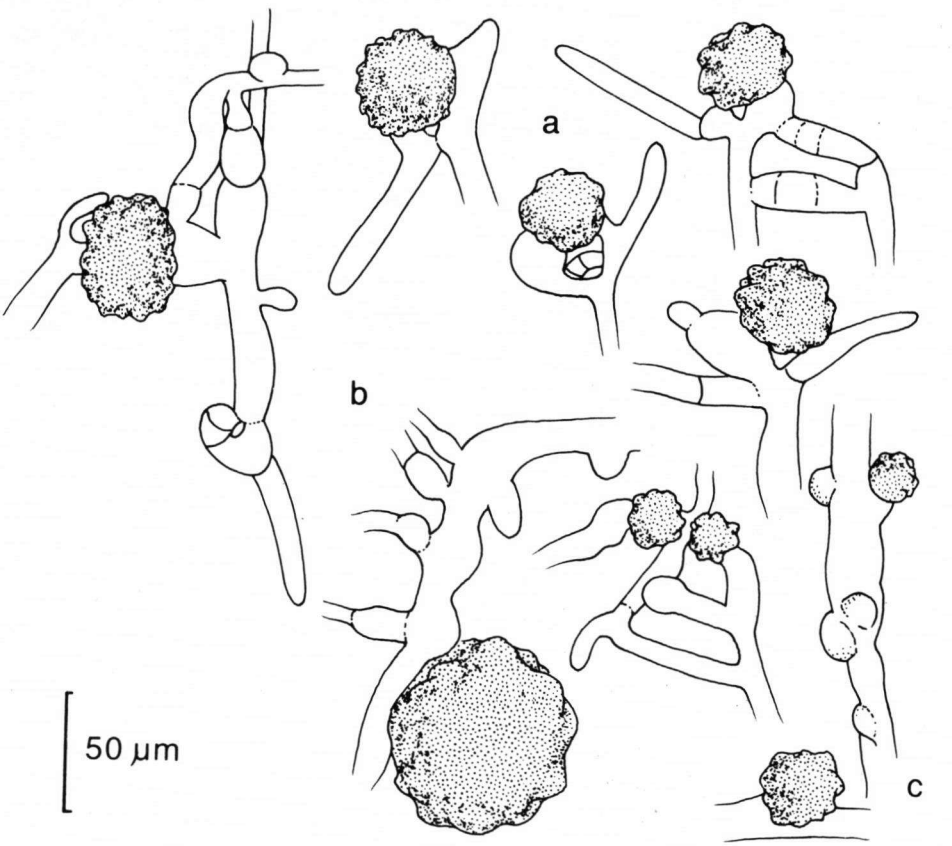

Fig. 3. Zygosporic stages in *Zygorhynchus japonicus*, CBS 154.69. — a. 'Typical' pattern. — b. Prevailing pattern. — c. Azygospores.

Zygorhynchus japonicus does not closely resemble any known *Mucor* species. There are some similarities with *Mucor bacilliformis* Hesseltine (1954). One of these is the attraction of several aerial hyphae by a single one of opposite sex resulting in an arachnoid appearance in undisturbed young colonies.

Zygorhynchus heterogamus (Vuill.) Vuill. & Z. macrocarpus Ling — Fig. 4c

Zygorhynchus heterogamus (Vuill.) Vuill. in Bull. trimest. Soc. mycol. Fr. 19: 116. 1903.
Zygorhynchus macrocarpus Ling in Revue gén. Bot. 42: 7. 1930.

Of both species some type material is still in existence:

The holotype material of *Z. heterogamus* = *Mucor heterogamus* Vuill. consists of slides prepared by Vuillemin in 1886. (Hesseltine & al. (1959) studied and described these slides).

CBS 215.27 = NRRL 2663 = type strain of *Z. macrocarpus*, ex soil of peatery, France.

The first description of *Z. heterogamus*, complemented with Vuillemin's drawings, and the observations of the original slides by Hesseltine & al. gave the following features: sporangia av. 50–60 μm diam.; columellae globose (on narrow base); sporangiospores globose to short ellipsoidal, 3–5.5 × 2.1–4 μm; zygospores 45–150, mostly over 100 μm diam, the larger suspensors subglobose.

These features differ slightly from those given by Hesseltine & al. (1959) as a composite after five isolates, identified with *Z. heterogamus* and cultured on SMA viz. sporangia mostly up to 35 (15–55) μm diam.; columellae applanate to almost globose; sporangiospores irregular short oval, a few globose, 2–4 × 4–6.5(5 × 8) μm; zygospores 30–70 μm diam.; the larger suspensors abruptly inflated, globose.

Material examined. CBS 215.27 = NRRL 2663 = type strain of *Zygorhynchus macrocarpus* Ling, ex soil of a peatery, France. — CBS 455.58 = NRRL 1616 = Harvard University Collection no. 202. — CBS 338.74, ex sediment in a drain pipe, Sweden. — CBS 580.83, ex sandy soil, potatofield, Haren, The Netherlands (1981). — CBS 594.83, ex soil, Páramo Cruz Verde, ca. 3000 m alt., Cundinamarca, Columbia, *H. Valencia*; isol. W. Gams.

The type strain of *Z. macrocarpus* fits the above description of *Z. heterogamus*. Ling Young (1930) noted the similarity of zygospores in both species, but omitted to indicate differences. Also fitting this characterization are the recent isolates CBS 580.83 and CBS 594.83.

Influence of temperature: at 30°C no growth in CBS 215.27, 580.83 and 594.83; at 27°C growth and sporangia in CBS 215.27, growth restricted or absent in CBS 580.83 and 594.83; at 15–24°C growth and sporulation, both asexual and sexual.

REMAINING SPECIES

The remaining species (strains) fit the original and Hesseltine & al.'s descriptions. This concerns:

Zygorhynchus californiensis Hesseltine & al.: CBS 402.58 = NRRL 2658 = IFO 6663, T, ex soil, California.

Zygorhynchus moelleri Vuill.: CBS 111.10 = NRRL 1497, rec. as *Z. vuilleminii* Namyslowski var. *agamus* Namyslowski, *B. Namyslowski*; 216.27 = IMI 21113, Y. Ling; 284.28, CLMR; 380.29, ex wood; 348.37, culture contaminant, CMI; 395.49, ex soil; 581.50, ex root *Gerbera* sp., 460.51 = IMI 47187, culture contaminant; 406.58 = NRRL 2660, ex soil, Wisconsin; 444.56 = ATCC 16388 = VKM F-1366, (T, *Mucor saximontensis* Rall), ex soil, USA, *G. Rall*; 501.66, ex soil, Austria.

Zygorhynchus psychrophilus Schipper & Hintikka 336.68, T, ex brown needles of windblown *Picea* sp., the needles being immersed in snow, Finland, *V. Hintikka.*

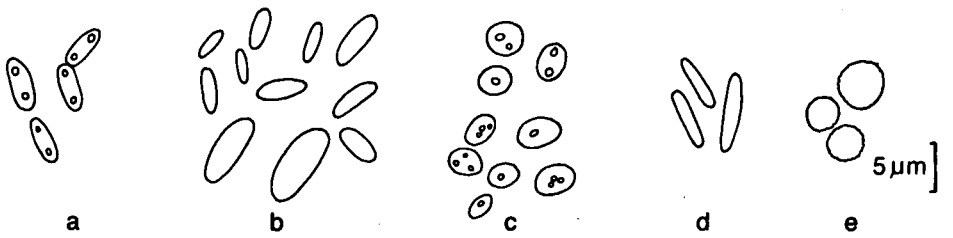

Fig. 4. Sporangiospores. — a. *Zygorhynchus moelleri*. — b. *Z. japonicus*. — c. *Z. heterogamus*. — d. *Z. psychrophilus*. — e. *Z. exponens*.

VARIABILITY OF FEATURES

Hesseltine & al. (1959) used the shape of the larger suspensor, shape of the columellae and shape and size of the sporangiospores as primary characteristics for species delimitation in the genus *Zygorhynchus*.

Columellae: Though applanate, globose and short ellipsoidal columellae are present in most strains, a particular shape prevails in each species.

Sporangiospores: Shape of the sporangiospores is constant in each species. Cylindrical-ellipsoidal sporangiospores, mostly with a droplet at either end, are distinctive for *Z. moelleri*; ellipsoidal-oval sporangiospores, very variable in size, are produced by *Z. japonicus*; in *Z. psychrophilus* sporangiospores are fusiform; globose (or subglobose) sporangiospores are produced in *Z. californiensis*, *Z. exponens*, and *Z. heterogamus*, verruculose in *Z. exponens* and smooth in *Z. californiensis*, and *Z. heterogamus* (Fig. 4).

Suspensors: Suspensors of a pyriform shape are present in *Z. californiensis* and *Z. moelleri*; abrupt enlargement, resulting in globose, pyriform or intermediate forms, has been observed in *Z. heterogamus* and *Z. psychrophilus*.

The CBS strains developed generally well on D-glucose and D-xylose as carbon source; no lipase activity was detected; amylase activity was weak or absent.

DISCUSSION

Zygorhynchus is closely related to *Mucor*. Differences are relative rather than absolute:

(i) The species of *Zygorhynchus* are always homothallic, whereas homothallism is rare in *Mucor*.

(ii) In *Zygorhynchus* the production of both zygospores and sporangia on the same hypha is usual, in *Mucor* this condition is rare.

(iii) In *Zygorhynchus* (inequal) gametes, borne on the same hypha, are always present; in *Mucor* gametes are on different hyphae.

Schipper & Stalpers (1980), discussing the various aspects of the mating system in Mucorales, stated that neither heterothallism nor homothallism are absolute conditions.

Homothallic strains are known to show a preference for a particular mating type, e.g. Satina & Blakeslee (1930) found a (+)tendency in strains of *Zygorhynchus heterogamus* and a (-)tendency in strains of *Z. moelleri* in mating behaviour; this tendency was confirmed by Werkman & Van den Ende (1974) in their studies on conversion of sex-hormone precursors; Schipper (1971) induced zygospore formation in the *Zygorhynchus* manner in agamic strains of *Z. moelleri* through contrasts with either (+) or (-) 'partners', but not both.

In homothallic *Mucor* species (+) and (-) zygophores are, at some distance, connected through the substrate mycelium. In the *Zygorhynchus* manner of zygospore formation, the location of (+) and (-) is much closer; also, with decreasing distance, the locations are less fixed (see e.g. Schipper & Stalpers, 1980). Still, the difference may be looked upon as a matter of degree.

The major reason for retention of the genus and its intermediate species is the fact that the *Zygorhynchus* pattern has never been observed in *Mucor*, and the stability of this pattern in the species with both the *Zygorhynchus* and the *Mucor* pattern of zygospore production. The species *Z. exponens* and *Z. japonicus* are rare. In the studied strains of the common *Z. moelleri* the *Mucor* pattern was not found, nor was it found in *Z. californiensis*, *Z. heterogamus/Z. macrocarpus*, or *Z. psychrophilus*.

Though some similar species exist in the two genera they are certainly not identical.

EXCLUDED SPECIES

Von Arx (1982) transferred three *Mucor* species to the genus *Zygorhynchus* on the assumption of a closer relationship to *Zygorhynchus* than to *Mucor*, but failed to specify the reasons.

The species considered, viz. *Mucor bacilliformis* Hesseltine (homothallic, suspensors equal), *Mucor amphibiorum* Schipper (heterothallic, suspensors equal) and *Mucor indicus* Lendner (heterothallic, suspensors inequal) produce copulating gametangia that originate from separate hyphae. A '*Zygorhynchus*' pattern of zygospore formation has never been observed in these species.

KEY TO THE SPECIES

1. Sporangiospores globose or subglobose 2
1. Sporangiospores cylindrical-ellipsoidal, ellipsoidal or fusiform 4
2. Sporangiospores rough; sporangial wall breaking at maturity; zygospore production mostly in *Mucor* manner, fewer in *Zygorhynchus* manner . . . . . *Z. exponens*
2. Sporangiospores smooth 3
3. Zygospores black, up to 80 µm diam. and beyond; sporangiospores globose, subglobose to short ellipsoidal . . . . . *Z. heterogamus*
3. Zygospores brown, up to 60 µm diam.; sporangiospores globose . . . . . *Z. californiensis*

4. Sporangiospores cylindrical-ellipsoidal, typically 4 × 2 (5 × 2.5) μm, with a droplet at either end; zygospores 50 μm in max. diam. . . . . *Z. moelleri*
4. Sporangiospores oval-ellipsoidal or fusiform 5
5. Sporangiospores fusiform, rather equal; no growth at 20°C . . . . . *Z. psychrophilus*
5. Sporangiospores oval-ellipsoidal, very variable in size, up to 10 μm in length; suspensors (in the *Zygorhynchus* stage) very near to each other; over 20°C no growth. . . . . *Z. japonicus*

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