

Six Proliferous Species of *Thraustochytrium* from Taiwan

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ABSTRACT: Six proliferous species of *Thraustochytrium* are described and illustrated, namely *T. proliferum* Sparrow, *T. motivum* Goldstein, *T. aureum* Goldstein, *T. multirudimentale* Goldstein, *T. kerguelensis* Bahnweg and Sparrow and *T. rossi* Bahnweg and Sparrow. The last three species are described as new records to Taiwan.

KEY WORDS: *Thraustochytrium*, Basal rudiment, Taiwan.

INTRODUCTION

Thraustochytrium is the largest genus in the family Thraustochytriaceae (Sparrow, 1973) and in the subclass Thraustochytriidae (Porter, 1989). Members of this genus occurring in marine water, mud, sediment, on algae and in plant debris, are traditionally isolated by pine pollen grains and baited in marine water or mud samples. They can grow extensively on nutrient liquid and solid synthetic media. Fifteen saprobic species are recognized in the present fungal taxa. On the basis of appearance of the internal proliferation of the sporangia, these species are generally classified into two groups, including eight proliferous species and seven non-proliferous species (Karling, 1981).

Volz *et al.* (1976) have firstly reported eight species of *Thraustochytrium* from the coastal areas in Taiwan. Recently, we isolated six proliferous species of *Thraustochytrium* collected from various marine water and mud samples. The morphological characteristics of these species are described and illustrated in this paper.

MATERIALS AND METHODS

Samples of marine water and mud were collected along shores of river and estuary, and from salt ponds in Taiwan. The samples were placed in sterile glass Petri dishes, baited with pine pollen grains, and incubated at 20°C in darkness in the laboratory. KMV agar medium (containing antibiotics) (Porter, 1987) was used for isolation and cultivation. Pure cultures were maintained in KMV slush agar medium (Porter, 1987) in screw cap tubes and transferred every month. All pure cultures are deposited at the mycological laboratory in Chia-Nan University of Pharmacy and Science.

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Morphological observations were made by placing pine pollen grains or KMV slush agar media under a light compound microscope. Developmental stages were recorded on Kodak T-MAX film by Leica interference microscope. Species identification is followed by Karling (1981).

TAXONOMY

Thraustochytrium proliferum Sparrow, Biol. Bull. 70: 259, 1936.

Figs. 1-8

In KMV slush: Sporangia proliferous, globose or pyriform, with few oil droplets when young, 41-74 μm long by 53-69 μm diam at maturity; hyaline, thin-walled; with a basal rudiment, 16-48 μm diam. Pseudorhizoids delicate, branched. Zoospores globose or reniform, 2.5-4 \times 3-5 μm , biflagellate, churning within the sporangium prior to discharge, liberated by deliquescence of upper part of sporangial wall.

On KMV agar: Colony weak salmon pink.

Specimens examined: keelung: Ho Ping Island, marine water collected from shore, Aug. 8, 1991, *NTNU H1-B*; **Tainan County:** Tsengwen River, marine water collected from estuary, Dec. 27, 1998, *CNU J01e*; Sep. 25, 1999, *CNU 902L*; Nov. 29, 1999, *CNU 1102L*; Dec. 30, 1999, *CNU 1202L*; Jan. 25, 2000, *CNU 102L*; Apr. 29, 2000, *CNU 402L*; Jun. 24, 2000, *CNU 602L*; July 13, 2000, *CNU 702M*; **Tainan City:** Sze-Tsao, marine water and mud collected from salt ponds, Feb. 5, 2001, *CNU 1A01-Sa*; Mar. 22, 2001, *CNU 1C02*, *2B02*. All specimens were isolated on pine pollen grains baited with marine water and mud samples.

Notes: This is the type species of the genus *Thraustochytrium* (Sparrow, 1936). It is identified mainly by the zoospores delimiting progressively from apex to base within a sporangium and liberated by deliquescence of upper part of sporangial wall after its maturation. *Thraustochytrium proliferum* was found all year around. However, it appears only at the site with salinity more than 3.0 ‰ and preferably isolated from estuary.

Thraustochytrium motivum Goldstein, Amer. J. Bot. 50: 273, 1963.

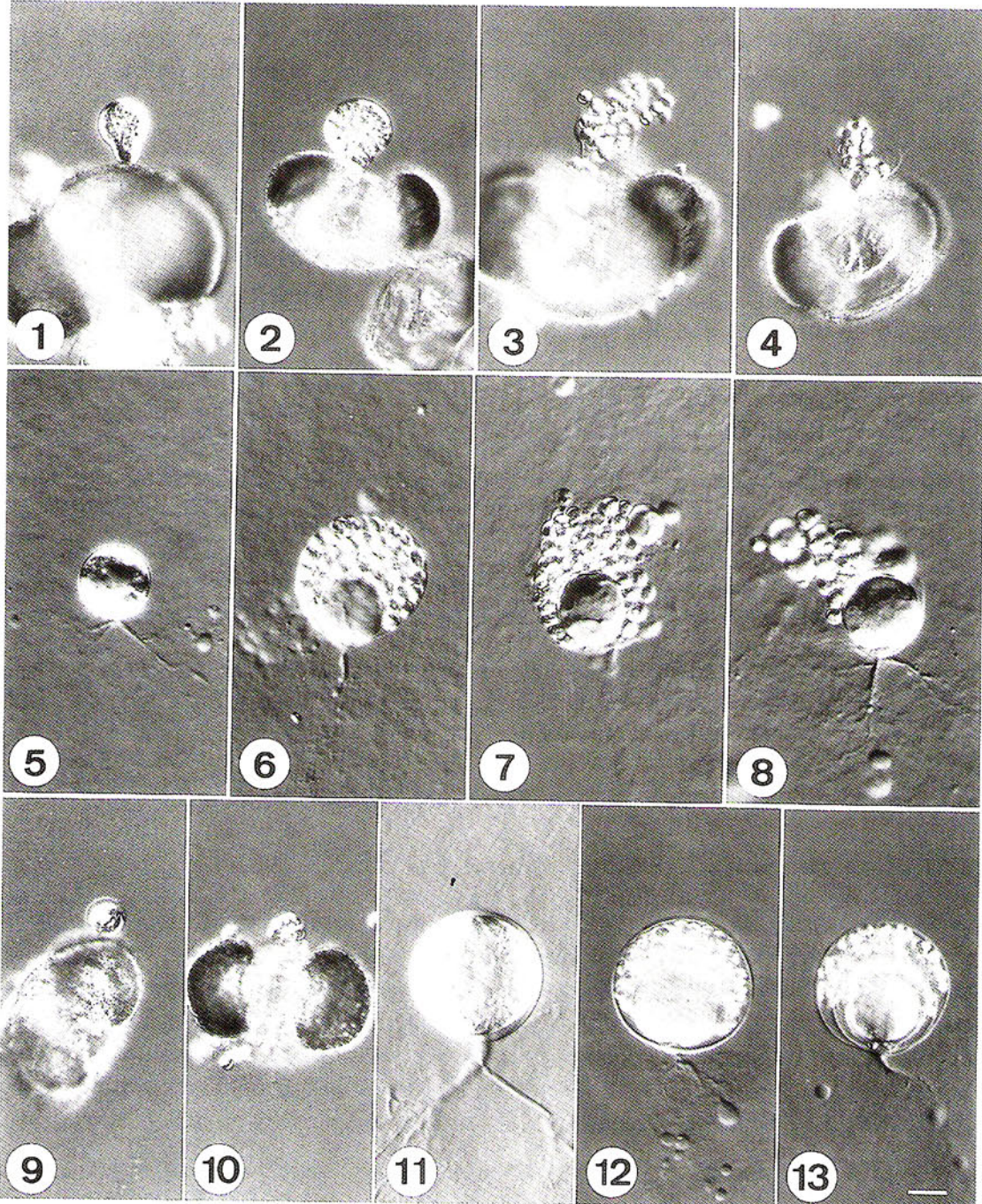
Figs. 9-13

In KMV slush: Sporangia proliferous, globose or pyriform, 31-62 μm long by 21-44 μm diam; smooth, hyaline, thin-walled; with a basal rudiment, globose, 8-23 μm diam. Pseudorhizoids delicate, branched. Zoospores delimited within the sporangium, releasing one by one through a fissure in the sporangial wall; spherical or ellipsoid, biflagellate, 2-2.5 \times 2.5-3 μm . Basal rudiment beginning division within primary sporangium, occasionally tertiary sporangium simultaneously appeared with primary and secondary sporangium.

On KMV agar: Colony opalescent, over the surface of agar with amoeboid type in shape.

Specimens examined: Keelung: Ho Ping Island, marine water collected from shore, Aug. 8, 1991, *NTNU H1-A*; **Tainan County:** Tsengwen River, marine water collected from estuary, Oct. 10, 1998, *CNU J01c*; Nov. 27, 1998, *CNU J01c*; Nov. 29, 1999, *CNU 1102M*; Feb. 22, 2000, *CNU 202L*; Apr. 4, 2000, *CNU 302L*; May 27, 2000, *CNU 501M*; Aug. 14, 2000, *CNU 802M*; **Tainan City:** Sze-Tsao, marine water and mud samples collected from salt ponds, Nov. 27, 1998, *CNU J01*; Feb. 28, 2001, *CNU 1C01*; Mar. 22, 2001, *CNU 3A02*, *CNU 3A02-Sa*; Apr. 26, 2001, *CNU 1B03*, *1C03*, *2B01*, *3A03*, *3A03-Ab*. All specimens were isolated on pine pollen grains baited with marine water and mud samples.

Notes: *Thraustochytrium motivum* is the most common species of this genus, which appears in most collection sites all year around. This species is easily recognized by the multiple fission of its basal rudiment and by the secondary sporangium which frequently delimited within the primary sporangium.



Figs. 1-8. *Thraustochytrium proliferum*. 1-4: On pine pollen. 5-7: In KMV slush. 1: Young pyriform sporangium. 2: Mature sporangium with angular zoospores and a basal rudiment. 3 and 4: Zoospores escaping through a sporangium with torn upper wall and basal rudiment in the base. 5: Young sporangium with delicate pseudorhizoids. 6: Mature sporangium with a basal rudiment. 7 and 8: Zoospores escaping from the torn upper wall of sporangium. bars = 10 μ m. Figs 9-13. *Thraustochytrium motivum*. 9-10: On pine pollen. 11-13: In KMV slush. 9: Basal rudiment developing into secondary sporangium with a centric oil droplet. 10: Mature secondary sporangium with a basal rudiment. 11: Young sporangium with several vesicles and conspicuous pseudorhizoids. 12: Mature sporangium with one basal rudiment. 13: Basal rudiment of the primary sporangium developing into a secondary sporangium. bars = 10 μ m.

Thraustochytrium aureum Goldstein, Arch. Mikrobiol. 45: 102, 1963. Figs. 14-19

In KMV slush: Sporangia proliferous, globose, subglobose or pyriform, with many oil droplets when young, 13-30 μm diam at maturity; hyaline, smooth-walled; with a basal rudiment, globose, 6.5-15 μm . Pseudorhizoids branched. Zoospores delimited and swimming freely within the sporangium, puncturing the distal portion of the sporangial wall; ellipsoid or fusiform, 2.5-4 \times 4-10 μm , laterally biflagellate; becoming quiescent, globose.

On KMV agar: Colony yellow to salmon-orange.

Specimens examined: **Keelung:** Ho Ping Island, marine water collected from shore, Aug. 8, 1991, *NTNU HI-C*; **Tainan County:** Tsengwen River, half-salty water collected from stream, Nov. 21, 1998, *CNU J05b*; Oct. 23, 1999, *CNU 1001L*; Nov. 29, 1999, *CNU 1101L*; May 27, 2000, *CNU 501M*; **Tainan city:** Sze-Tsao, marine water collected from salt pond, Feb. 20, 2001, *CNU 1C01*, *3A01*. All specimens were isolated on pine pollen grains baited with marine water samples.

Notes: The motility of the zoospores in the sporangium prior to discharge through an opening in the persistent sporangial wall is the major diagnostic character of this species.

Thraustochytrium multirudimentale Goldstein, Amer. J. Bot. 50: 273, 1963. Figs. 20-25

In KMV slush: Sporangia proliferous, globose or subglobose, with a large eccentric oil droplet when young, about 18-34 μm diam at maturity; hyaline, smooth, thin-walled; with 2-4, occasionally 6, basal rudiments, 6.5-14 μm diam. Pseudorhizoids stranded, branched or few branched. Zoospores liberated through 1, or 2 fissures in the sporangial wall, at first globose then becoming reniform, 4-5 \times 7.5-10 μm , laterally biflagellate.

On KMV agar: Colony white.

Specimens examined: **Keelung:** Ho Ping Island, marine water collected from shore, Aug. 8, 1991, *NTNU HI-F*; **Tainan County:** Chiku, marine water collected from sea water fish pond, Dec. 27, 1998, *CNU J01f*; Tsengwen River, marine water collected from estuary, Nov. 29, 1999, *CNU 1101M*; Dec. 30, 1999, *CNU 1202L*; Jan. 25, 2000, *CNU 102L*; **Tainan city:** Sze-Tsao, mud from salt ponds, Feb. 20, 2001, *CNU 2A01-Sa*; Mar. 22, 2001, *CNU 3A02-Sc*. All specimens were isolated on pine pollen grains baited with marine water and mud samples.

Notes: The prominent characteristics of this species are sporangium with 2-4 basal rudiment, occasionally 6, within the base of residual sporangial wall and zoospores liberated by 1 or 2 fissure at upper part of a sporangium.

Thraustochytrium kerguelensis Bahnweg and Sparrow, Amer. J. Bot. 61: 762, 1974.

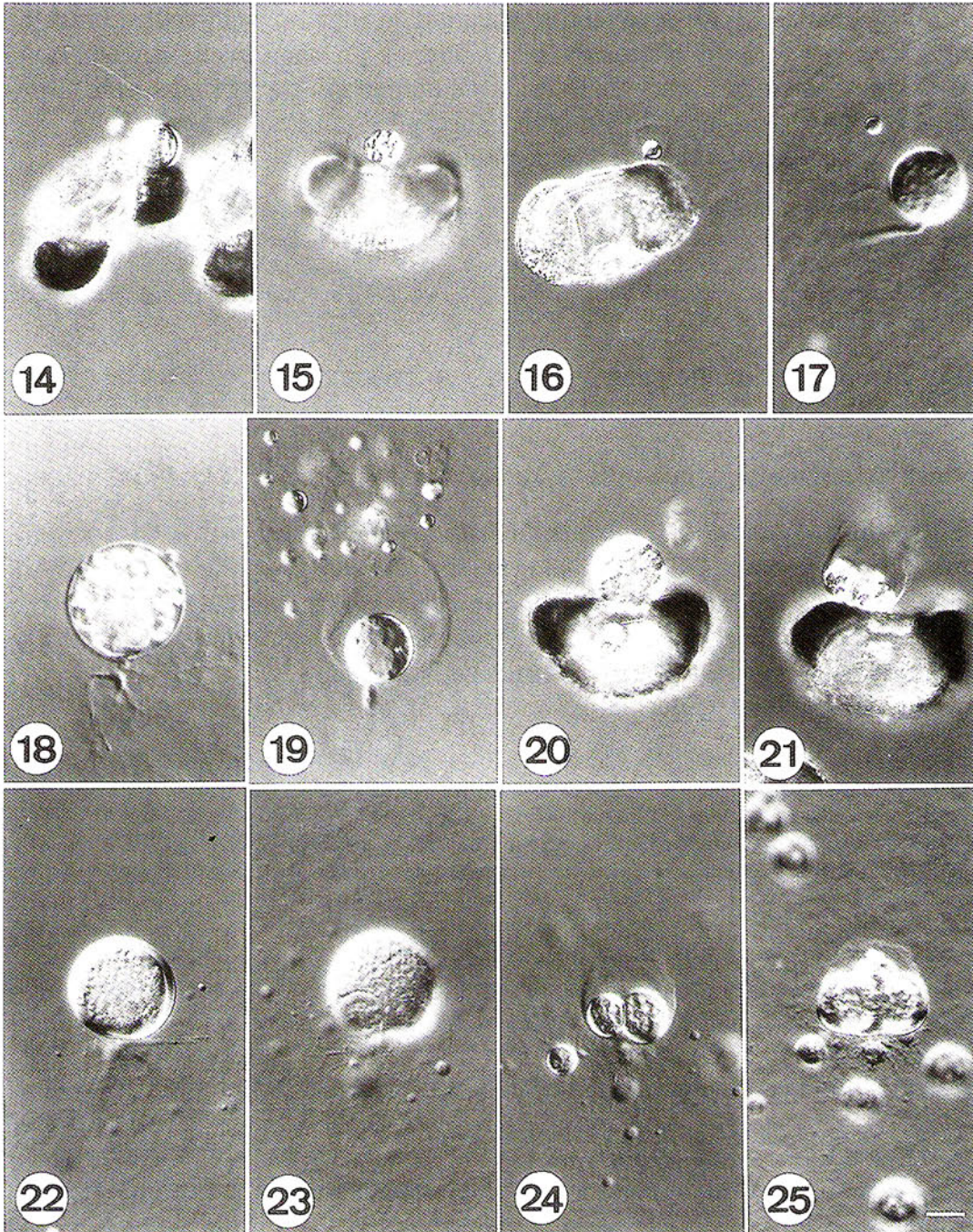
Figs. 26-31

In KMV slush: Sporangia proliferous, globose or subglobose, with a large vesicle when young, about 26-54 μm diam at maturity; hyaline, smooth, thin-walled; with 3-10, usually 6, basal rudiments, 10-21 μm diam within a sporangium. Pseudorhizoids stranded, branched. Zoospores liberated by complete deliquescence of a sporangial wall, globose then becoming reniform, 3.5-5 μm diam, laterally biflagellate.

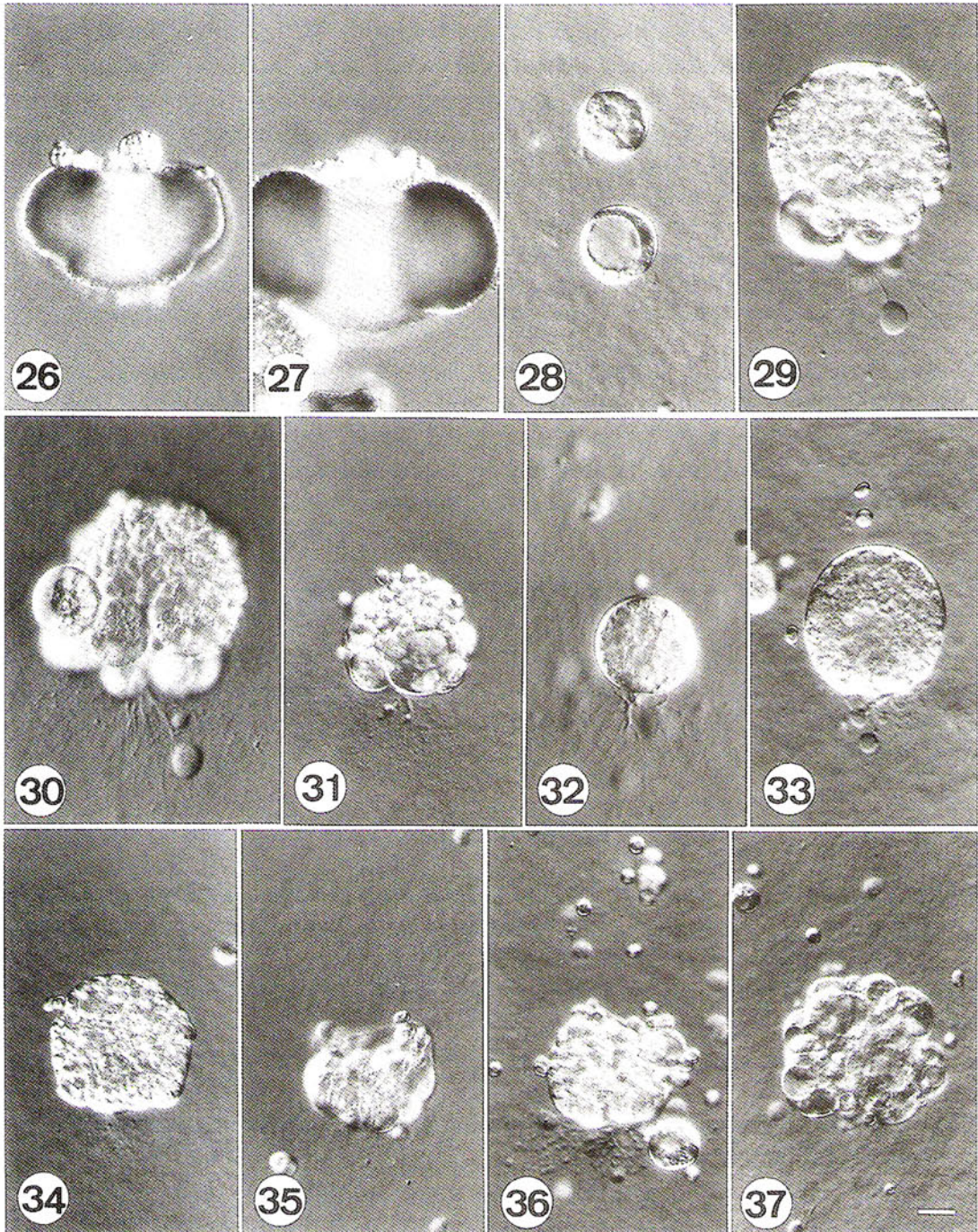
On KMV agar: Colony white.

Specimens examined: **Tainan hsien:** Chiku, marine water collected from salt pond, Dec. 27, 1998, *CNU J02e*; **Tainan city:** Sze-Tsao, marine water collected from salt pond, Dec. 27, 1998, *CNU J02e*; marine water and mud collected from salt ponds, Mar. 22, 2001, *CNU 2B02*, *4A01-Sc*. All specimens were isolated on pine pollen grains baited with marine water and mud samples.

Notes: The major characteristics of this species are a sporangium with 3-10, usually 6 rudiments, and the complete deliquescence of sporangial wall during zoospore liberation.



Figs. 14-19. *Thraustochytrium aureum*. 14-16: On pine pollen. 17-19: In KMV slush. 14: Young sporangium. 15: Mature sporangium. 16: Empty sporangium with one basal rudiment. 17: Young sporangium. 18: Mature sporangium with one basal rudiment. 19: Zoospores escaping from the upper pore of sporangial wall. bars = 10 μ m. Figs. 20-25. *Thraustochytrium multirudimentale*. 20-21: On pine pollen. 22-25: In KMV slush. 20: Developing sporangium. 21: Four rudiments within the base of residual sporangial wall. 22: Young sporangium. 23: Developing sporangium with two basal rudiments. 24 and 25: Two and four rudiments within the base of residual sporangial wall. bars = 10 μ m.



Figs. 26-31. *Thraustochytrium kerguelegsis*. 26-27: On pine pollen. 28-31: In KMV slush. 26: Developing sporangium. 27: Sporangium residue with six basal rudiments. 28: Young sporangium with a large vesicle. 29 and 30: Mature sporangium with six basal rudiments. 31: Disintegration of sporangial wall, with some released zoospores and basal rudiments. bars = 10 μ m. Figs. 32-37: *Thraustochytrium rossi*. In KMV slush. 32: Young sporangium. 33: Zoospores delimited progressively from base to apex. 34: Mature sporangium. 35: Part of sporangial wall disintegrating and a portion of the zoospores escaping. 36 and 37: The sporangium after complete disintegration of wall, with more than ten basal rudiments remained. bars = 10 μ m.

Thraustochytrium rossi Bahnweg and Sparrow, Amer. J. Bot. 61: 762, 1974. Figs. 32-37

In KMV slush: Sporangia proliferous, globose or subglobose, about 37-53 μm long by 32-63.5 μm wide at maturity; hyaline, thin-walled; with 4-20 globose, basal rudiments of 11-26.5 μm diam. Pseudorhizoids delicate, branched with swelling structure. Zoospores ellipsoid, 2.5-3.5 \times 2.5-5 μm ; initially part of sporangial wall deliquesce and a portion of zoospores emerging, finally the sporangial wall disintegrated completely and leaving a cluster of individual rudiments behind which develop into new sporangia.

On KMV agar: Colony white.

Specimens examined: **Keelung:** Ho Ping Island, marine water collected from shore, Aug. 8, 1991, *NTNU H1-D*; **Tainan city:** Sze-Tsao, marine water collected from salt ponds, Feb. 5, 2001, *CNU 1A01*; Mar. 22, 2001, *CNU 4A02*. All specimens were isolated on pine pollen grains baited with marine water samples.

Notes: This species differs from *T. multirudimentale* and *T. kerguelensis* in having greater number of rudiments, and in the manner the sporangial wall deliquesce. The species of *Thraustochytrium* with multi-rudiments as listed above namely: *T. multirudimentale*, *T. kerguelensis* and *T. rossi*, are conspicuously appeared seasonally, so far they were frequently found during winter and early spring, but rare in summer.

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臺灣產六種增生性破囊壺菌 (*Thraustochytrium*)

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摘 要

本文描述臺灣產六種具有增生性游孢子囊(zoo sporangium)的破囊壺菌(*Thraustochytrium*)，即：增生破囊壺菌(*T. proliferum* Sparrow)，動孢破囊壺菌(*T. motivum* Goldstein)，金黃破囊壺菌(*T. aureum* Goldstein)，多基底增殖破囊壺菌(*T. multirudimentale* Goldstein)，格貴破囊壺菌(*T. kerguelensis* Bahnweg and Sparrow)及羅氏破囊壺菌(*T. rossi* Bahnweg and Sparrow)。其中後三種在單一游孢子囊(zoo sporangium)中具有多數個基底增殖體，且被列為臺灣新記錄種。

關鍵詞：破囊壺菌屬、基底增殖體、臺灣。

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