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Short Communication

Isolation and identification of *Neosartorya* species from house dust as hazardous indoor pollutants

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Isolation of ascomycetous microfungi from 58 house dust samples from detached and apartment dwellings around Kobe City revealed that species of *Talaromyces*, *Eurotium* and *Neosartorya* were common ascomycetous propagules in the house dust. *Neosartorya* species accounted for 8.3% of the total identified isolates, of which *N. pseudofischeri* was the main constituent, indicating that it may be a common potential fungal pathogen in the dwelling environment. Based on the specimens collected, *N. pseudofischeri* is described and illustrated as new to Japan.

Key Words——ascomycetes; hazardous fungi; indoor environment; *Neosartorya*; *Neosartorya pseudofischeri*.

In our routine work on the relationship between damp and hazardous molds in the indoor environment, some members of the ascomycetous genus *Neosartorya* have frequently been isolated from Nagoya City. For example, *N. quadricincta* (E. Yuill) Malloch et Cain was isolated from molded plaster board for ceiling material in 1982 and *Neosartorya* sp. (later identified as *N. pseudofischeri* Peterson) from an air sample of a living room in a detached dwelling in 1986. Little attention has been paid to ascomycetous fungi in the indoor environment as a potential hazard to respiratory health, although xerophytic ascomycetes belonging to the genus *Eurotium* have proved to be very common in house dust, some of which were shown to be associated with the ecology of house dust mites as an origin of house-dust allergen (Lustgraaf, 1977; Lustgraaf and Bronswijk, 1977; Samson and Lustgraaf, 1978; Samson, 1985; Beguin, 1995). Pollution by ascomycetous fungi of common materials in the dwelling environment has been overlooked because they fail to grow or are overgrown by other fast-developing fungi in routine surveillances of domestic fungi by the usual detection methods (Brundrett and Onions, 1980; Hunter et al., 1988; Miller et al., 1988; Hamada and Morita, 1990; Summerbell et al., 1992). Thus a floral approach to associate nonxerophytic ascomycetes with the domestic environment has not been reported. The objective of this study was to determine whether undesirable nonxerophytic ascomycetes such as *Neosartorya* species are present in the mycoflora of household wastes.

Materials and Methods

House dust samples Fifty-eight house dust samples were collected during March to May of 1990 and July of 1991. These were 42 samples from detached dwellings and 16 samples from apartment dwellings located around Kobe City, western Japan. The dust was obtained using the home-owner's vacuum cleaner fitted with a sterilized collection bag. The samples were stored separately in polyethylene bags on a dry place at 4°C until used to isolate microfungi.

Detection of microfungi by selective isolation method Sieved dust sample (3 g per sample) was placed into Erlenmeyer flasks and immersed in 65% alcohol for 8 min. The alcohol was then discarded, and the processed sample was washed several times by sterile distilled water. The processed sample was diluted by adding 20 ml of sterile distilled water and heated at 60°C for 30 min in a water bath (Warcup and Baker, 1963). Each sample of 1 ml was added to potato-dextrose agar (PDA) containing 100 mg/L chloramphenicol, poured into a Petri dish (95 mm in diam) and incubated at 25°C for 14 d. The colonies of microfungi were counted and identified based on their macroscopic and microscopic morphology.

Species identification Ascomycetous fungi isolated from the PDA plates were subcultured on Czapek, Czapek-yeast extract (CYA), malt extract (MEA) and oatmeal agars as an aid to species identification. The morphology of their ascospores was usually studied with scanning electron microscopy (SEM). For SEM, ascospores were fixed in 2% osmium tetroxide, dehydrated in a graded alcohol series, critical-point dried, and sputter coated with platinum-palladium.

Results and Discussion

Ascomycetous flora of house dust Mycoflora of house dust was analyzed by an isolation method involving preheating of samples, which resulted in the death of most mycelial fungi and the germination of dormant ascomycete spores. Thus about 1,000 cultures of thermotolerant microfungi were isolated in total from the 58 house dust samples analyzed in the present survey. As expected, of the samples plated on PDA, ascomycetes comprised 84% (796 isolates) of the total of 948 growing colonies, whereas deuteromycetes represented only 7.5% (Table 1). The species identification of these isolates is shown in Table 2. Species of *Talaromyces*, *Eurotium* and *Neosartorya* and *Paecilomyces variotii* were prominent. Although the predominant ascomycetes isolated from house dust were species of *Talaromyces* (anam. *Penicillium*), two teleomorphic genera of *Aspergillus*, namely, *Eurotium* and *Neosartorya*, were found that have more definite health implications as potential causatives of infection and allergy. Recent observations suggest that *Eurotium* species and some other xerophytic fungi may be among the effective causative agents of human allergies, particulary bronchial asthma (S. Torii, personal communication). Here *Neosartorya* species are worthy of further comments.

Isolation of *Neosartorya* and its significance The genus *Neosartorya* was introduced by Malloch and Cain (1972) for members of the *Aspergillus fischeri* series in the *Aspergillus fumigatus* group of Raper and Fennell (1965). The series is characterized by white or yellowish colonies, globose, nonostiolate ascomata with a membranaceous peridium, globose to ovoid, unitunicate asci and hyaline, lenticular ascospores with two or more equatorial crests. The anamorphs, characterized by columnar, light blue-green to dark green conidial heads, conidiophores with a flask-shaped vesicle, uniseriate aspergilla and globose or ellipsoidal, small conidia, are almost identical with *Aspergillus fumigatus* Fres. Most species are thermotolerant, being able to grow well at 37°C or higher temperatures.

Table 1. The percentage isolation of microfungi from house dust samples after preheating treatment.

Organisms	No. of isolates	Isolation %
Ascomycetes	796	84.0
Deuteromycetes	71	7.5
Unidentified fungi	81	8.5
Total	948	100.0

Aspergillus fumigatus is the chief etiological agent of aspergillosis, which is variously subclassified into several clinical entities. Its occurrence in the environment principally causes allergic bronchopulmonary aspergillosis hypersensitivity pneumonitis and immunoglobulin E mediated asthma (Mishra et al., 1992). *Aspergillus neoellipticus* Kozakiewicz, which differs from *A. fumigatus* in having elliptical and smooth conidia rather than globose and lobate-reticulate ones, is also known to be a human pathogen. Both species are strictly anamorphic, having no known teleomorph. A close relationship between *A. neoellipticus* and *Neosartorya fischeri* (Wehmer) Malloch et Cain, however, has been demonstrated by Ishizaki et al. (1995) using data based on mitochondrial DNA restriction fragment length polymorphism. They suggested that *A. neoellipticus* was assignable to the anamorphic state of *N. fischeri*. Although there are occasional reports describing *Neosartorya* species as human pathogens, SEM examination of conidia is required for definitive identification of clinical isolates of *A. fumigatus* (Kozakiewicz, 1989). Otherwise, clinical isolation of anamorphic *Neosartorya* may be dismissed as "*A. fumigatus*" without further identification.

One of the most interesting fungi encountered here is *N. pseudofischeri* (Peterson, 1992). This fungus had been isolated at autopsy from fungal lesions of human neck vertebrae and sent for identification from the U.S. Centers for Disease Control, Atlanta, to the ARS Culture Collection, the National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, Illinois. Ascospores of *N. pseudofischeri* clearly align the

Table 2. The identity of microfungi isolated from house dust samples by selective isolation method.

Species	No. of isolates	Isolation %	Species	No. of isolates	Isolation %
Ascomycetes			*Eupenicillium brefeldianum*	3	0.3
Talaromyces flavus	211	22.3	*Eupenicillium* spp.	19	2.0
T. trachyspermus	128	13.5	*Gelasinospora* spp.	7	0.7
T. macrosporus	18	1.9	*Chaetomium globosum*	3	0.3
T. wortmannii	7	0.7	*Pseudeurotium zonatum*	1	
T. helicus	1		Deuteromycetes		
Eurotium amstelodami	185	19.5	*Paecilomyces variotii*	53	5.6
E. herbariorum	124	13.1	*Trichocladium pyriforme*	8	0.8
E. rubrum	17	1.8	*Gilmaniella humicola*	5	0.5
Neosartorya pseudofischeri	51	5.4	*Penicillium* spp.	3	0.3
N. glabra	17	1.8	*Aspergillus fumigatus*	1	
N. quadricincta	4	0.4	*Periconia* sp.	1	

species with the *N. fischeri* series. The convex ascospore walls, which are ornamented with triangular flaps of tissue or short, nonanastomosing ridges (Fig. 2-C), serve to distinguish them from those of *N. fischeri* and other species with highly ornamented ascospore walls. A fundamental knowledge of the ecology of *N. pseudofischeri* in human dwellings is of remarkable significance for the environmental control of opportunistic infection and allergenically mediated aspergillosis, because all of the *Neosartorya* strains in the ARS Culture Collection which were recorded as being of clinical origin were morphologically and genetically identical to *N. pseudofischeri*. These strains have been obtained in several cases from debilitated patients, or those suffering from other diseases (Gerber et al., 1973; Peterson, 1992). Although *Neosartorya* accounted for 8.3% of the total identified isolates, the detection of *N. pseudofischeri* as a main constituent of the *Neosartorya* species and its known association with human opportunistic diseases suggest that *N. pseudofischeri* may be a common potential pathogen in the indoor environment and its recovery from clinical materials must not be discounted as contamination.

Description of *N. pseudofischeri* A distinctive *Neosartorya* collected from an air sample of a living room in Nagoya City, first thought to represent a new species (unpublished report by Tsubouchi, 1986), has now been identified as *N. pseudofischeri*. The recent description of *N. pseudofischeri* by Peterson (1992) matched the Nagoya specimen in the shape, size and ornamentation of the ascospores. The abundance of the subsequent materials in the house dust has provided an opportunity to describe *N. pseudofischeri* for the first time from Japan.

Neosartorya pseudofischeri Peterson, Mycol. Res. **96**: 549. 1992. Figs. 1, 2

St. anam. *Aspergillus thermomutatus* (Paden) Peterson, Mycol. Res. **96**: 549. 1992.

Basionym. *Aspergillus fischeri* Wehmer var. *thermomutatus* Paden, Mycopathol. Mycol. Appl. **36**: 161. 1968.

Colonies on Czapek agar spreading broadly, attaining a diameter of 54–56 mm in 7 d at 25°C, 85 mm within 7 d at 37°C, more or less floccose, thin, loose-textured, White to Pale Yellow (M. 4A3 after Kornerup and Wanscher, 1978) or Buff (Rayner, 1970); ascomata slowly produced; conidiogenesis inconspicuous, not sufficiently produced to influence the colony appearance; reverse Pale Yellow (M. 4A2) or Primrose (R). Colonies on CYA spreading broadly, attaining a diameter of 85 mm in 7 d at 25°C, velvety, more or less radially sulcate, consisting of a thin mycelial felt intermixed with numerous ascomata in a granular appearance, Yellowish White to Pale Yellow (M. 4A2-3) or Primrose (R); conidiogenesis scattered in submarginal areas but inconspicuous; exudate small, clear; reverse Pale Yellow (M. 3A3) to Buff (R). Colonies on CYA at 37°C growing more rapidly than at 25°C, velvety, radially sulcate, zonate, consisting of a thin basal felt; ascomata tardily produced; conidiogenesis heavy, Dull Green to Purplish Grey (M. 25E3-14F2) or Fuscous Black (R); reverse Pale Yellow or Pale Luteous (R). Colonies on MEA spreading broadly, attaining a diameter of 85 mm within 7 d at 25°C, more or less radially sulcate, thin, loose-textured, consisting of a uniform layer of white ascomata adjacent to the agar surface; conidiogenesis limited, not sufficiently produced to influence the colony appearance; exudate small, clear; reverse Yellowish White to Pale Yellow (M. 3-4A3) or Pale Luteous (R).

Ascomata nonostiolate, initiated from coiled hyphae, superficial, scattered, white to pale yellow, globose to subglobose, 150–450 μm in diam, surrounded by hyaline aerial hyphae; peridium thin, 12.5–20 μm thick, pseudoparenchymatous, multilayered; outer layer consisting of textura epidermoidea, irregular, thick-walled cells measuring 4–7.5 μm wide; inner layers of textura angularis, thin-walled, flatten cells. Asci 8-spored, borne in short chains, globose to ovoid, 12.5–15 × 11–13 μm, evanescent. Ascospores hyaline, lenticular, 6–7.5 × 4–5 μm (4.5–5 μm in diam excl. crests), sometimes larger up to 9 × 7.5 μm, provided with two equatorial crests which are prominent and well-separated; convex surfaces ornamented with triangular flags or short linear ridges.

Conidial heads small, loosely radiate. Conidiophores arising from the basal mycelium, hyaline or often purplish in the lower part, straight or sometimes sinuous; stipes 120–450 × 5–7.5 μm at the middle, smooth-walled, septate; vesicles clavate to flask-shaped, 12.5–20 μm in diam. Aspergilla uniseriate; phialides cylindric, 5–8.5 × 2–3 μm, covering the upper half to two-thirds of the vesicle. Conidia hyaline, green in mass, subglobose to broadly ovoid, 2.5–4 μm in diam, smooth-walled.

Specimens examined: NCI 2043, a culture of an isolate from the air in the living room of a detached dwelling, Nakagawa-ku, Nagoya-shi, Japan, collected by H. Tsubouchi, 3 October 1986; SUM 3041-3044, cultures of isolates from house dust, Chuo-ku, Kobe-shi, Japan, collected by N. Toyazaki, March-May 1990 and July 1991.

Recently, the reduction of ventilation rates in many Japanese dwellings, especially in modern residential buildings, has resulted in serious problems of water condensation and fungal growth on common materials in the dwelling environment. Carpets, humidifiers, and air-conditioning systems have come into wide use, and this tendency means that, even in winter, the indoor environment may afford conditions that allow fungi to develop. In Japan, yearly increases in allergic disease cases in which indoor pollutions are a considerable contributing factor, combined with increasing tendency to home nursing of people with abnormally high susceptibility to allergens, indicate that a comprehensive survey on domestic fungal propagules would be valuable as a basis for improving standards of hygiene in dwellings (Udagawa, 1994; Samson et al., 1994; Beguin, 1995). Perhaps the greatest significance of this study lies in the provision of data that broadens our ecological knowledge of potentially hazardous ascomycetes in the domestic environment, and in pointing out an unexplored area of environmental

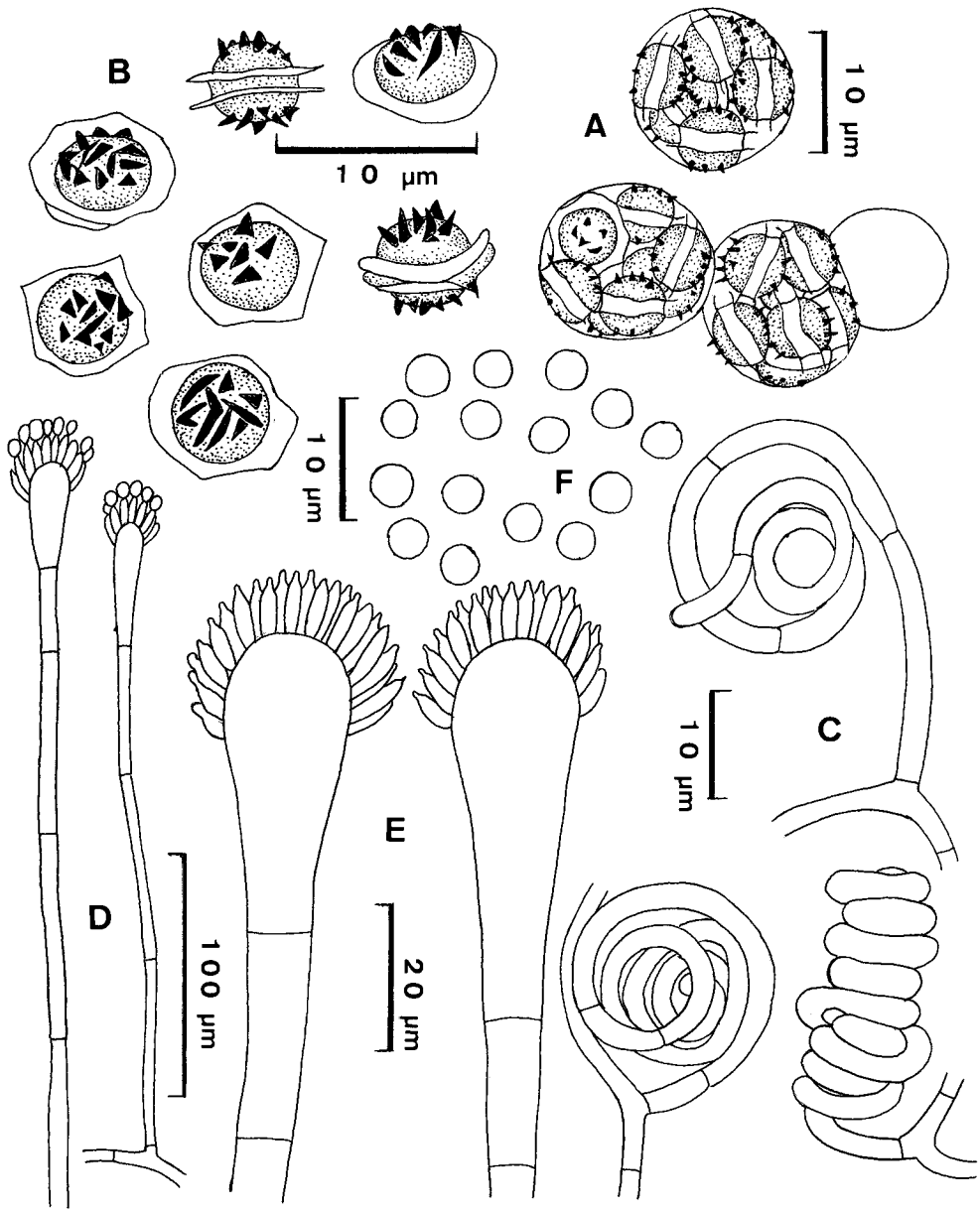

Fig. 1. *Neosartorya pseudofischeri*, NCI 2043.
A. Asci. B. Ascospores. C. Ascomatal initials. D, E. Aspergilla. F. Conidia.

mycology in relation to human health.

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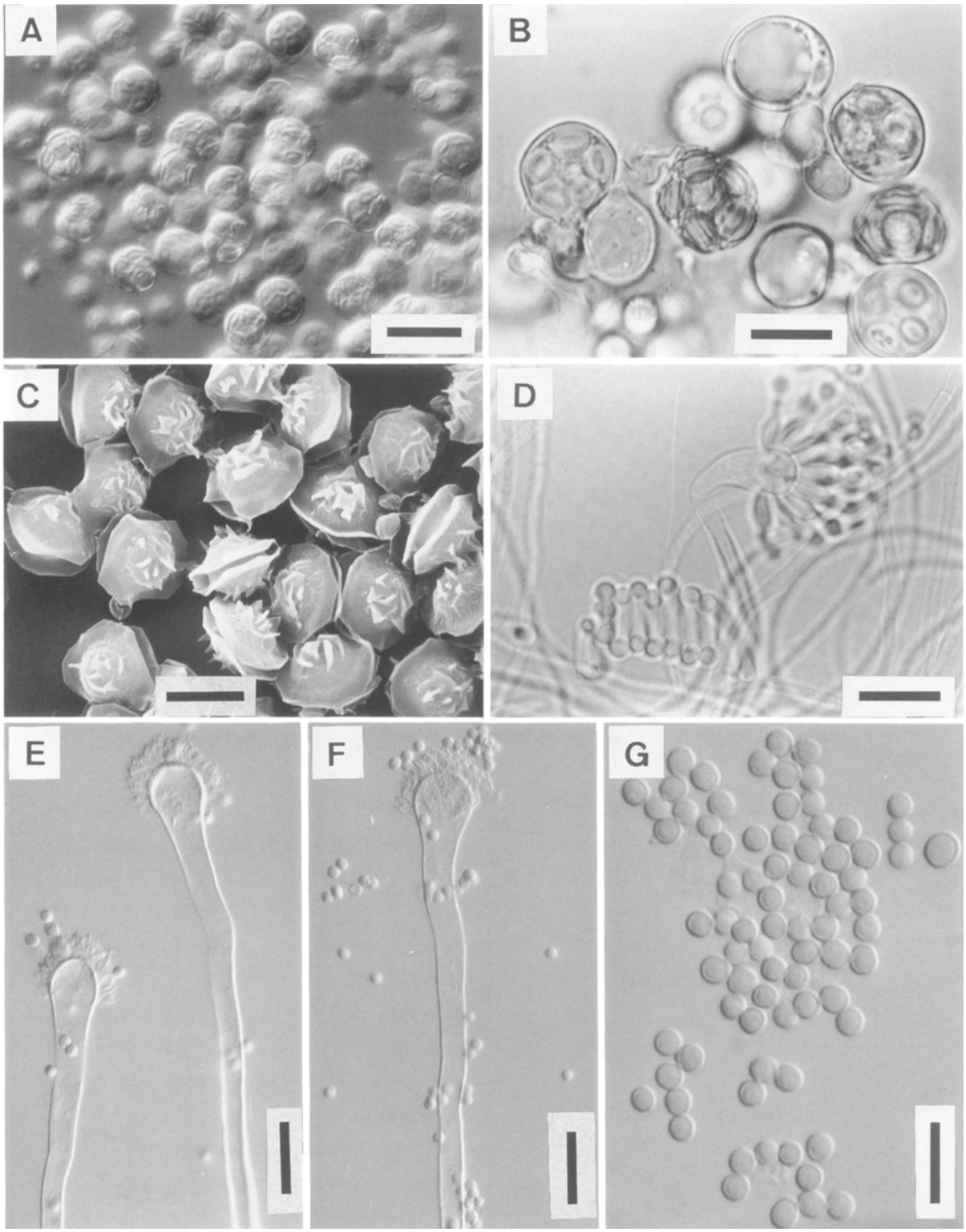

Fig. 2. *Neosartorya pseudofischeri*, NCI 2043.
A, B. Asci. C. Ascospores (SEM). D. Ascomatal initial and aspergillum. E, F. Aspergilla. G. Conidia.
Scale bars: A = 20 μm; B = 10 μm; C = 5 μm; D = 10 μm; E, F = 20 μm; G = 10 μm.

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