

Monograph of Eight Simple-club Shape Clavarioid Fungi from Nam Nao National Park Based on Morphological and Molecular Biological Data

*Ammanee Maneevun*¹

Niwat Sanoamuang^{2*}

Abstract

Eight taxa from 2 genera of simple-club shape clavarioid fungi from Nam Nao National Park, Thailand collected during rainy season in 2008-2009 were investigated and illustrated. Identification to species level was based on morphological and molecular characteristics. Monographs of macroscopic fruiting bodies, light and scanning electron microscopic detail of spores, basidia and hyphae were illustrated. A phylogenetic tree was created by analysis of DNA fingerprint using Amplified Ribosomal DNA Restriction Analysis technique (ARDRA) on ITS1-5.8S-ITS2 rRNA gene. All found species were *Clavaria falcata* Persoon, *Clavaria rosea* Dalman, *Clavaria vermicularis* Swartz, *Clavaria aurantiocinnabarina* Schweinitz, *Clavaria miyabeana* S. Ito in S. Imai., *Ramariopsis fusiformis* (Sowerby) R.H. Petersen, *Ramariopsis helvola* (Pers. Ex. Fr.) R.H. Petersen and *Ramariopsis laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen. The ARDRA technique was properly used to classify the simple-club shape clavarioid fungi into specific levels. Two species of these, *C. falcata* Persoon and *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen are new records for Thailand.

Keywords: clavarioid fungi, monograph, phylogeny, *Clavaria*, *Clavulinopsis*, *Ramariopsis*

¹Graduate Student, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University

²Associate Professor, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, and the Applied Taxonomy Research Center, Khon Kaen University

*Corresponding author, e-mail: niwat@kku.ac.th

Introduction

In this research, simple-club coral fungi include the ones with single stem without branches, which can be commonly found in Thailand, locally called Hed-Pa-Ka-Rang-Khem, Hed-Pa-Ka-Rang-Nham or Hed-Pa-Ka-Rang-Non (Chandrasrikul et al., 2008). Either Hed-Pa-Ka-Rang-Khem or Hed-Pa-Ka-Rang-Nham has pointed tips while those of Hed-Pa-Ka-Rang-Non are rounded. Both, however, are similar in terms of their single stems fruiting bodies emerging from the ground which could be a single stem or twisted and straight up with very few or no branch. Their fruiting bodies are colorful, especially the one in yellow. These types of fungi can be found anywhere in the world (Dentinger and McLaughlin, 2006). They are classified in Phylum Basidiomycota, Class Agaricomycetes, Sub-class Phallomycetidae (Hibbett et al., 2007; Kirk et al., 2008), Order Cantharellales and several Families such as Family Clavariaceae.

Within the Hed-Pa-Ka-Rang-Nham or Hed-Pa-Ka-Rang-Non's genera, there are *Clavaria*, *Clavulina*, *Clavulinopsis* and *Ramariopsis*. Nevertheless, there is still some confusion on the clear characteristics between *Clavaria*, *Clavulinopsis* and *Ramariopsis*. Petersen (1978), therefore, used the following classifications: 1) Characteristics of the hilar appendix; 2) Color pigment tendency to have carotene or other color; 3) Existence of phenol which turned into green when colored with ferric sulfate solution; 4) Spore surface which could be smooth or uneven; and 5) Difference in the amount of nuclei in basidia and spores. Those in the *Clavulinopsis* genera were combined with the *Clavaria* or *Ramariopsis* genera. The *Clavulinopsis* genus was then demoted to be subgenus, with some controversy over the issue (Olariaga, 2009).

Since it can be rather difficult to clearly classify by considering only the morphological characteristics which can be done only by field experts, the molecular characteristics are currently playing an important role in being part of the consideration. For example, the Amplified Ribosomal DNA Restriction Analysis (ARDRA) is used to clearly identify the differences among taxa by increasing the amount of DNA in rRNA gene with specific primers for each rRNA gene.

Nam Nao National Park, with the total area of 966 square kilometers or 603,750 Rais, is located on the area covering Amphur Muang, Amphur Lom Sak, Amphur Nam Nao, in Petchaboon Province, and Amphur Kon San, in Chaiyapoom Province. It sits on the Petchaboon Mountain Range which separates the Northeast from the North regions. The area is mostly covered with forest; Dry Dipterocarp Forest or Deciduous Dipterocarp Forest, Mixed Deciduous Forest, Tropical Rain Forest, Coniferous Forest, Pine Forest, and Open Grass Lands (Department of National Parks, Wildlife and Plant Conservation, 2009). This national park is still rich and abundant, suitable for the study of fungi species in Thailand, on which a very few researches has been conducted, especially on simple-club shape clavarioid fungi. The study was done using the analysis of morphological and molecular characteristics for complete information.

Methodology & Equipments

Samples of simple-club shape clavarioid fungi were collected from the Nam Nao National Park during rainy season, June to September of 2008 to 2009. Fungi's macroscopic morphological characteristics include shape, size, surface, fresh, and changing of color when touched. An experiment

on chemical reactions was conducted by using 10% FeSO_4 and 10% KOH on the surface. The microscopic morphological characteristics were tested and recorded after staining the spores with cotton blue and Melzer's reagent (shape, size, and surface) basidia (shape, size, clamp-connection, number of spores per sterigma) and hyphae at magnification of 1,000 times. In addition, a Scanning Electron Microscopic (SEM) detail of spore surface were done by cutting fruiting body tissues on stub then coated with gold (Hahn and Christan, 2002), then investigated their detailed surface of spores using Scanning Electron Microscope (LEO model 1450 VP).

A phylogenetic tree was created by analysis of DNA fingerprint using Amplified Ribosomal DNA Restriction Analysis technique (ARDRA) with SDS lysis buffer (400mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1% sodium dodecyl sulfate), according to the method applied from Liu et al. (2000). The targeted DNA amount was increased by using polymerase chain reaction (PCR) in ITS1-5.8s-ITS2 by using specific primer ITS1F and ITS4 (Gardes and Bruns, 1993; White et al., 1990; Olariaga, 2009). The PCR procedures used were adapted from method by Olariaga (2009). The first step was the targeted DNA pre-denaturation, at 94 degree Celsius for 5 minutes. Then the next step was to increase DNA by denaturation at 94 degree Celsius for 30 seconds, alternating with annealing process at 54.5 degree Celsius for 30 seconds and extension process at 72 degree Celsius for 30 seconds. This was done in total of 35 replications. PCR product digested with specific enzyme *Alu* I, *Eco*R I, *Hinf* I and *Taq* I were then distinguished by gel electrophoresis on

2% agarose gel. Differences on DNA bands were used to create a phylogenetic tree for simple-club shape clavarioid fungi relationship analysis with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), on NTSYSps version 2.0.

Results

Variation on Taxa and Morphological Characteristics

Clavaria falcata Persoon, Neues Mag. Bot. 1: 118 (1794)

The fruiting body is smooth, slim, club-like and straight up without branches. It is 1.7-7.8 cm. in height and 0.2-0.4 cm. in diameter. Its cylindrical stub can be both isolated or stay in cluster, 1-1.5 cm. in height and 0.1-0.2 cm. in diameter, mostly clear and white or cream in color. The top part is flat with long groove in the middle, and solid white or cream in color. The end is flat and rather rounded, with brownish yellow in color. The inner fresh is hollow, dry, and white in color (Figure 1A). It smells like garlic, and in this experiment, was not tested for the chemical reactions.

Spore shape is broadly ovate to narrowly ellipsoid, with smooth tiny lobe of 8.3x6.6 microns in size (7-12 microns long and 4-9 micron wide), shown in Figure 1B. It's basidia has clavate shape with 9-12 microns wide and 45-67 microns long. It was found with 4 sterigmata per basidia (Figure 1C) while a sterigma was 2-5 microns long, and the clamp connection on both hyphae and basidia base were not found.

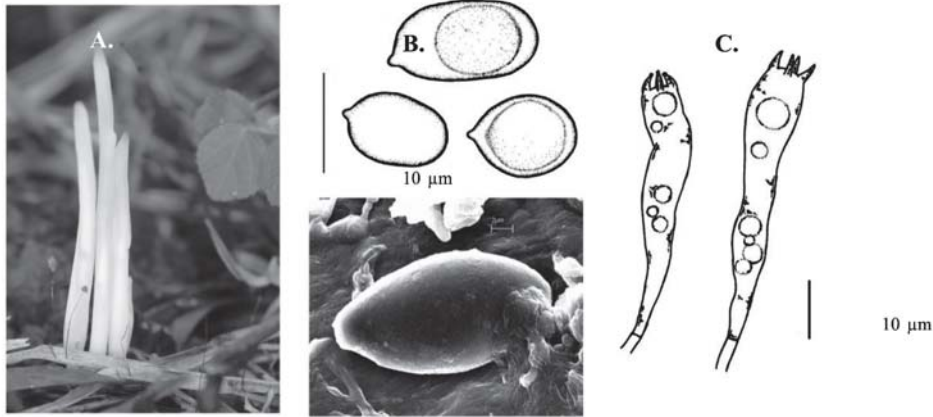


Figure1. *Clavaria falcata* Persoon: A. Fruiting Bodies, B. Spore, C. Basidium

***Clavaria rosea* Dalman, Vetensk. Akad. Handl. 32: 157 (1881)**

The fruiting body is a long, straight, fragile, cylindrical club pointing upward, with no branches. It has a size of 3-4.5 cm. in height and 0.2-0.3 cm. in width. The surface is smooth and they tend to stay together in a cluster, 0.3-0.5 cm. high and 0.1-0.15 cm. in diameter. It tends to be small at base and grows bigger toward the end. The color is clear red, shiny and transparent. As it grows bigger in the top part, in a cylindrical shape, the color is solid red with white patches or whitish powder around. The end is rounded and red or dark red (Figure 2A).

Inner fresh is firm, moist, and gelatin-like. The color is clear red. In this experiment, they were not tested for the chemical reactions.

The spore has a shape of subglobose or ellipsoid, with smooth tiny lobe in an average size of 5.7x3.2 microns. It is 4.5-6 microns in length and 2.5-4 microns in width (Figure 2B). The basidium has a club shape, 6-8 microns in width and 30-45 microns in length. 2-4 sterigma per basidia were found, shown in Figure 2C. The sterigma is 2.5-4.5 microns long. There is no clamp connection in both hyphae and basidia.

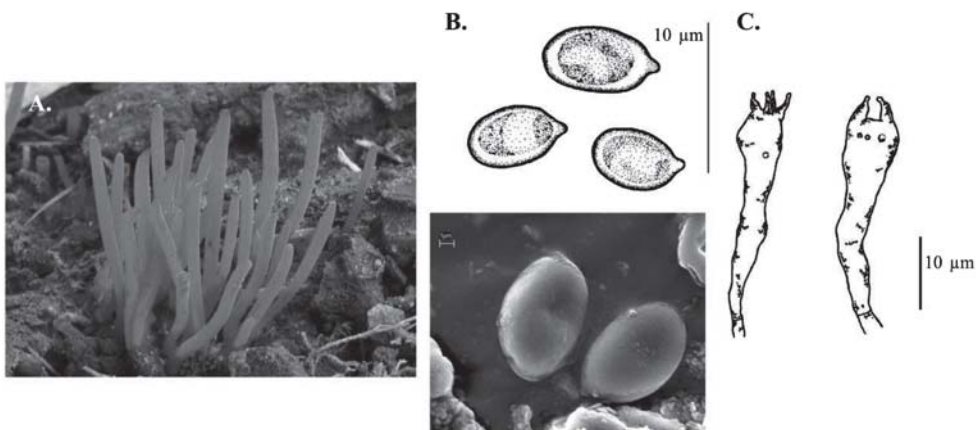


Figure2. *Clavaria rosea* Dalman: A. Fruiting Bodies, B. Spore, C. Basidium

***Clavaria vermicularis* Swartz, Kungl. Svenska Vetenskapsakad. Handl. 32: 159 (1811)**

The fruiting body is a long, straight, fragile, cylindrical club pointing upward, with no branches. It has a size of 2.6-5 cm. in height and 0.1-0.2 cm. in width. They tend to grow individually, 0.3-0.5 cm. high and 0.1 cm. in diameter. It tends to be thin and grows larger toward the end. The color is clear white, shiny and transparent. As it grows bigger in the top part, in a cylindrical shape, the color is solid white with patches or white dusty around. The fruiting tip is rounded and white or brownish yellow (Figure

3A). The inner fresh is firm, dry, and white. In this experiment, they were not tested for the chemical reactions.

The spore has a shape of ellipsoid, with smooth tiny lobe in an average size of 7.1x3.2 microns. It is 5-9 microns in length and 3-4 microns in width (Figure 3B). The basidium has a club shape, 6-9 microns in width and 30-40 microns in length. Four sterigmata per basidium were found, shown in Figure 3C. The sterigma is 2-3 microns long. There is no clamp connection in both hyphae and basidia.

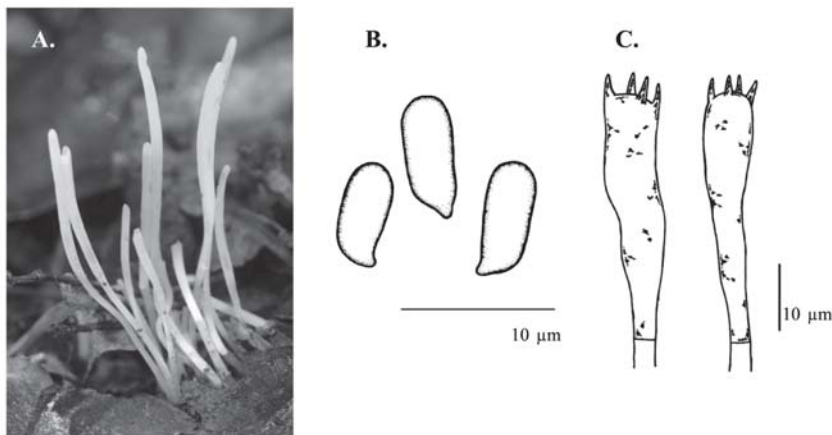


Figure 3. *Clavaria vermicularis* Swartz: A. Fruiting Bodies, B. Spore, C. Basidium

***Clavaria aurantiocinnabarina* Schweinitz, Ann. Bot. Mem. 1: 358 (1950)**

The fruiting body can be a long and straight or twisted cylindrical club pointing upward, with no branches. It has a size of 1-5 cm. in height and 0.1-0.2 cm. in width. They grow individually but in clusters, 0.3 cm. high and 0.1 cm. in diameter. Its color is light orange, covered with white hair. The upper part is cylindrical, some has orange or reddish orange groove in the middle (Figure 4A). The inner fresh can be firm or hollow, dry, and reddish orange

in color. The fruiting body do not change color when tested with 10% FeSO_4 or 10% KOH.

The spore has a shape of globose or subglobose, with smooth tiny lobe in an average size of 6.7x5.9 microns. It is 5-8 microns in length and 4-7 microns in width (Figure 4B). The basidia has a club shape, 7-9 microns in width and 45-62 microns in length. Four sterigmata per basidium were found, shown in Figure 4C. The sterigma is 4-7 microns long. Clamp connections were found in both hyphae (Figure 4D) and basidia.

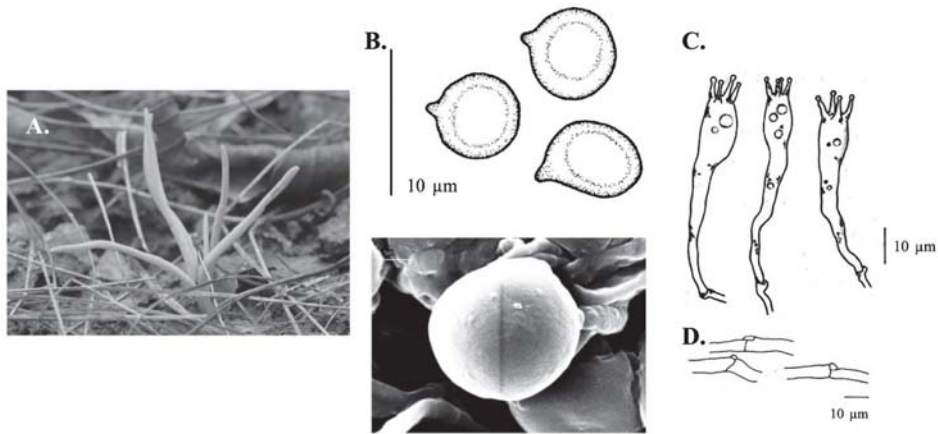


Figure 4. *Clavaria aurantiocinnabarina* Schweinitz: A. Fruiting Bodies, B. Spore, C. Basidium, D. clamp connection on hypha

***Clavaria miyabeana* S. Ito in S. Imai, Trans. Sapp. Nat. Hist. Soc. 11:72 (1930)**

The fruiting body can be a flat, long and point straight upward or twisted cylindrical and worm-like, with no branches. It has a size of 7.8-13.5 cm. in height and 0.4-0.5 cm. in width. The surface can be smooth or a bit uneven. They grow individually but stay in clusters, 1-1.5 cm. high and 0.5 cm. in diameter. Its color is orange or carrot-like orange. The upper part can be pointed or a bit rounded, orange or carrot-like orange in color (Figure 5A). The inner fresh is dry and hollow, and orange or carrot-like orange in color. The fruiting body slowly

changed into green when tested with 10% FeSO_4 and also changed into black when tested with Melzer's reagent. However, it did not respond to 10% KOH.

The spore has a shape of globose or subglobose, with smooth tiny lobe in an average size of 6.4x5.2 microns. It is 5-8 microns in length and 4-6 microns in width (Figure 5B). The basidium has a clavate shape, 6-9 microns in width and 35-52 microns in length. Four sterigmata per basidium were found, shown in Figure 5C. The sterigma is 3-4 microns long. Clamp connections were found in both hyphae (Figure 5D) and basidia.

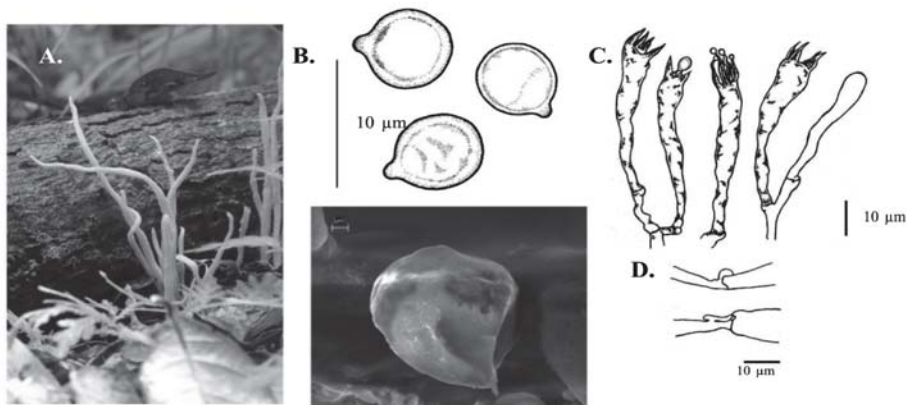


Figure 5. *Clavaria miyabeana* S. Ito in S. Imai: A. Fruiting Bodies, B. Spore, C. Basidium, D. clamp connection on hypha

***Ramariopsis fusiformis* (Sowerby) R.H. Petersen,
Mycologia 70(3): 668 (1978)**

The fruiting body can be a long and straight or slightly twisted cylindrical club pointing upward, with no branches. It has a size of 2-7 cm. in height and 0.1-0.2 cm. in width. It is smooth and solid. The cylindrical bases stay in clusters, 0.5-0.8 cm. high and 0.1-0.2 cm. in diameter, and light orange in color. The upper part is somewhat flat, and golden yellow or yellowish orange. It has a pointed end and yellow or brownish yellow in color (Figure 6A). The inner fresh is firm and dry, and light yellow in

color. The fruiting body did not respond to 10% KOH.

The spore has a shape of globose or subglobose, with smooth tiny lobe in an average size of 6x5.2 microns. It is 5-5.7 microns in length and 4.5-6 microns in width (Figure 6B). The basidia has a clavate shape, 6-9 microns in width and 35-52 microns in length. Four sterigmata per basidium were found, shown in Figure 6C. The sterigma is 3-4 microns long. Clamp connections were present in both hyphae (Figure 6D) and basidia.

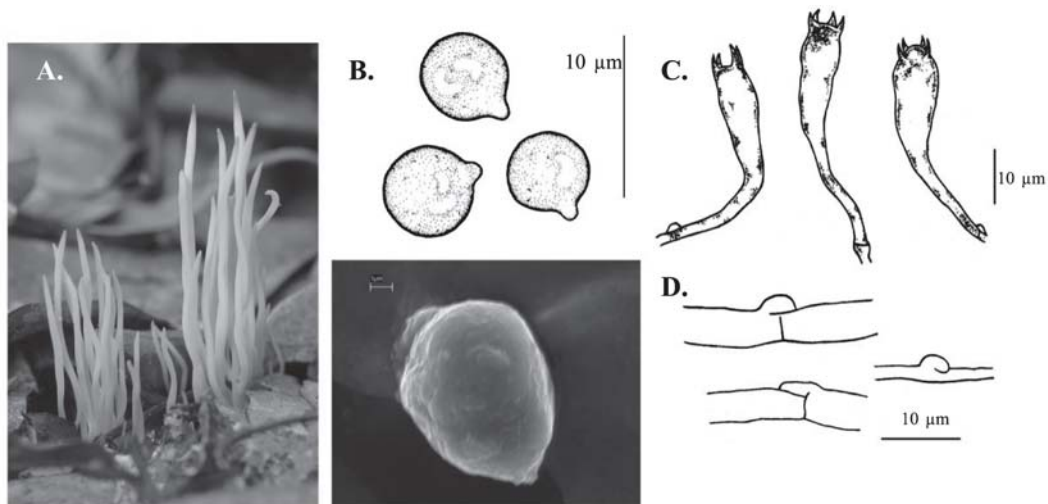


Figure 6. *Ramariopsis fusiformis* (Sowerby) R.H. Petersen: A. Fruiting Bodies, B. Spore, C. Basidium, D. clamp connection on hypha

***Ramariopsis helvola* (Persoon) R.H. Petersen,
Mycologia 70(3): 668 (1978)**

The fruiting body is a long and straight cylindrical club pointing upward, with no branches. It has a size of 1-5 cm. in height and 0.1-0.4 cm. in width. The single and smooth cylindrical bases is 0.5-0.8 cm. high and 0.1 cm. in diameter, and yellow in color. The upper part is bigger than the base, somewhat flat and has a yellow groove in the middle.

It has a slightly flat and rounded end in yellow or brown (Figure 7A). The inner fresh is hollow and dry in yellow. The fruiting body changed into orange when tested with 10% KOH.

The spore has broadly ovoid or subglobose shape, with tiny lobes with sharply angular or echinulate warts, in an average size of 6.5x5.2 microns. It is 5-9 microns in length and 4-6 microns in width (Figure 7B). The basidia has a clavate

shape, 6-10 microns in width and 30-55 microns in length. Four sterigma per basidia were found, shown in Figure 7C. The sterigma is 4-5 microns long.

Clamp connections were found in both hyphae (Figure 7D) and basidia.

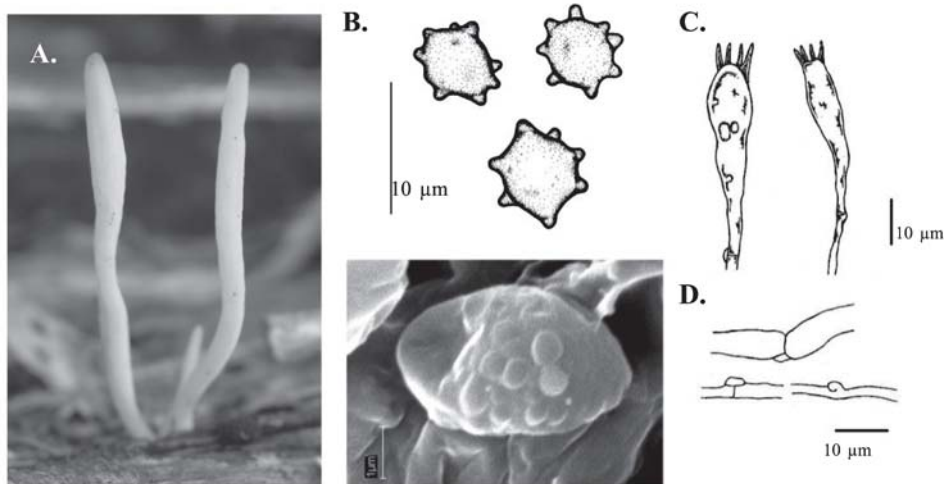


Figure 7. *Ramariopsis helvola* (Persoon) R.H. Petersen: A. Fruiting Bodies, B. Spore, C. Basidium, D. clamp connection on hypha

***Ramariopsis laeticolor* (Berkeley & M.A. Curtis)
R.H. Petersen, *Mycologia* 70(3): 668 (1978)**

The fruiting body can be long and pointing straight up or slightly twisted, cylindrical or slightly flat, club with no branches. It has a size of 1-3.5 cm. in height and 0.1-0.3 cm. in width. The surface is sometimes smooth or a bit uneven. It has a single base growing in cluster, 0.5 cm. in height and 0.1 cm. in diameter, and yellowish white or light yellow in color. There are white hair covering the surface. The upper part is cylindrical or slightly flat and yellow or golden yellow. It has a pointed or a bit rounded end in yellow or brownish yellow (Figure

8A). The inner fresh is hollow and dry in yellow. The fruiting body slowly changed into green when tested with 10% FeSO_4 and changed into green when tested with 10% KOH.

The spore has broadly ovate or ovate or pyriform shape, with smooth tiny lobes, in an average size of 5×3.9 microns. It is 3.5-7 microns in length and 3.5-5 microns in width (Figure 8B). The basidium has a clavate shape, 5-7 microns in width and 38-50 microns in length. Four sterigmata per basidium were found, shown in Figure 8C. The sterigma is 3-6 microns long. Clamp connections were found in both hyphae (Figure 8D) and basidia.

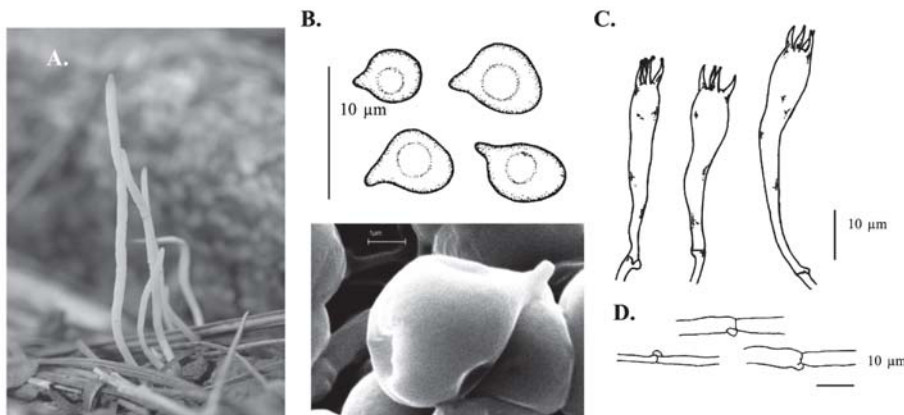


Figure 8. *Ramariopsis laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen: A. Fruiting Bodies, B. Spore, C. Basidium, D. clamp connection on hypha

The Phylogenetic Tree

A phylogenetic tree was created by analysis of DNA fingerprint using Amplified Ribosomal DNA Restriction Analysis technique (ARDRA) on ITS1-5.8S-ITS2 rRNA gene. The following species: *Clavaria falcata* Persoon, *Clavaria rosea* Dalman, and *Clavaria vermicularis* Swartz, had the size of 680 bp, whereas *Clavaria aurantiocinnabarina* Schweinitz and *Clavaria miyabeana* S. Ito in S. Imai, were at 620 bp. On the other hand, *Ramariopsis fusiformis* (Sowerby) R.H. Petersen, *Ramariopsis helvola* (Pers. Ex. Fr.) R.H. Petersen, and *Ramariopsis laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen were found to be 660 bp in size. From the DNA pattern digested with 4 specific enzymes which were *Alu* I, *EcoR* I, *Hinf* I,

and *Taq* I (Figure 9), a phylogenetic tree was created to classify the 8 types of simple-club shape clavarioid fungi into specific levels, at 61 percent similarity coefficient. As a result, they can be classified into 2 groups. Group 1 consisted of *C. falcata* Persoon, *C. rosea* Dalman, and *C. vermicularis* Swartz. Group 2 were *C. aurantiocinnabarina* Schweinitz, *C. miyabeana* S. Ito in S. Imai, *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen, *R. helvola* (Persoon) R.H. Petersen, and *R. fusiformis* (Sowerby) R.H. Petersen, at 93% similarity coefficient. The distinctions between species were obvious, except for *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen and *R. helvola* (Persoon) R.H. Petersen, which were extremely similar (Figure 10).

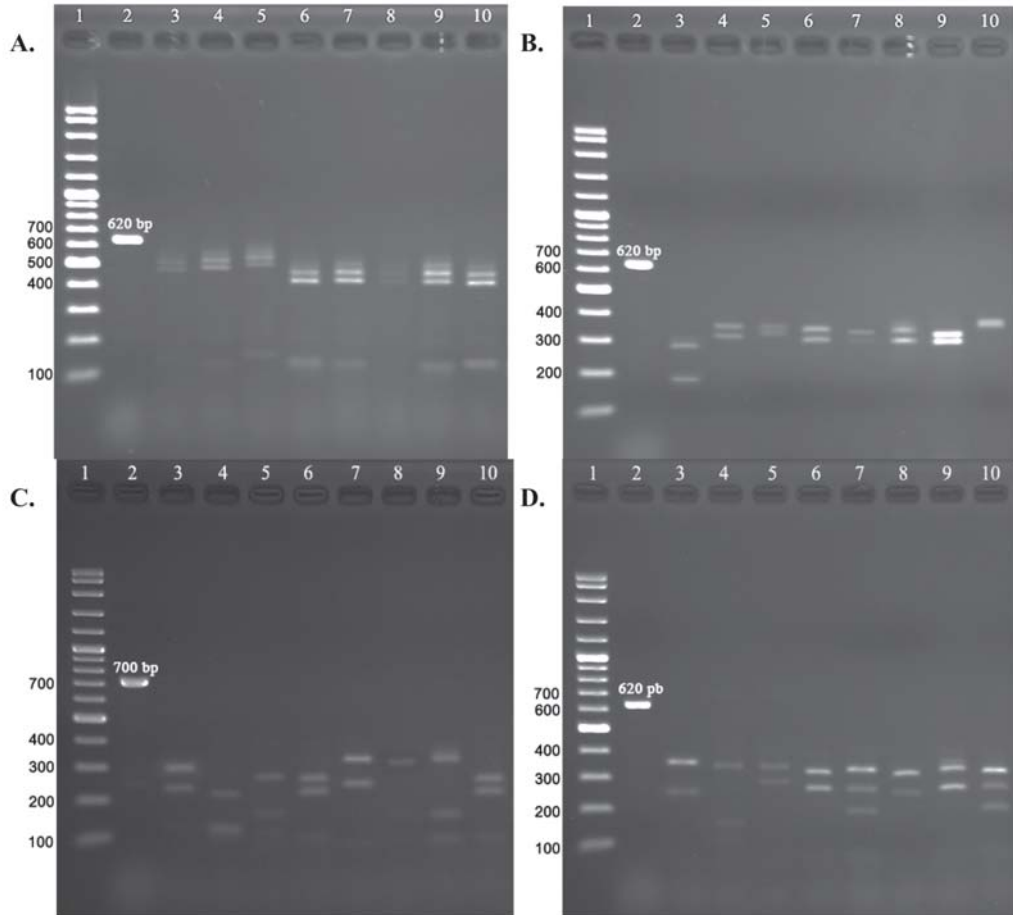


Figure 9. DNA patterns of simple-club shape clavarioid fungi digested with specific enzymes A. *Alu* I Enzyme, B. *EcoR* I Enzyme, C. *Hinf* I Enzyme, D. *Taq* I Enzyme. The 1st column was Marker 100 bp, the 2nd was uncut, the 3rd was *Clavaria falcata* Persoon, the 4th was *Clavaria rosea* Dalman, the 5th was *Clavaria vermicularia* Swartz, the 6th was *Clavaria aurantiocinnabarina* Schweinitz, the 7th was *Ramariopsis fusiformis* (Sowerby) R.H. Petersen, the 8th was *Ramariopsis helvola* (Persoon) R.H. Petersen, the 9th was *Ramariopsis laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen, and the 10th was *Clavaria miyabeana* S. Ito in S. Imai.

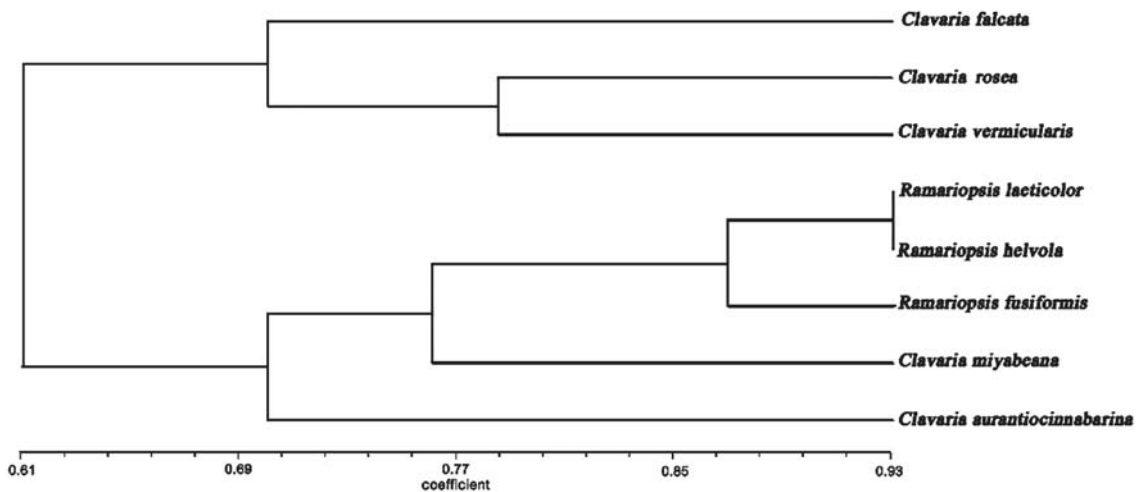


Figure10. A Phylogenetic tree illustration relationships of simple-club shape clavarioid fungi when applying DNA patterns digested with specific enzymes: *Alu* I, *EcoR* I, *Hinf* I and *Taq* I. Analysis by the UPGMA method.

Discussion

From the study of various species of simple-club shaped clavarioid fungi in Nam Nao National Park, 2 taxa and 8 genera were found, of which some were previously found in Thailand (Royal Institute of Thailand, 2007; Chandrasrikul et al., 2008). However, morphologically, there were only records of sizes of the fruiting bodies and shapes of spores; none on characteristics of basidia and clamp-connection, which indeed were necessary for species classification. Moreover, molecular biological characteristics were not recorded, hence additional data found from this study would be useful for the distinction of each species, especially in the distinction of *C. falcata* Persoon from *C. vermicularis* Swartz. Additionally, it could help in the distinction of *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen from *R. fusiformis* (Sowerby) R.H. Petersen and *R. helvola* (Persoon) R.H. Petersen, which were very

similar. It was also the first time for *C. falcata* Persoon and *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen to be found and recorded in Thailand.

The morphological and molecular characteristics among the *Clavaria* taxa were mostly identical in percentage, while clearly distinctive from *Ramariopsis* taxa. *C. falcata* Persoon, *C. rosea* Dalman, and *C. vermicularis* Swartz were extremely similar and classified in Subgenus *Clavaria* (Petersen, 1978), while *C. aurantiocinnabarina* Schweinitz and *C. miyabeana* S. Ito in S. Imai were in Subgenus *Clavulinopsis* (Petersen, 1978). Nevertheless, they were highly related to *Ramariopsis* taxa, which were genetically identical, including *R. fusiformis* (Sowerby) R.H. Petersen, *R. laeticolor* (Berkeley & M.A. Curtis) R.H. Petersen, and *R. helvola* (Persoon) R.H. Petersen, and were classified in Subgenus *Donkella* and Subgenus *Ramariopsis* (Petersen, 1978), respectively. In summary, the results from the study of various species of simple-club shaped clavarioid fungi in

Nam Nao National Park, in both morphological characteristics and phylogenetic tree, were satisfactory and in accordance with Petersen (1978).

Acknowledgement

The author would like to thank the staff of Nam Nao National Park, Petchaboon, for their kind suggestions, guidance, and support to the research team. We also would like to thank the Applied Taxonomy Research Center, Khon Kaen University for entirely funding this research study.

References

- Chandrasrikul, A., Suwanarit, P., Sangwanit, U., Morinaka, T., Nishizawa, Y. and Murakami, Y. 2008. **Diversity of Mushrooms and Macrofungi in Thailand**. Kasetsart University Press. Bangkok, Thailand.
- Dentinger, B.T.M. and McLaughlin, D.J. 2006. Reconstructing the Clavariaceae using nuclear large subunit rDNA sequences and a new genus segregated from *Clavaria*. **Mycologia** 98(5): 746-762.
- Department of National Parks, Wildlife and Plant Conservation. 2009. **Nam Nao National Park**. [Online]. [Cite 10 July 2009]. From: <http://www.dnp.go.th/parkreserve/asp/style1/default.asp?npid=36&lg=1>.
- Gardes, M. and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. **Molecular Ecology** 2: 113-118.
- Hahn, C. and Christan, J. 2002. *Ramaria chocoensis* sp. nov., a gomphoid member of *Ramaria* sect. *Dendrocladium* from Colombia, El Chocó, with special regards to rhizomorph anatomy. **Mycological Progress** 1(4): 383-398.
- Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf S., James, T., Kirk, P.M., Lucking, R., Lumbsch, H.T., Lutzoni, F., Matheny, B.P., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, C.M., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., S.M. Standridge, F., Oberwinkler, Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schuüßler, A., Sugiyama, J., Thorn, G.R., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., and Zhang, N. 2007. A higher-level phylogenetic classification of the fungi. **Mycological Research** 111: 509-547.
- Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J.A. 2008. **Dictionary of the Fungi, 10th Edition**. CAB International. Wallingford Oxon, UK.
- Lui, D., Coloe, S., Baird, R., and Pedersen. J. 2000. Rapid mini-preparation of fungal DNA for PCR. **Journal of Clinical Microbiology** 38(1): 471.
- Olariaga, I. 2009. **The order Cantharellales in the Iberian Peninsula and the Balearic**

Islands. Ph. D. Thesis in Universidad del País Vasco, Spain.

Petersen, R.H. 1978. Notes on clavarioid fungi. XV. Reorganization of *Clavaria*, *Clavulinopsis* and *Ramariopsis*. **Mycologia** 70(3): 660-671.

Royal Institute of Thailand. 2007. **Mushrooms in Thailand.** T Film Ltd. Company. Nonburi, Thailand.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics, pp. 315-322. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), **PCR Protocols: A Guide to Methods and Applications.** Academic Press: San Diego.