

***Phaeoclavulina* and *Ramaria* (Gomphaceae, Gomphales) from Nam Nao National Park, Thailand**

AMMANEE MANEEVUN¹, JOLYON DODGSON² AND NIWAT SANOAMUANG^{1,3*}

¹Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, THAILAND

²Faculty of Science, Maharakam University, Maha Sarakham 44150, THAILAND

³Applied Taxonomic Research Center, Khon Kaen University 40002, THAILAND

* Corresponding author. E-mail: niwatsanoa@gmail.com

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ABSTRACT.— *Phaeoclavulina* and *Ramaria* are two related genera of coral fungi that have highly branched basidiomata. Most of them are edible and they are commonly found in Nam Nao National Park, Phetchaboon, Thailand. This paper describes samples collected during 2008-2009 in order to expand our current knowledge of the species composition of Thai coral fungi. Collected specimens were identified by macroscopic and microscopic morphological characteristics including scanning electron microscopy analysis of spore details, from which two genera and 11 species were found. Of these 11 species, eight are new records for Thailand (*Ramaria botrytoides*, *R. conjunctipes*, *R. cystidiophora* var. *fabiolens*, *R. flava*, *R. rubripermanens*, *R. sanguinipes*, *R. sino-conjunctipes* and *R. velocimutans*). The taxonomy of all 11 species and a key to the two genera and 11 species are provided. A phylogenetic tree for the genetic relationship of the 11 species, based upon the amplified ribosomal DNA restriction analysis (ARDRA) of the ITS1-5.8S-ITS2 rRNA gene fragment, revealed a coefficient of 93% for distinguishing the identity of each species. Interestingly, the two *Phaeoclavulina* species did not group together and separately from the *Ramaria*, but rather grouped apart from each other and within two of the *Ramaria* groups.

KEY WORDS: Coral fungi, *Phaeoclavulina*, *Ramaria*, phylogenetic tree, ARDRA

INTRODUCTION

Phaeoclavulina Blinkmann and *Ramaria* Fr. ex Bonord. are two genera of fungi that are characterized by having highly branched basidioma, which are usually described as being divided into stipe, branches and apices (Exeter et al., 2006). The sporal characters, including color, shape and ornamentation, are important in differentiating specimens to genera (Villegas et al., 2005). *Phaeoclavulina* were formerly included in the genus *Ramaria* as they all share a similar basidiomal morphology. However, according to Beug (2004), Nasim et al. (2008) and Giachini et al. (2010), the *Phaeoclavulina* have subsequently been separated from the *Ramaria* by the

presence or absence of clamp connections, spore ornamentation and their basidiomata morphology. Their spore ornamentations are echinulate, verrucose, subreticulate or reticulate where the basidiomata are fan-shaped, funnel-shaped or ramarioid. The *Ramaria* in turn formerly belonged to a subgenus of the *Echinoramaria* and some of the *Lentoramaria* had been redescribed as *Phaeoclavulina* (Giachini, 2004; Giachini et al., 2010). Currently, only three subgenera, *Laeticolora*, *Lentoramaria* and *Ramaria*, are recognized within the genus *Ramaria*. Both genera, *Phaeoclavulina* and *Ramaria*, are grouped in the Gomphales, Gomphaceae (Giachini et al., 2010; Hosaka et al., 2006; Hibbett et al., 2007) based on the combined sequence analysis of the nuclear 25S-rDNA

and mitochondrial 12S-rDNA and ATP6 DNA fragments.

The Nam Nao National Park covers the area of Umphur Lomsak, Umphur Nam Nao in Petchaboon province and Umphur Kornarn in Chaiyaphum province, Thailand. It is located on the Petchaboon Mountains and its total area covers roughly 966 square kilometers or 603,750 acres. Geologically the area is mountainous and is comprised of dry or deciduous dipterocarp, mixed deciduous, tropical and coniferous mountainous forests plus grass fields (National Park, Wildlife and Plant Conservation Department, 2009). The entire forest area is considered to be very abundant in coral fungi. However, very little information of coral fungi in Thailand is available, being essentially comprised of the book written by Chantrasrikul et al. (2008) and the paper on simple - club clavarioid fungi in Nam Nao National Park by Maneevun and Sanoamuang (2010). In particular, reports on the diversity of *Phaeoclavulina* and *Ramaria* coral fungi have not yet been published.

MATERIALS AND METHODS

Morphology.— Observation, photography and sample collection, from Nam Nao National Park, was performed during the rainy seasons (August-October) of 2008 and 2009. Macroscopic morphological characteristics, including the stalk, branches, end of branches and body, plus the staining reactions when bruised and when chemically tested by exposure to 10% (w/w) FeSO₄, 10% (w/v) KOH and Melzer's reagent, were examined by eye or light microscope. Microscopic characteristics, including the spore type and basidium clamp connection of the coral fungi (Exeter

et al., 2006) were observed by scanning electron microscopy (SEM). For SEM, the dry tissue at the stub area of the fungi was cut, coated with gold (Hahn and Christan, 2002) and then observed under the scanning electron microscope (LEO model 1450 VP). All specimens examined were kept as dried material at the Science Museum, Khon Kaen University.

Molecular analyses.— DNA extraction was performed in SDS lysis buffer (400 mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1% (w/v) sodium dodecyl sulfate) using a method adapted from Liu et al. (2000). Ribosomal DNA fragments containing the ITS1-5.8S-ITS2 region were replicated via the polymerase chain reaction (PCR) using the general fungi primers ITS-1F and ITS-4 (Gardes and Bruns, 1993; White et al., 1990). PCR reactions were performed in a 25 µl final volume comprised of 5 µl of the genomic DNA (100 ng/ µl), 5 µl of 5X Buffer, 2 µl of 25 mM MgCl₂, 0.2 µl of 100 mM dNTPs, 0.5 µl each of 100 pmol/µl primer (ITS-1F and ITS-4), 0.125 µl of 5 U/µl Taq DNA Polymerase (Promega) and dH₂O 11.675 µl. PCR was carried out in a Biometra, Model T-personal thermal cycle machine using parameters adapted from Olariaga (2009) as follows: 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 54.5 °C for 30s and 72 °C for 30 s. The reaction was then stopped by cooling to 4 °C. The DNA amplicon was cleaned up and then aliquoted into four equal amounts to each of which in a 10 µl final volume was digested by 5 µl of PCR product, 2 µl of 10X buffer and 2 µl of 10 U/µl of one *Alu* I, *Eco*RI, *Hinf* I and *Taq* I restriction enzymes, digested by 37 °C for 6 h. before being co-resolved alongside a 100 bp ladder by electrophoresis on a 2% (w/v) agarose-TBE gel. The band patterns,

scored as present or absent dominant bands, were used to produce a phylogenetic tree for the coral fungi by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method using the NTSYSps version 2.0 software.

RESULTS AND DISCUSSION

(A.) *Phaeoclavulina* Blinkmann (formerly *Ramaria* subgenus *Echinoramaria*)

1. *Phaeoclavulina cokeri* (R.H. Petersen) Giachini (*Ramaria cokeri* R.H. Petersen, 1976)

Six samples were randomly selected for data evaluation. The basidiomata branches dichotomously out to form a coral-like bush shape of 3-12 cm tall and 1-7.5 cm wide that is frail and easily broken. When left for a long period, it becomes rigid. The surface is slightly bumpy and wavy to smooth at the ends. When bruised, it becomes dark purple or blackish brown. It has a single stalk or a

group of single stalks. The stipe is ~2-3 cm tall with a diameter of 0.4-0.5 cm, with white tissue near the ground becoming orange-yellow or brownish yellow higher up. Many branches break out from the stipe and then branch out dichotomously around 7-9 times. Each branch is of a similar size, being narrow (0.3-0.4 cm in diameter) and long (1.5-2 cm), parallel to the stipe. Smaller basidiomata are orange-yellow in color while larger basidiomata are yellow or brownish yellow. Yellow powder can be found covering the surfaces and forks of branches. The tips of the branches are either sharp or blunt. Branches of small basidiomata are orange, with white tips (Fig. 1a). The inner flesh has a firm texture and dry surface. The color is cloudy white or grey, but when left for long periods it becomes purplish grey.

Chemical reactions: When tested with 10% (w/v) KOH, the surface color turns red-orange and the inner flesh turns orange-yellow, whilst with 10% (w/v) FeSO₄ both turn green rapidly. Treatment with 10%

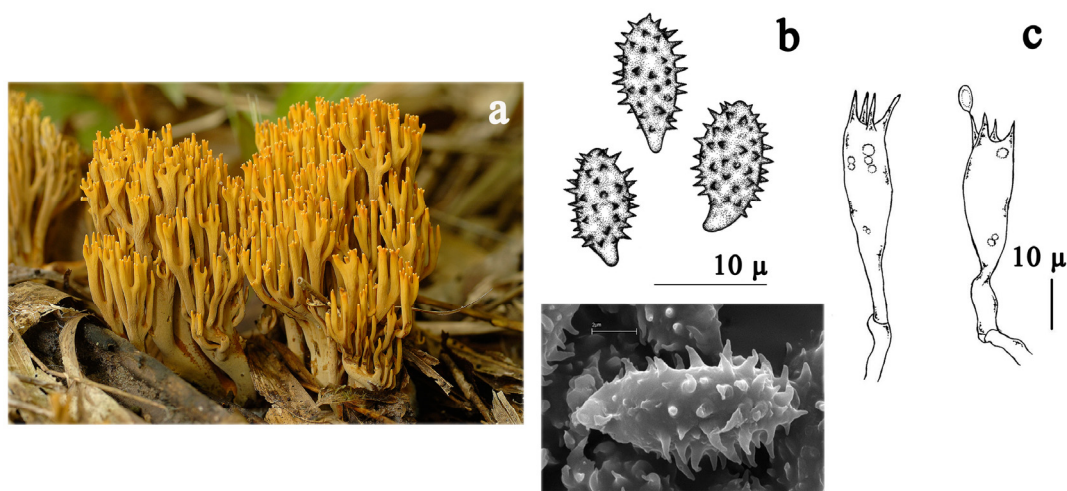


FIGURE 1. *Phaeoclavulina cokeri* (R.H. Petersen) Giachini showing the (a) basidioma, (b) spore and (c) basidium.

(v/v) H_2SO_4 causes the surface to turn red-orange, while Melzer's reagent causes it to turn blackish blue.

The spore shape is ellipsoid, 4-5.5 μ wide and 9.5-13.5 μ long, with small spiky nodes or echinulates at the tips of the branches (Fig. 1b). Spore prints are yellow or brownish yellow. Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium around 7-10 μ wide and 40-60 μ long. The ratio of basidium to sterigmata is 1:4 (Fig. 1c). The size of the sterigma is ~3-4 μ long and clamp connections were found at the fibers and at the base of the basidium.

2. *Phaeoclavulina cyanocephala* (Berk. & M.A. Curtis) Giachini (*Ramaria cyanocephala* (Berkeley & M.A. Curtis) Corner, 1950)

Nine samples were randomly selected for data evaluation. The basidioma branches dichotomously out resembling a coral-like

shape, 5.5-17 cm tall and 3-7 cm wide, that is frail and easily broken. When left for a long period, it becomes rigid. The surface is slightly bumpy and wavy while smooth at the ends. When bruised, it becomes dark purple or blackish brown. It has a single thick stipe. The brown or dark brown stalk is ~2-3 cm tall and 1-2 cm wide. Many branches break out from the stalk then branch out dichotomously around 6-7 times. Each brown or dark brown branch is of a similar size, being narrow (0.4-1 cm diameter) and long (2-4 cm), parallel to the stipe. Yellowish brown powder can be found covering the surface and forks of branches. The sharp or slightly blunt tips of the branches break into two serrations like a fork, with the ends of the branches being blue, dark blue and white at the tips (Fig. 2a). The inner flesh is firm and has a dry surface. The color is grey, but when left for long periods it becomes purplish grey.

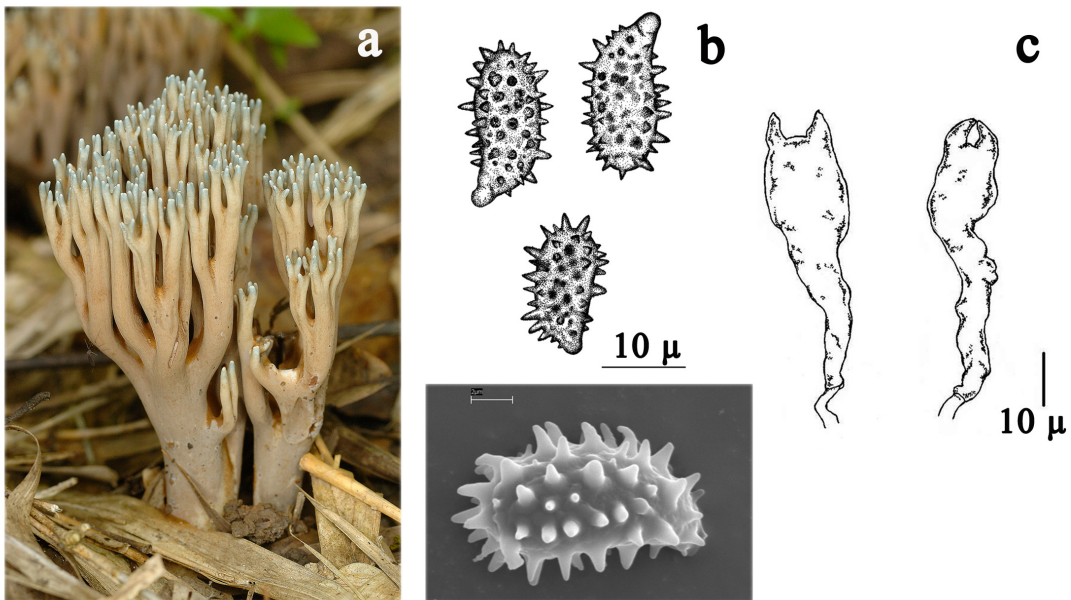


FIGURE 2. *Phaeoclavulina cyanocephala* (Berk. & M.A. Curtis) Giachini showing the (a) basidioma, (b) spore and (c) basidium.

Chemical reactions: When tested with 10% (w/v) FeSO_4 , the surface and inner flesh turns green rapidly. Melzer's reagent causes the surface to turn black. The inner flesh turns yellow when tested with 10% (w/v) KOH.

The spore shape is ellipsoid, 4.5–6 μ wide and 6–10 μ long, with small spiky nodes (echinulates) at the tips of the branches (Fig. 2b). Spore patterns are brownish yellow. Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium ~4.5–6 μ wide and 40–65 μ long. The ratio of basidium to sterigmas is 1:2 (Fig. 2c). The size of the sterigma is around 3–4 μ long and clamp connections were found at the fibers and at the base of the basidium.

(B.) *Ramaria* Fr. ex Bonord.

Subgenus *Ramaria*

3. *Ramaria rubripermanens* Marr & D.E. Stuntz, *Ramaria* of Western Washington: 43 (1974)

Nine samples were randomly selected for data evaluation. The basidioma is ~9–12.5 cm tall and 8–10.5 cm wide with a smooth or bumpy surface with small waves and is sticky. When scratched, the color does not change. It has a sweet flavored scent. It has a large, thick, cylindrical, single, light pinkish-white stipe, ~4.3–5 cm tall and 3–4.5 cm diameter. The tips of the light pinkish-white or light pink branches break into 2–4 serrations with blunt tips (Fig. 3a). The inner flesh is white, firm and has a dry surface.

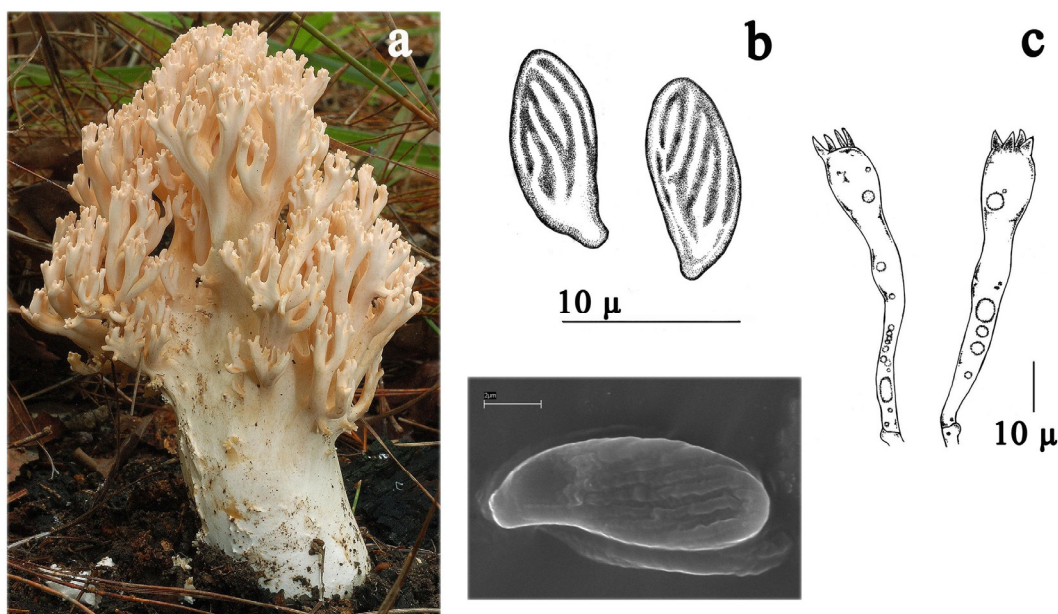


FIGURE 3. *Ramaria rubripermanens* Marr & D.E. Stuntz showing the (a) basidioma, (b) spore and (c) basidium.

Chemical reactions: The surface and inner flesh turns orange-yellow and yellow, respectively, when tested with 10% (w/v) KOH, whilst they rapidly turn green and blue-green, respectively, when tested with 10% (w/v) FeSO₄.

The spore shape is ellipsoid, 4-5.5 μ wide and 9-12 μ long, with small bumpy nodes and a striate surface at the ends of the branches (Fig. 3b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium, 9-12.5 μ wide and 48-70 μ long. The ratio of basidium to sterigmas is 1:4 (Fig. 3c). The size of the sterigma is around 3-5 μ long and clamp connections were found at the fibers and at the base of the basidium.

Subgenus *Lentoramaria*

4. *Ramaria concolor* (Corner) R.H.

Petersen, Biblioth. Mycol. 43: 54 (1975)

Fifteen samples were randomly selected for data evaluation. The basidioma is a wide, expanding, thick and opaque bush shape similar to coral, 7-14 cm tall and 4-12 cm wide. It is frail and easily broken. The surface is smooth or slightly bumpy. When scratched, the mark becomes blackish brown. It has a small, thin, brownish-yellow, single stalk of 2-2.5 cm tall and 0.8-1 cm diameter, from which 2-3 branches break out and then divide into sub-branches dichotomously around 5-7 times forming a thick bush. The brown, purplish-brown or dark brown branches are thin, straight and flat or cylindrical, 2 cm tall and 0.3-0.5 cm in diameter. Brown powder can be found at the surface and forks. The ends of the branches break into two serrations, like forks with sharp tips. The ends of the branches have a light yellowish-brown or light brown color. The tips have a tint of

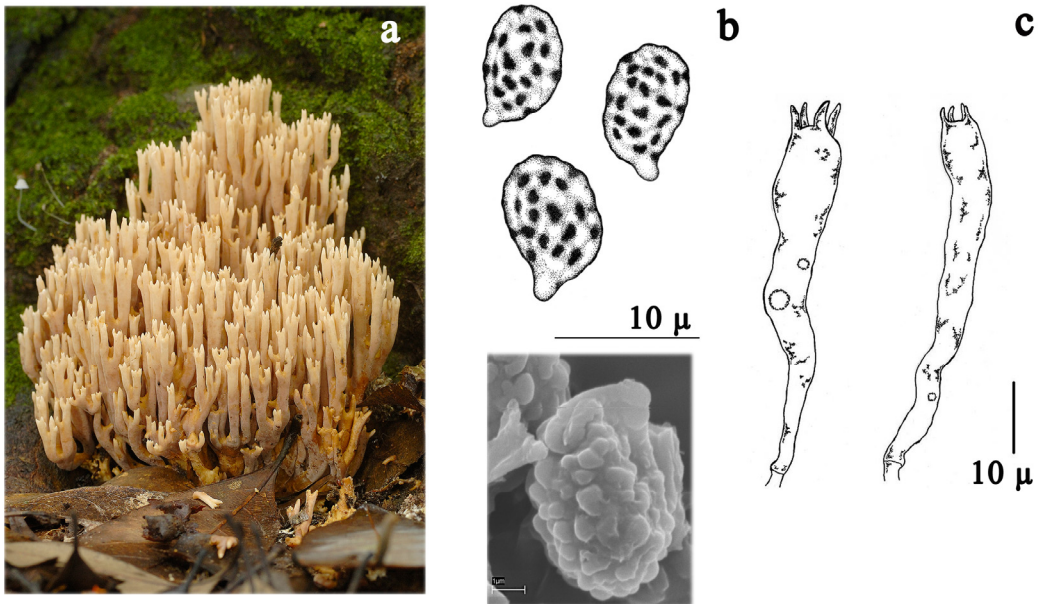


FIGURE 4. *Ramaria concolor* (Corner) R.H. Petersen showing the (a) basidioma, (b) spore and (c) basidium.

white or are light brown (Fig. 4a). The inner flesh is brownish-yellow, firm and with a gelatin-like surface.

Chemical reactions: The surface color turns blue-green when tested with 10% (w/v) FeSO_4 , red-brown when tested with 10% (w/v) KOH and purplish black when tested with Melzer's reagent. The inner flesh turns brown slowly when tested with 10% (w/v) KOH.

The spore shape is ellipsoid, 6-9 μ wide and 10-15 μ long, with a verrucose surface at the ends (Fig. 4b). Spore patterns are brown colored. Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium 5.5-9 μ wide and 40-65 μ long. The ratio of basidium to sterigmata is 1:4 (Fig. 4c). The size of the sterigma is ~3-6 μ long and clamp connections were found at the fibers and at the base of the basidium.

Subgenus *Laeticolora*

5. *Ramaria botrytoides* (Reck.) Quél, Vademecum für Pilzfreunde: 253 (1918)

Eighteen samples were randomly selected for data evaluation. The basidioma has a wide expanding bush shape, 3.5-9 cm tall and 1-7.5 cm wide, similar to coral, whilst small ones are similar to a large cauliflower shaped coral. It is frail and easily broken. The surface is smooth or bumpy and slightly wavy. When scratched, the mark does not change color. It has a single large, thick, white or pinkish-white stalk, 1-1.5 cm tall and 1.8-2 cm diameter, from which many branches break out and then branch out dichotomously around 7-8 times. Small basidiomas are short and fat while large basidiomas have long, thick branches that are slightly fat or cylindrical, 1.2-1.8 cm tall and 0.5 cm in diameter. The color is light pink or light pinkish-white. The ends of the branches separate into 2-4 small dark pink, pink or light reddish-pink serrations (Fig. 5a). The inner flesh is yellowish-white, firm and dry with a gelatin-like surface.

Chemical reactions: The surface turns

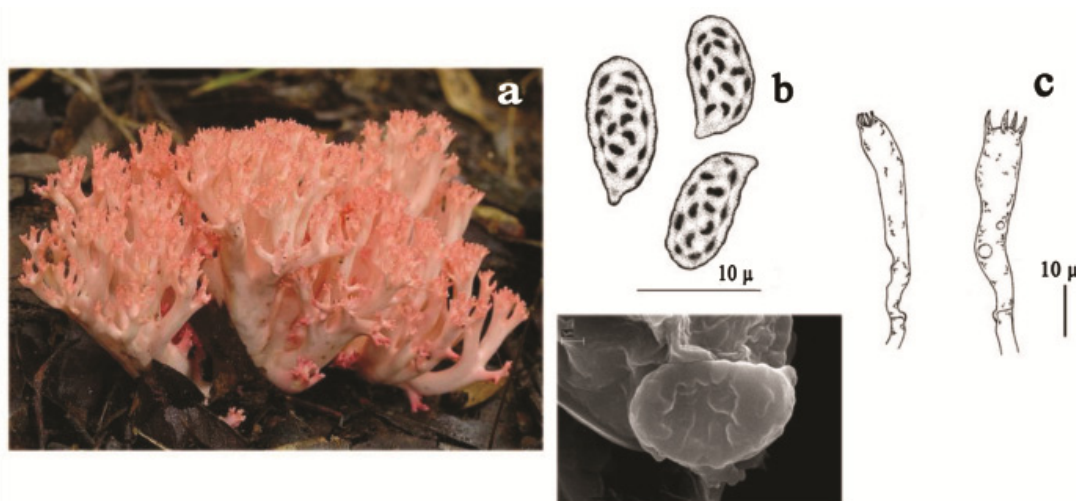


FIGURE 5. *Ramaria botrytoides* (Reck.) Quél showing the (a) basidioma, (b) spore and (c) basidium.

orange-yellow when tested with 10% (w/v) KOH and turns blue-green rapidly when tested with 10% (w/v) FeSO_4 .

The spore shape is ellipsoid, $3.5\text{--}5\ \mu$ wide and $7\text{--}9\ \mu$ long, with a verrucose surface at the ends (Fig. 5b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium, $\sim 6\text{--}10\ \mu$ wide and $35\text{--}45\ \mu$ long. The ratio of basidium to sterigmas is 1:4 (Fig. 5c). Clamp connections could not be found at the fibers and basidium base.

6. *Ramaria conjunctipes* (Coker) Conner,
Ann. Bot. Mem. 1: 567 (1950)

Three samples were randomly selected for data evaluation. The basidioma has a wide expanding bush shape similar to coral, $3\text{--}7\ \text{cm}$ tall and $3\text{--}6\ \text{cm}$ wide, with a smooth

and dry surface. It is frail and easily broken. When scratched, the color does not change. It is hollow. The light orange stalk is comprised of many branches combined together, $2\text{--}3.5\ \text{cm}$ tall and $1\text{--}1.5\ \text{cm}$ diameter, from which two to three branches break out from the stalk then divide into sub-branches dichotomously around 4–5 times. The orange or orange-yellow branches are slim, long, slightly flat, $1.5\text{--}2\ \text{cm}$ tall and $1.2\text{--}1.5\ \text{cm}$ diameter. The end of the branches break out into two blunt, orange tips similar to a fork (Fig. 6a). The inner flesh is hollow, succulent, gelatin-like and clear white.

Spore shape is ellipsoid or broadly ellipsoid, $4.5\text{--}5.5\ \mu$ wide and $8\text{--}9\ \mu$ long, with a verrucose surface at the ends (Fig. 6c). Spores dyed with cotton blue

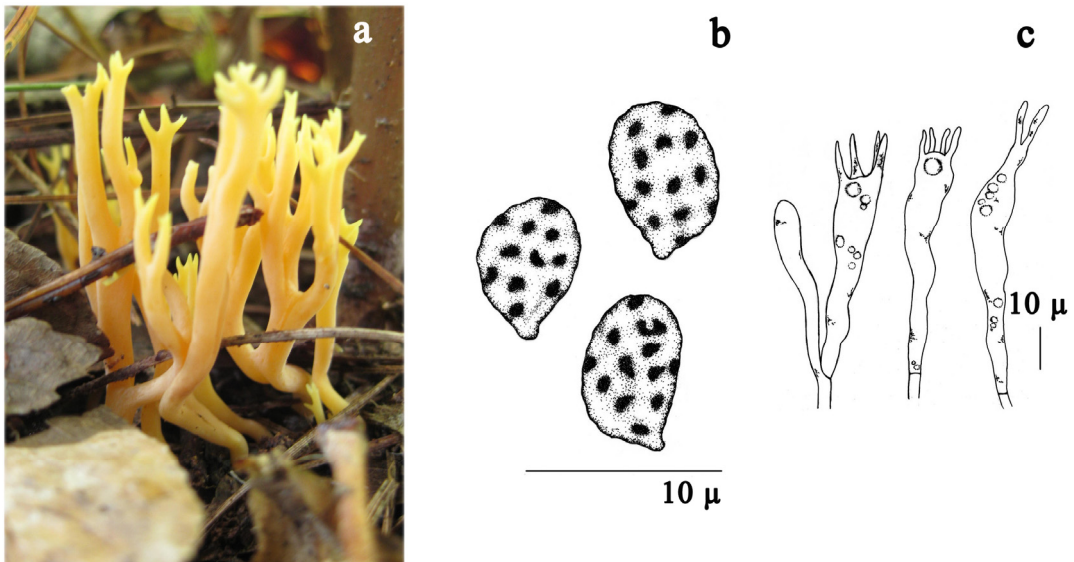


FIGURE 6. *Ramaria conjunctipes* (Coker) Conner showing the (a) basidioma, (b) spore and (c) basidium.

(cyanophilous) have a clavate shaped basidium, 6-9 μ wide and 30-45 μ long in size. The ratio of basidium to sterigmas is 1:2 to 4 (Fig. 6c). No clamp connections were found at the fibers and basidium base.

7. *Ramaria cystidiophora* var. *fabiolens*, Marr & D.E. Stuntz, *Ramaria* of Western Washington, (1974)

Eighteen samples were randomly selected for data evaluation. The basidioma has a wide expanding bush shape similar to coral, 7.5 cm tall and 8.5 cm wide, with a smooth and dry surface. It is frail and easily broken. When scratched, it does not change color. It has a single thick fat stipe, 2.5 cm tall and 2.5 cm in diameter. Small basidiomas are yellow and large are white or light orange-white. Three to four sub-branches break out from the stipe and divide

6-7 times into more branches. The branches are orange, short and fat, 0.7 cm tall and 0.5 cm diameter, and divide at the end into 2-4 orange, sharp tips like small nodes (Fig. 7a). The inner flesh is firm and dry.

Chemical reactions: Surface color rapidly changes to green when tested with 10% (w/v) FeSO_4 and brownish-yellow when tested with 10% (w/v) KOH.

Spore shape is ellipsoid, 3-4.5 μ wide and 7-11 μ long, with a verrucose surface at the ends (Fig. 7b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium around 7-10 μ wide and 35-60 μ long in size. The ratio of basidium to sterigmas is 1:4 (Fig. 7c). A small number of clamp connections and gloeoplerous hypha were found at the fibers. Clamp connections were also present at the base of the basidium.

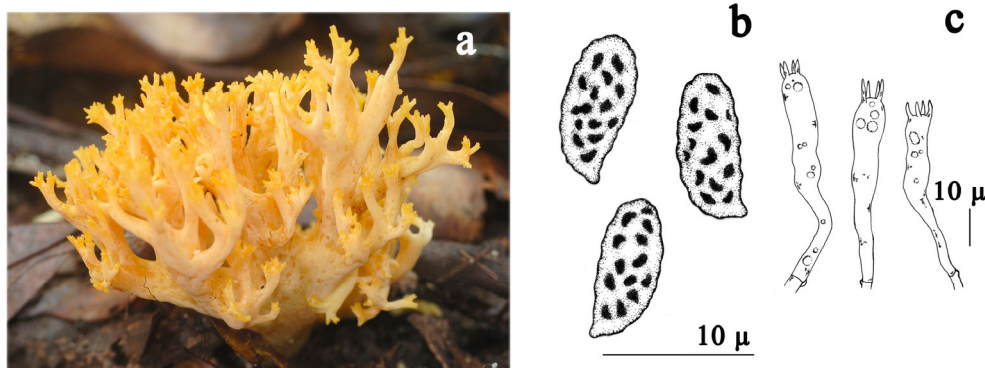


FIGURE 7. *Ramaria cystidiophora* var. *fabiolens*, Marr & D.E. Stuntz showing the (a) basidioma, (b) spore and (c) basidium.

8. *Ramaria flava* (Fr.) Corner, Flore Mycol. de France: 466 (1888)

Three samples were randomly selected for data evaluation. The basidioma, 4.5-7 cm tall and 4.5-9.5 cm wide, is a wide expanding coral-like bush shape. Small basidiomas are similar to cauliflower spreading out like a fan. Surface is smooth. It is frail and easily broken. When scratched, it does not change color. The white or light yellowish-white stipe is large (2 cm tall) and fat (diameter), from which many sub-branches break out from the stipe then divide dichotomously around 7-8 times to the side like a fan. The first branch is big and fat with following branches being narrow, short and thick. The branch shape is cylindrical, 0.5-2 cm tall and 0.5-2 cm in

diameter, and the end of the branches break into 2-4 serrations, with small, blunt tips. Small basidiomas are yellow, large basidiomas are light yellow (Fig. 8a). The inner flesh is white, firm and has a dry surface.

Chemical reactions: Surface color changes to green when tested with 10% (w/v) FeSO_4 .

Spore shape is ellipsoid, 3.5-4.5 μ wide and 7.5-11 μ long, with a verrucose surface at the ends (Fig. 8b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium, ~6.5-10 μ wide and 42-65 μ long in size. The ratio of basidium to sterigmata is 1:4 (Fig. 8c). Sterigma size is 3-4 μ long. Clamp connections were found at the fibers and base of the basidium.

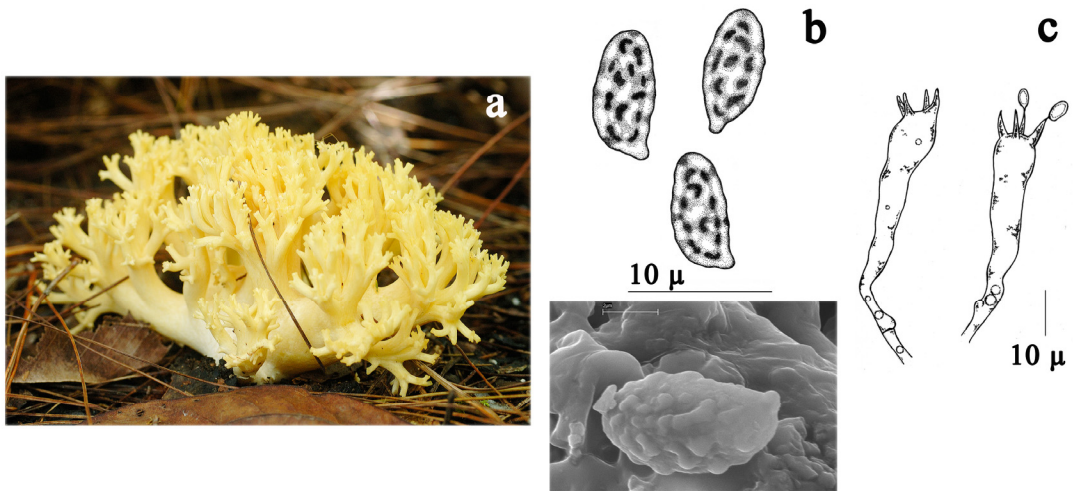


FIGURE 8. *Ramaria flava* (Fr.) Corner showing the (a) basidioma, (b) spore and (c) basidium.

9. *Ramaria sanguinipes* R.H. Petersen & M. Zang, Acta Bot. Yunnan. 8(3): 289 (1986)

Eighteen samples were randomly selected for data evaluation. The basidioma is a wide expanding bush shape similar to coral, 7-12.5 cm tall and 2.3-10 cm wide with a smooth and sticky surface. When bruised, the color changes to red or red-brown. It has a single, thick, light yellowish-white or tinged with red stalk, 2 cm tall and 1-2 cm diameter, from which two to three sub-branches break out and then divide dichotomously 7-8 times forming a bush shape. The light yellowish-white branches are equally sized, 1-3 cm tall and 0.3-0.5 cm diameter, and grow straight, upwards with cylindrical shaped branches. The ends of the

branches break out into many serrations with short and blunt tips. Small caps are yellow and large basidiomas are yellowish-white (Fig. 9a). The inner flesh is white, firm and has a dry surface.

Chemical reactions: Basidioma surface and inner flesh turns yellow when tested with 10% (w/v) KOH and both turn green rapidly when tested with 10% (w/v) FeSO₄.

Spore shape is ellipsoid, 3-5 μ wide and 7-11.5 μ long, with a verrucose surface at the ends (Fig. 9b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium, ~8-10 μ wide and 30-40 μ long. The ratio of basidium to sterigmas is 1:4 (Fig. 9c). Sterigma size is 4-6 μ long. Clamp connections could not be found at the fibers and basidium base.

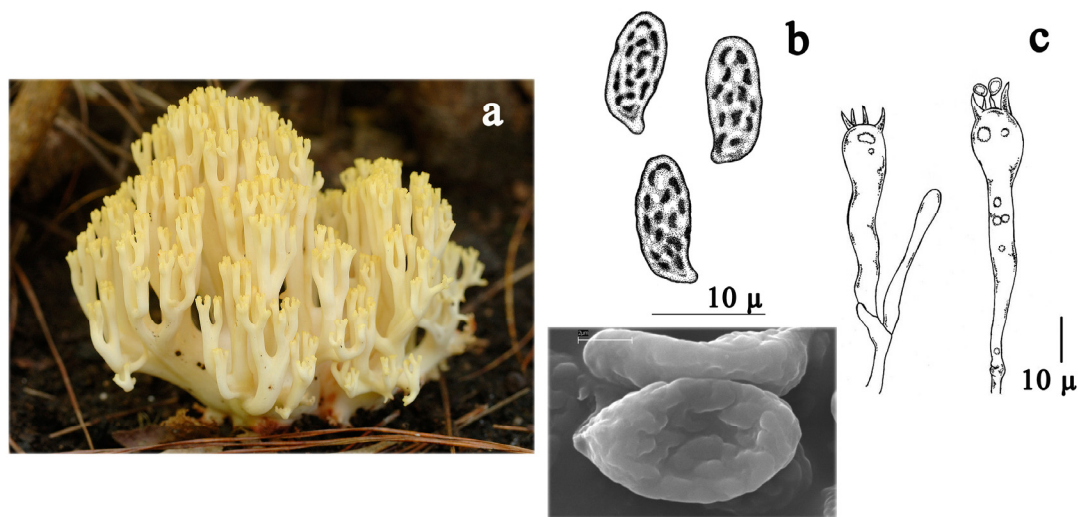


FIGURE 9. *Ramaria sanguinipes* R.H. Petersen & M. Zang showing the (a) basidioma, (b) spore and (c) basidium.

10. *Ramaria sino-conjunctipes* R. H. Petersen & M. Zang. Acta Bot. Yunnan. 12(1): 50 (1990)

Eighteen samples were randomly selected for data evaluation. The basidioma is a wide expanding bush, 4.5-12 cm tall and 1-6 cm wide, shaped similar to coral, with a smooth surface. It is frail and easily broken. When left for a while, it becomes rigid. The color does not change when scratched. It is hollow. The white or light orangey-white thick and fat stipe is comprised of many branches combined together, and is 1-3.5 cm tall and 3-3.5 cm diameter. When bruised it becomes tinged with purple spots. Two to three sub-branches break out from the stalk and divide dichotomously around 7-8 times. Branches are slim and long, cylindrical or slightly flat, 1.5-2 cm tall and 0.2-0.3 cm diameter. The end of each branch breaks into two sharp tips like a fork. Small basidioma are light orange-white or

light yellowish-orange, large fully grown basidiomas are brownish-orange or orangey-yellow (Fig. 10a). Some basidiomas have brownish-orange powder on the forks or surface. The inner flesh is white, hollow, succulent and has a dry surface.

Chemical reactions: Surface color turns green rapidly when tested with 10% (w/v) FeSO_4 and dark green slowly when tested with Melzer's reagent. It turns brownish-yellow when tested with 10% (w/v) KOH.

Spore shape is ellipsoid or broadly ellipsoid, 4-5.5 μ wide and 6.5-9 μ long, with a verrucose surface at the ends (Fig. 10b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium around 8-10 μ wide and 45-65 μ long. The ratio of basidium to sterigmata is 1:4 (Fig. 10c). Sterigma size is 4-7 μ . Clamp connections could not be found at the fibers and basidium base.

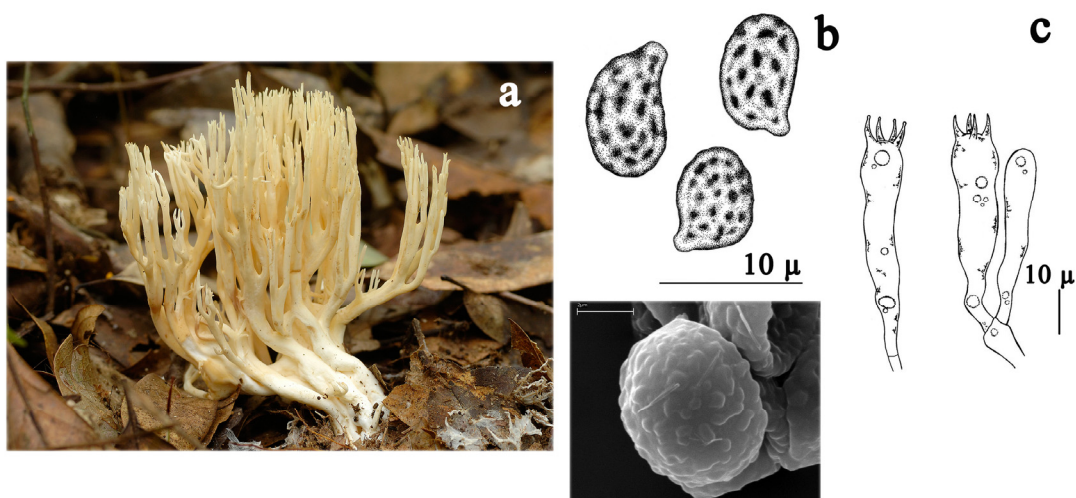


FIGURE 10. *Ramaria sino-conjunctipes* R. H. Petersen & M. Zang showing the (a) basidioma, (b) spore and (c) basidium.

11. *Ramaria velocimutans* Marr & D.E. Stuntz, *Ramaria* of Western Washington: 124 (1974)

Eighteen samples were randomly selected for data evaluation. The basidioma is a wide expanding bush, 4-15 cm tall and 5.5-12 cm wide, shaped similar to coral and with a rough, slightly wavy or smooth and slightly sticky surface. When scratched, it does not change color. It has a single, creamy-white or brownish-creamy-white, thick and fat stalk, 1.5-3 cm tall and 2.5-3.5 cm diameter, from which many sub-branches break out and then divide polychotomously or dichotomously around 6-8 times to form the wide bush. The cylindrical shaped creamy-white, light white or light brownish-creamy-white branches are short, thick and fat, 1-1.5 cm tall and 0.3-0.8 cm in diameter. Some basidiomas will have light yellowish-brown powder on the surface or forks. The ends of the

branches break into 2-4 small, light brownish-creamy-white or light brownish-orange tips (Fig. 11a). The inner flesh is firm with a dry surface, and is white except for a clear brown semi-gelatin zone at the base.

Chemical reactions: The surface color rapidly turns blue-green when tested with 10% (w/v) FeSO_4 and turns yellow-orange when tested with 10% (w/v) KOH. The inner flesh turns green slowly when tested with 10% (w/v) FeSO_4 .

Spore shape is ellipsoid to sub-cylindrical, 3-4.5 μ wide and 8-12 μ long, with a verrucose surface at the ends (Fig. 11b). Spores dyed with cotton blue (cyanophilous) have a clavate shaped basidium around 6-8 μ wide and 45-50 μ long in size. The ratio of basidium to sterigmas is 1:4 (Fig. 11c). Sterigma size is 7-15 μ . Clamp connections were found at the fibers and base of the basidium.

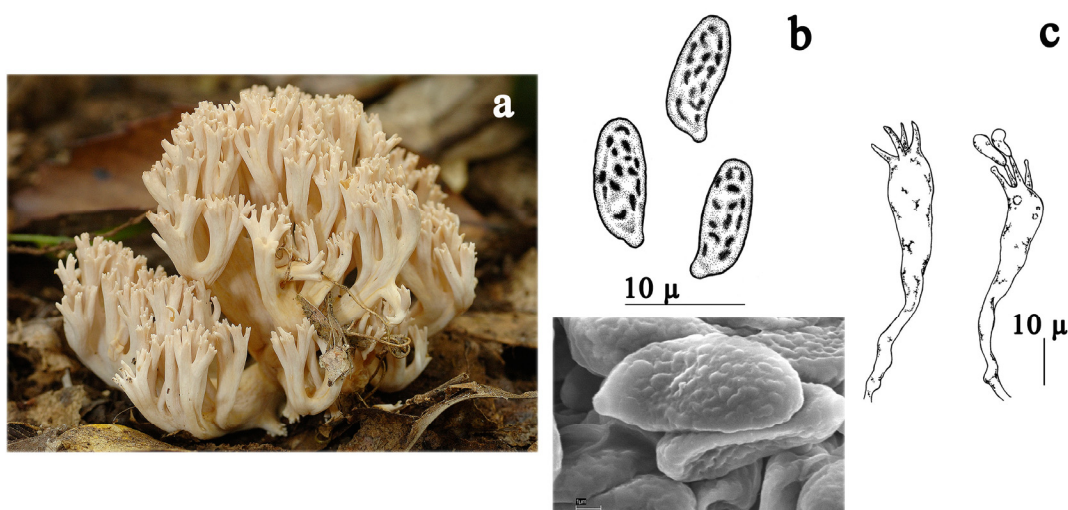


FIGURE 11. *Ramaria velocimutans* Marr & D.E. Stuntz showing the (a) basidioma, (b) spore and (c) basidium.

Keys to species of *Phaeoclavulina* and *Ramaria* coral fungi found in Nam Nao National Park.

- 1 Basidiomata branching dichotomously, dark toned colored, grows on the ground or on decomposing trees, clamp connections present, spore surface echinulate (*Phaeoclavulina* Blinkmann).....2
- 1 Basidiomata branching dichotomously, brightly colored, clamp connections absent or present, spore surface verrucose or striate (*Ramaria* Fr. ex Bonord.).....3
- 2 Basidiomata yellow to brownish-yellow, branches yellow to brownish-yellow, height up to 12 cm, spore size $9-3.5 \times 4-5.5 \mu$, basidium length more than 40μ
Phaeoclavulina cokeri (R.H. Petersen) Giachini
- 2 Branches blue to blue-green, height up to 23 cm, spore size $4.5-6 \times 6-10 \mu$, spore to basidium ratio 2:1, basidium size more than 40μ*Phaeoclavulina cyanocephala* (Berk. & M.A. Curtis) Giachini
- 3 Spore surface striate, basidioma color light pink or light purplish-pink, height up to $4-5.5 \times 9-12 \mu$, basidium length more than 48μ
Ramaria rubripermanens Marr & D.E. Stuntz
- 3 Spore surface verrucose4
- 4 Found on tree logs or areas with decomposing leaves, clamp connections present, branches yellow-brown to dark brown, when scratched, color changes to dark brown, spore size $\sim 6-9 \times 10-15 \mu$, basidium size more than 40μ
Ramaria concolor (Corner) R.H. Petersen
- 4 Clamp connections present or none, categorized as mycorrhiza or grows on the ground..... 5
- 5 Clamp connections absent at the basidium base..... 6
- 5 Clamp connections present at the basidium base.....8
- 6 Orangey-white to light orange basidioma, spore size less than or equal to 9μ7
- 6 Pink basidioma, height up to 9 cm., gelatin-like inner flesh, spore size $\sim 3.5-5 \times 7-5 \mu$, basidium size less than 35μ
Ramaria botrytoides (Reck.) Quél
- 7 Orange or yellow-orange basidioma, height up to 7 cm, inner flesh hollow, succulent and gelatin-like, spore size $4.5-5.5 \times 8-9 \mu$, basidium length less than 40μ
Ramaria conjunctipes (Coker) Conner
- 7 Orangey-white to light brownish-orange basidioma, height up to 12 cm, inner flesh hollow, succulent but dry surface, spore size $4-5.5 \times 6.5-9 \mu$, basidium length more than 45μ
Ramaria sino-conjunctipes R. H. Petersen & M. Zang
- 8 Basidiomata yellow or orange.....9
- 8 Basidioma creamy white or brownish-creamy-white, height up to 15 cm., inner flesh of the stalk brown with white streak, semi-gelatin like, spore size $3-4.5 \times 8-12 \mu$, basidium size more than 45μ
Ramaria velocimutans Marr & D.E. Stuntz
- 9 Basidioma yellow.....10
- 9 Basidioma orange, height up to 7.5 cm., spore size $3-4.5 \times 7-11 \mu$, basidium length more than 35μ , gloeoplerous hypha present at the fibers.....
Ramaria cystidiophora var. *fabiolens* Marr & D.E. Stuntz
- 10 Basidioma spreads out like a fan, stout stalk, fat and white, yellow branches, height up to 7 cm, spore size $3.5-4.5 \times 7.5-11 \mu$, basidium length more than 35μ
Ramaria flava (Fr.) Corner
- 10 Light yellow or whitish-yellow basidioma, the color changes to red or brownish-red when bruised, height up to 12.5 cm., spore size $3-5 \times 7-11.5 \mu$, basidium size more than 30μ*Ramaria sanguinipes* R.H. Petersen & M. Zang

Evolutionary relationships.— By studying the evolutionary relationships using the ARDRA technique, it was found that the PCR amplicons of the ITS1-5.8S-ITS2 region fragment of DNA (defined by the ITS1-F/ITS-4 primers) of coral fungi in the *Phaeoclavulina* genera have DNA fragments sized around 680-700 bps, whilst that for the DNA fragments from ramarioid-coral fungi were different but partially overlapping at around 620-790 bps (data not shown). After single restriction enzyme

digesting with (separately) *Alu* I, *EcoR* I, *Hinf* I and *Taq* I (Fig. 12), the restriction fragment band profile was scored and used to form a phylogenetic tree to compare the evolutionary relationships between these coral fungi (Fig. 13) for 2 representative samples per species previously classified by morphological analysis, as detailed above. Using a similarity coefficient of 93% all ramarioid-coral fungi found in the Nam Nao National Park, Thailand, could be divided into 10 individual groups.

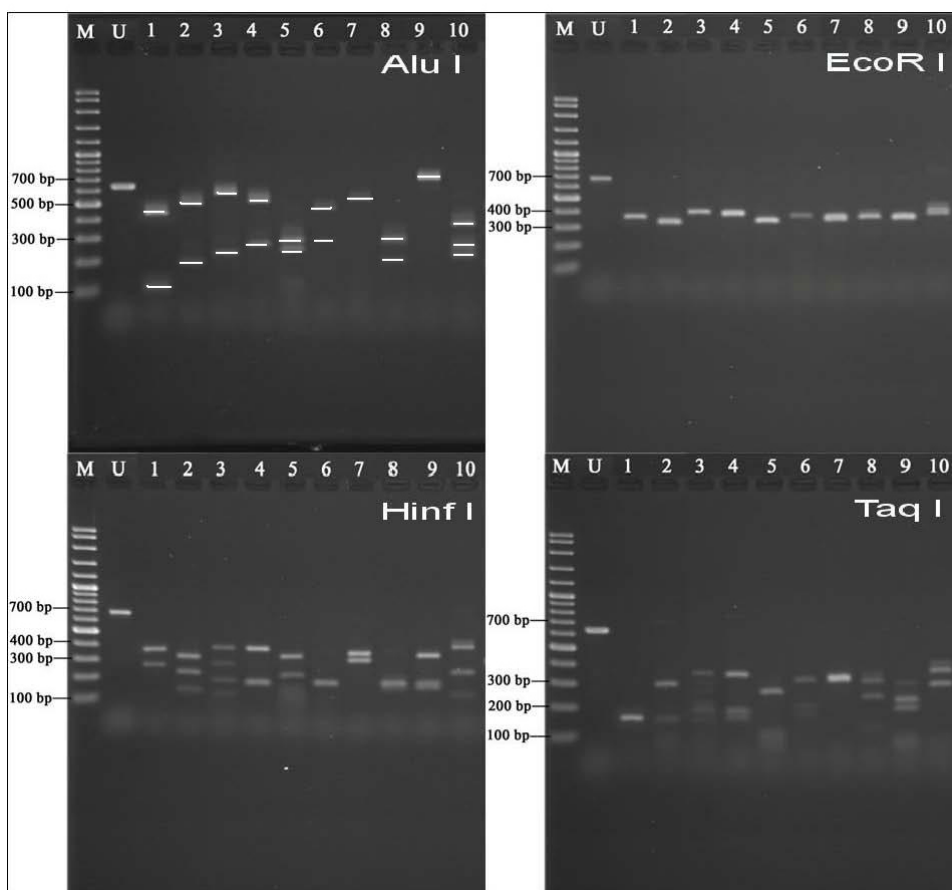


FIGURE 12. DNA restriction patterns from digesting the ITS1-5.8 rDNA-ITS2 amplicon fragment with one of the indicated four restriction enzymes (*Alu* I, *EcoR* I, *Hinf* I and *Taq* I). Lanes: **M**, 100 bp ladder marker; **U**, Unrestricted PCR amplicon from *Ramaria botrytoides*; and then restricted amplicons from **1**, *Phaeoclavulina cokeri*; **2**, *P. cyanocephala*; **3**, *R. botrytoides*; **4**, *R. concolor*; **5**, *R. cystidiophora* var. *fabiolens*; **6**, *R. flava*; **7**, *R. rubripermanens*; **8**, *R. sanguinipes*; **9**, *R. sino-conjunctipes* and **10**, *R. velocimutans*.

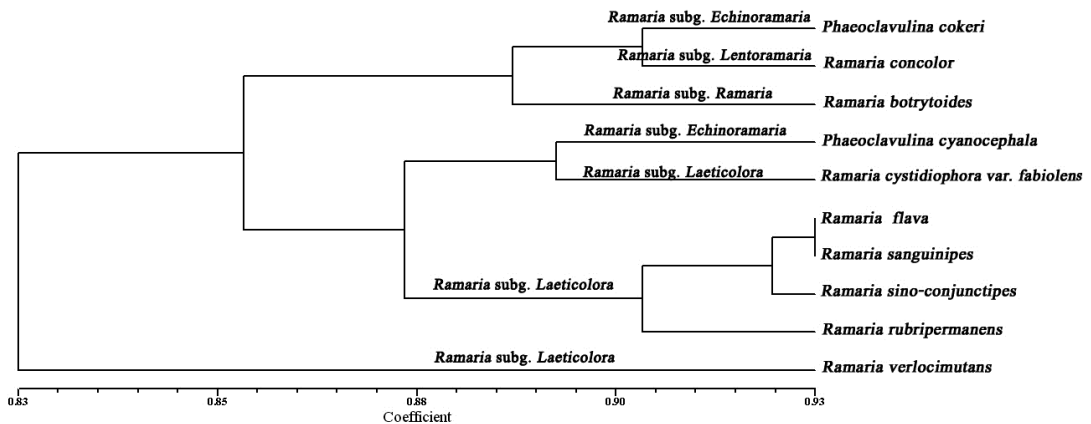


FIGURE 13. Phylogenetic tree of all Ramarioid fungi from Nam Nao National Park drawn the from UPGMA analysis of the *Alu* I, *EcoR* I, *Hinf* I and *Taq* restriction fragment DNA patterns of the ITS1-5.8 rDNA-ITS2 amplicon fragment.

The two species in the genus *Phaeoclavulina* do not get placed together and separate from those in the genus *Ramaria*, but rather the two *Phaeoclavulina* species are placed within *Ramaria*, one within the *Ramaria lentoramaria* subgenus and sister clade to the *Ramaria ramaria* subgenus, and the other within a sister clade of *Ramaria laeticolora* subgenus. This is probably due to that the number of restriction enzymes employed in this research was technically improper for cluster analysis as shown in a sister clade among *Clavaria miyabeana* and *Ramariopsis* spp. (Maneevun and Sanoamuang, 2010). To perform the expected phylogenetic relationships i.e. to separate *Phaeoclavulina* completely from *Ramaria*, combined sequences e.g. nuclear large subunits rDNA, mitochondrial small subunit rDNA, and mitochondrial *atp6* DNA demonstrated by Giachini et al., (2010).

CONCLUSION

By studying the diversity of coral shaped fungi in Nam Nao National Park, two genera and 11 species were found as follows:

1. *Phaeoclavulina* genera (two species): *P. cokeri* (R.H. Petersen) Giachini and *P. cyanocephala* (Berk. & M. A. Curtis) Giachini
2. *Ramaria* genera (nine species), which are divided into the following three subgenera:
 - 2.1) subgenus *Laeticolora* (seven species): *R. conjunctipes* (Coker) Corner, *R. cystidiophora* var. *fabiolens* Marr & D.E. Stuntz, *R. flava* (Fr.) Corner, *R. rubripermanens* Marr & D.E. Stuntz, *R. sanguinipes* R.H. Petersen & M. Zang, *R. sino-conjunctipes* R.H. Petersen & M. Zang and *R. velocimutans* Marr & D.E. Stuntz
 - 2.2) subgenus *Lentoramaria* (one species): *R. concolor* (Corner) R.H. Petersen
 - 2.3) subgenus *Ramaria* (one species): *R. botrytoides* (Reck.) Quél

P. cokeri and *P. cyanocephala* were formerly categorized in the *Ramaria* family in subgenus *Echinoramaria* but were moved to the *Phaeoclavulina* genera because the fungi color was dark in tone, such as green, blue, brown, reddish-orange, etc. It grows on the ground or on decomposing rotten

wood. Fiber system type is monomitic. Clamp connections could be found at the fibers. Spores are spiky or verrucose (Giachini, 2004), but generally the former name and category of coral fungi are still widely used.

The ITS1-5.5S rDNA-ITS2 PCR amplicon size, as defined by the ITS-1F/ITS-4 primer pair, of *Phaeoclavulina* and *Ramaria* coral fungi is around 620-700 bp (data not shown). Using the ARDRA technique to study the evolutionary relationships with a similarity coefficient of 93%, coral fungi could be categorized efficiently. From the 135 samples collected and examined, 11 species of coral fungi were found in this study of which eight species are reported for first time in Thailand, specifically: *R. botrytoides*, *R. concolor*, *R. conjunctipes*, *R. cystidiophora* var. *fabiolens*, *R. rubripermanens*, *R. sanguinipes*, *R. sino-conjunctipes* and *R. velocimutans*.

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