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Coprophilous Ascomycetes from Phu Luang Wildlife Sanctuary and Khao Yai National Park in Thailand

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ABSTRACT

The diversity and distribution were studied in samples of dung fungi collected from four species of wildlife and domestic animals (barking deer, buffalo, cow and elephant) in the Khao Yai National Park, Nakhon Ratchasima province and the Phu Luang Wildlife Sanctuary, Loei province. Different isolation methods, such as the moist chamber, Warcup's direct plating, soil plate, dilution plate and heat and alcohol treatments were used. Identification of the fungal isolates was based on the morphological characteristics of colony growth on agar media, fruiting bodies and spore ornamentation using stereo, light and scanning electron microscopes. Forty-nine isolates of coprophilous Ascomycetes, comprising 16 genera and 20 species were found, including *Ascobolus*, *Cercophora*, *Chaetomium*, *Coprotus*, *Emericella*, *Eurotium*, *Eupenicillium, Gelasinospora*, *Hamigera*, *Neosartorya*, *Podospora*, *Saccobolus*, *Sordaria*, *Sporormiella*, *Talaromyces* and *Xylaria*. The isolation of *Podospora setosa* provided a new record of coprophilous Ascomycetes for Thailand.

Key words: diversity, distribution, taxonomy, coprophilous fungi, Ascomycetes

INTRODUCTION

Coprophilous Ascomycetes are commonly found on the dung of many herbivorous animals (Bell, 1983; 2005). Studies on the secondary metabolites of some coprophilous Ascomycetes, with potent antifungal and antibacterial activities, have been reported, such as *Podospora communis* and *P. pleiospora* (Che *et al*., 2004; Kuwahara and Enomoto, 2005; Weber *et al*., 2005). The diversity, distribution and decomposition of coprophilous Ascomycetes have been recorded in many countries (Nyberg and Persson, 2002; Elshafie, 2005; Masunga *et al*., 2006; Richardson, 2001; 2008). In Thailand, Manoch *et al*. (1999) reported 19 coprophilous Ascomycetes from 12 dung samples from the Huay Kha Khang Wild Life Sanctuary, Uthai Thani province. Somrithipol (2004) summarized approximately 26 genera, including 36 species of coprophilous ascomycetes from the Khao Yai National Park, Nakhon Ratchasima province. Jeamjitt *et al*. (2007) recorded 12 genera and 15 species of coprophilous Ascomycetes, including *Ascobolus*, *Ascodesmis*, *Cercophora*, *Chaetomium*, *Emericella*, *Gelasinospora*, *Podosordaria*, *Podospora*, *Saccobolus*, *Sordaria*, *Sporormiella* and *Zopfiella* from the Huay Kha Khang Wild Life Sanctuary and the Khao Yai National Park in Thailand. It is very important to study coprophilous Ascomycetes in Thailand because these fungi are poorly known and only significant records have been detailed. Hence, more investigations on coprophilous Ascomycetes

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need to be carried out in this tropical region for the discovery of new taxa.

The objectives of this research were to study: 1) the morphology of Ascomycetes found on various dung samples; and 2) the diversity and distribution of Ascomycetes associated with different dung types from the Khao Yai National Park, Nakhon Ratchasima province and the Phu Luang Wildlife Sanctuary, Loei province.

MATERIALS AND METHODS

Fifty-six dung samples from four animals (barking deer, buffalo, cow and elephant) were collected from the Khao Yai National Park, Nakhon Ratchasima province and the Phu Luang Wildlife Sanctuary, Loei province in Thailand. All samples were placed in a moist chamber near a window, where they were incubated for 2-7 d at 28°C. A direct isolation method was carried out from the dung surface under a stereomicroscope (SZ-PT Olympus). The ascomata were transferred directly onto water agar (WA), squashed to release the ascospores, which were incubated for 48 h. If the ascospores did not germinate, a fine needle was used to transfer the fruiting body with the remaining ascospores from WA, which were then placed in a test tube containing 60-80°C distilled water, 65% ethyl alcohol and 3% KOH for 30, 15 and 3-5 min respectively. The treated ascospores were spread on WA in a Petri dish and incubated for 12-24 h. A hyphal tip from a single spore was transferred to a slant of potato dextrose agar (PDA) and kept as pure culture in the Kasetsart University Fungal Collection (KUFC). Dry specimens of dung samples were kept in the herbarium at Kasetsart University (KUH). The soil plate, dilution plate, heat, alcohol treatments and Gochenaur's glucose ammonium nitrate agar (Gochenaur, 1964) (NH_4NO_3 1 g, K_2HPO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, rose bengal 0.03 g, yeast extract 1 g, glucose 5 g, agar 15 g, streptomycin solution 4 ml, distilled water 1 l) as well as 2% malt extract agar were used to isolate the dung fungi. Macroscopic features were studied, including colony growth pattern, color and texture on different agar media. Microscopic characters were observed on a slide preparation using sterile distilled water, with lactophenol as the mounting medium and examined under a light microscope (Olympus BH-2 with Normaski Interference Contrast). Camera lucida drawings were employed. Photomicrographs of fungal structures were taken under stereo, light and scanning electron microscopes.

For the procedure for SEM photomicrographs (Sharples and Moss, 2000), ripened ascomata and ascospores of Eurotiales and Sphaeriales from dry culture agar media were transferred with a fine needle and placed onto double-stick Scotch tape on aluminum stubs. The specimens were coated with gold for 5-7 min and examined under a JEOL JSM 6400 scanning electron microscope (Manoch *et al.,* 2007).

RESULTS AND DISCUSSION

Using various methods, 49 isolates of coprophilous Ascomycetes were found from the 56 dung samples of four animal species from the Khao Yai National Park, Nakhon Ratchasima province and the Phu Luang Wildlife Sanctuary, Loei province. Sixteen genera and 20 species of coprophilous Ascomycetes were recorded, including 8 Pyrenomycetes (40%), 8 Plectomycetes (40%) and 4 Discomycetes (20 %) (Table 1). The moist chamber method yielded the highest number of coprophilous Ascomycetes (6 spp.), followed by the alcohol treatment (5 spp.), heat treatment (5 spp.), soil plate method (3 spp.) and dilution plate method (1 spp.). The results indicated that deer dung yielded the highest number of fungal species (14 spp.), followed by elephant (11 spp.), cow (5 spp.) and buffalo (3 spp.). All taxa were cultivated on PDA. The pure cultures were provided for maintenance in the culture collection at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart

University, Bangkok (KPFC) for further study.

Thirty-one isolates of coprophilous Ascomycetes comprising 12 genera and 14 species were found, comprised of: *Ascobolus albidus*, *Chaetomium globosum*, *Emericella nidulans*, *Eupenicillium parvum*, *Eupenicillium* sp., *Eurotium amstelodami*, *Hamigera avellanea*, *Neosartorya fischeri*, *Saccobolus glaber* (Figures 1E-F), *Sordaria fimicola* (Figure 1H), *Sporormiella minima*, *Talaromyces bacillisporus*, *T. flavus* and *Xylaria* from barking deer, elephant, cow and buffalo dung from the Khao Yai National Park. Dung samples from barking deer and elephant from the Phu Luang Wildlife Sanctuary showed fifteen isolates comprising 12 genera and 15 species, namely: *Cercophora* sp. (Figure 1G), *Coprotus* sp.1 (Figure 1A-B), *Coprotus* sp.2 (Figure 1C-D), *Chaetomium globosum* (Figure 2C-D), *Eupenicillium parvum*, *Eupenicillium* sp. *Eurotium amstelodami*, *Gelasinospora indica* (Figure 2G-H), *Gelasinospora* sp. (Figure 2E-F), *Hamigera avellanea*, *Neosartorya fischeri*, *Podospora setosa* (Figure 2A-B), *Sordaria fimicola*, *Sporormiella minima* and *Talaromyces flavus* (Table 1).

Table 1 Occurrence (%) of coprophilous ascomycetes species on different dung types (the number of samples is indicated in brackets [#]).

Fungal species	Number of fungal isolates						Total isolate
	Khao Yai National park				Phu Luang Wildlife sanctuary		
	Barking deer (12)[#]	Elephant (10)[#]	Cow (9)[#]	Buffalo (8)[#]	Barking deer (10)[#]	Elephant (7)[#]	
*Ascobolus albidus**	-	1	-	-	-	-	**1**
Cercophora sp.***	-	-	-	-	-	1	**1**
Coprotus sp.1*	-	-	-	-	1	-	**1**
Coprotus sp.2*	-	-	-	-	-	1	**1**
*Chaetomium globosum****	2	-	1	-	1	1	**5**
*Emericella nidulans***	1	-	-	-	-	-	**1**
*Eupenicillium parvum***	1	-	-	-	1	-	**2**
Eupenicillium sp. **	1	-	-	1	2	-	**4**
*Eurotium amstelodami***	1	-	-	-	-	1	**2**
*Gelasinospora indica****	-	-	-	-	1	-	**1**
Gelasinospora sp.***	-	-	-	-	1	-	**1**
*Hamigera avellanea***	1	2	-	-	1	-	**4**
*Neosartorya fischeri***	-	-	1	1	1	-	**3**
*Podospora setosa****	-	-	-	-	-	1	**1**
*Saccobolus glaber**	-	1	-	-	-	-	**1**
*Sordaria fimicola****	1		2	-	1	1	**5**
*Sporormiella minima****	3	1	2	-	1	-	**7**
*Talaromyces bacillisporus***	1	2	-	-	-	-	**3**
*T. flavus***	2	-	-	1	1	-	**4**
Xylaria sp. ***	-	-	1	-	-	-	**1**
Total isolates	**14**	**7**	**7**	**3**	**12**	**6**	**49**
% Occurrence	**28.57**	**14.29**	**14.29**	**6.12**	**24.49**	**12.24**	**100**

* Class Discomycetes, ** Class Plectomycetes, *** Class Pyrenomycetes.

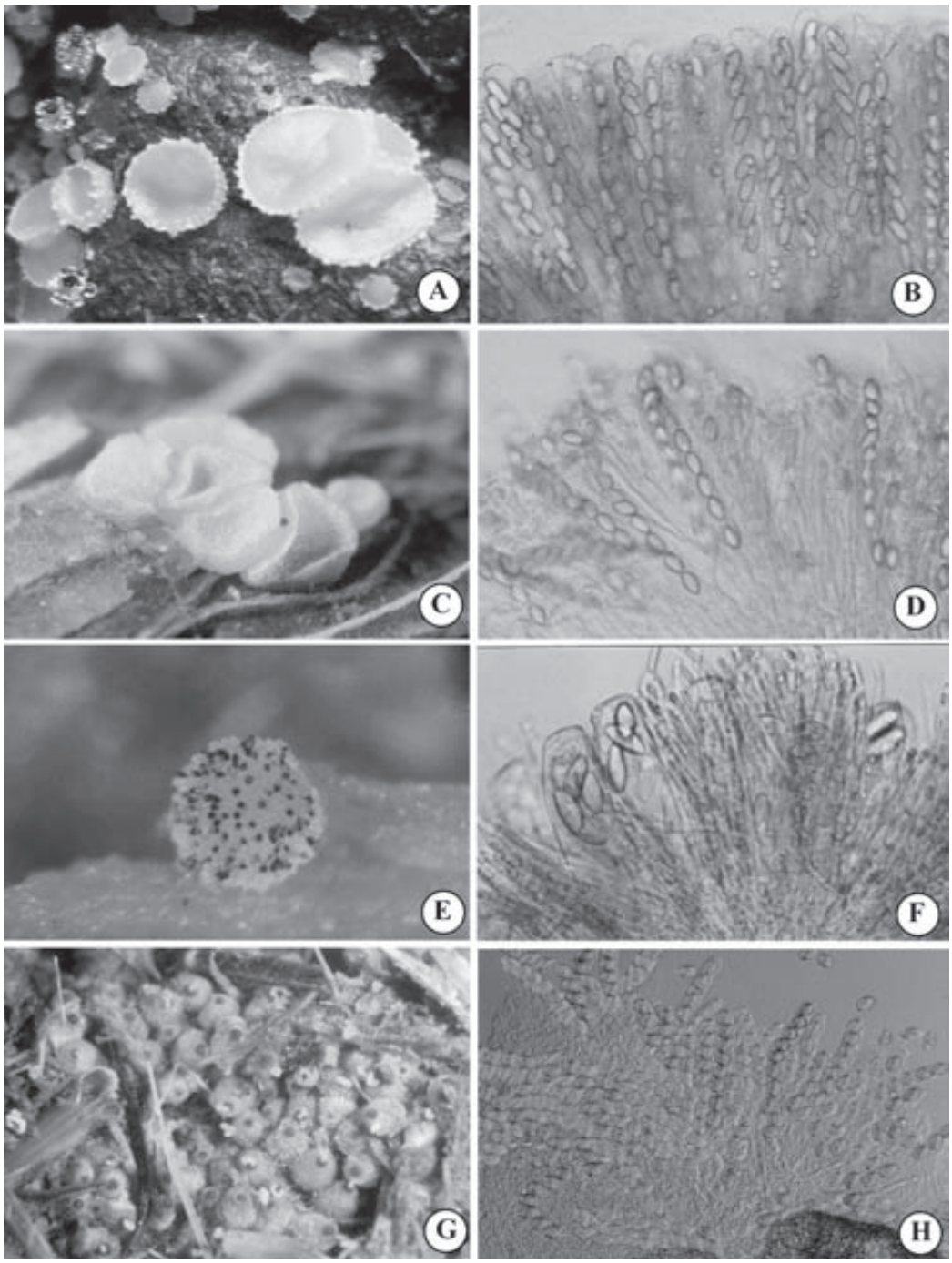

Figure 1 Light and stereo photomicrographs of ascomata, asci and ascospores:
A-B. *Coprotus* sp.1; C-D. *Coprotus* sp.2; E-F. *Saccobolus glaber*; G. *Cercophora* sp.; and H. *Sordaria fimicola*.

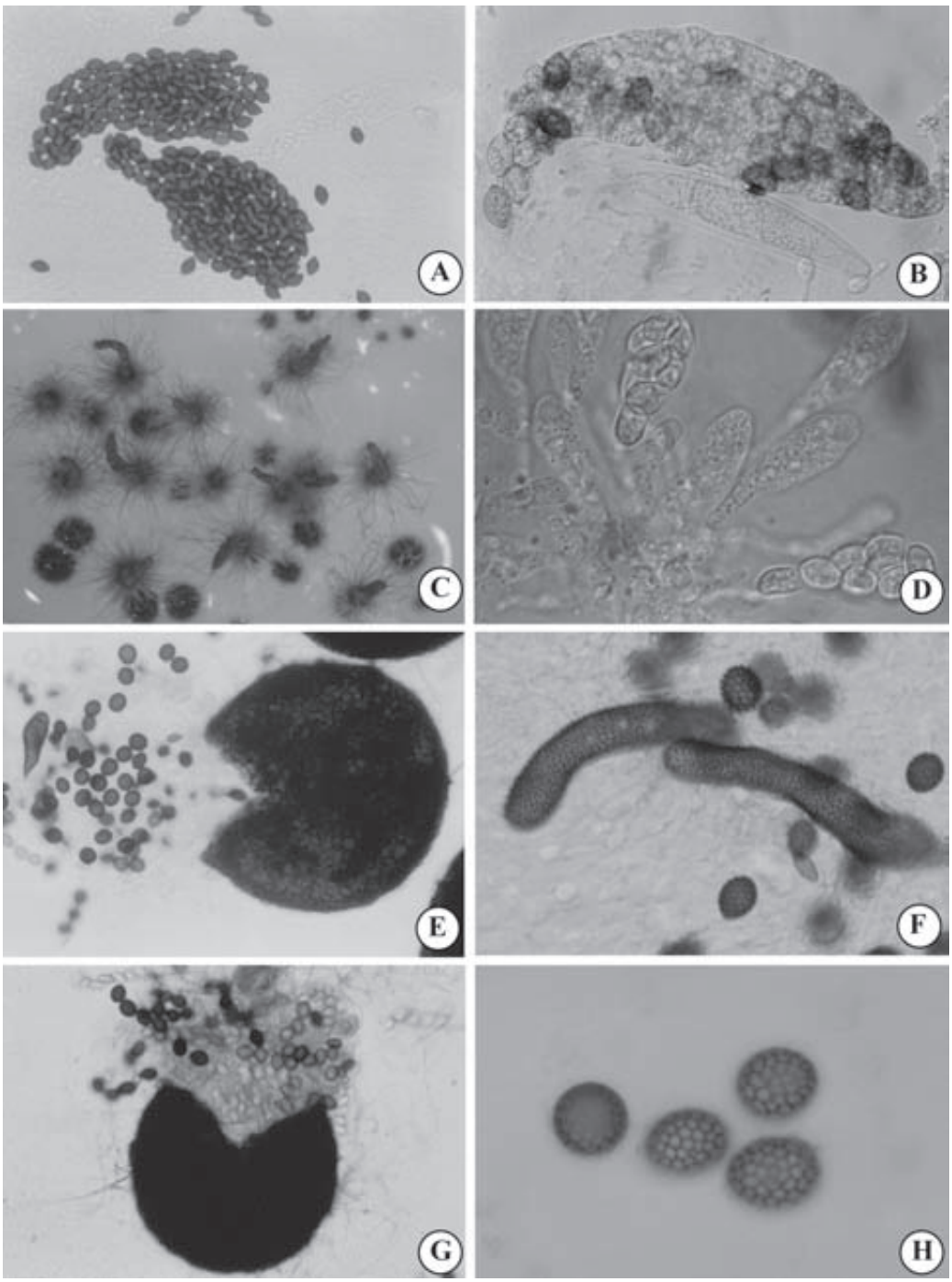

Figure 2 Light and stereo photomicrographs of ascomata, asci and ascospores: A-B. *Podospora setosa*; C-D. *Chaetomium globosum*; E-F. *Gelasinospora* sp.; and G-H. *Gelasinospora indica*.

Observation of the dung samples from the different locations indicated that some species occurred on only one type of dung sample, including *Ascobolus albidus*, *Cercophora* sp., *Coprotus* sp.1, *Coprotus* sp.2, *Emericella nidulans*, *Gelasinospora indica*, *Gelasinospora* sp., *Podospora setosa*, *Saccobolus glaber* and *Xylaria* sp. (Table 1). Moreover, the data showed that fungal species, such as *Chaetomium globosum*, *Eupenicillium parvum*, *Eupenicillium* sp., *Eurotium amstelodami*, *Hamigera avellanea*, *Neosartorya fischeri*, *Sordaria fimicola*, *Sporormiella minima*, *Talaromyces bacillisporus* and *T. flavus* were common and occurred in both locations. *Sporormiella minima* was the most abundant species, with seven isolates found from dung samples, followed by *Sordaria fimicola* (five isolates) and *Chaetomium globosum* (five isolates).

Podospora setosa was found on elephant dung from the Phu Luang Wildlife Sanctuary collected in the cold season. From the collections in the present study, *P. setosa* represented a new record for Thailand. Goff and Begueret (2004) studied the ribosomal protein of *Podospora setosa* that were compared by electrophoretical and immunological methods.

Van Geel *et al*. (2007) reported that the ascospores of the coprophilous *Sordaria*, *Sporormiella* and *Podospora* were very useful in the multidisciplinary paleoecological examination of a fossil vegetation buried under Dawson tephra (25,300 ^{14}C years BP) in northwestern Canada.

CONCLUSIONS

Sixteen genera and 20 species of coprophilous Ascomycetes were recorded from the Khao Yai National Park, Nakhon Ratchasima province and the Phu Luang Wildlife Sanctuary, Loei province. All taxa could be cultivated in pure culture. Among them, *Ascobolus albidus*, *Cercophora* sp., *Coprotus* sp.1, *Coprotus* sp.2, *Emericella nidulans*, *Gelasinospora indica*, *Gelasinospora* sp., *Podospora setosa*, *Saccobolus glaber* and *Xylaria* sp. were found on only one type of dung. The most common species were *Sporormiella minima*, *Sordaria fimicola* and *Chaetomium globosum*. The diversity of coprophilous fungi depended on the type and number of dung samples, habitats, collecting sites and isolation techniques. *Podospora setosa* represented a new record for Thailand of coprophilous Ascomycetes.

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