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# Cercospora and allied genera from Laos – 1: Notes on Zasmidium (Stenella s. lat.)

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**Abstract** – Leaf spots or lesions on living plants were collected from the Vientiane area and Bolikhamxay Province of Laos. Any cercosporoid taxa present were recorded, their macroscopic characters observed, cultures were isolated from single spores, and microscopic characters were observed under the microscope. Five *Zasmidium* species have presently been identified and five of these are new species. In this paper we introduce the taxonomic novelties *Zasmidium aporosae*, *Z. jasminicola*, *Z. pavettae* and *Z. suregadae*. Furthermore, *Stenella manihotis*, *S. meynae-laxiflorae* and *S. pseudoramularia* are assigned to *Zasmidium*.

anamorphic fungi / cercosporoid hyphomycetes / South East Asia / taxonomy / new species

#### INTRODUCTION

Studies on the biodiversity of the previously poorly studied fungi from various habitats in northern Thailand and Laos have been carried out (e.g. insects: Aung et al., 2008; leaf litter: Duong et al., 2008; wood: Kodsueb et al., 2008a, b; monocotyledons; Pinruan et al., 2007; Thongkantha et al., 2008). There is considerable interest in the cercosporoid fungi and recently there have been several publications from Australasia and Asia (Braun & Crous, 2007; Kirschner & Chen, 2007; Nakashima et al., 2007). Cercosporoids have been shown to be a polyphyletic group (Crous et al., 2007a) comprising numerous genera with partly unsettled phylogeny and taxonomy (Crous et al. 2007b). In this paper we deal with Stenella-like hyphomycetes from the Lao PDR (Lao People Democratic Republic). The genus Stenella was described by Sydow (1930) and recognized by Ellis (1971, 1976), who reduced Biharia Thirum. & Mishra (Thirumalachar &

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Mishra, 1953) to synonymy with this genus, Deighton (1979) followed this concept of Stenella and differentiated it from Mycovellosiella based on the formation of verruculose superficial hyphae and usually rough-walled, catenate conidia. Up to the present time about 150 species have been assigned to Stenella. The main characters of this genus are: formation of verruculose superficial secondary mycelium; conidia formed singly or catenate, amero- to scolecosporous, mostly verruculose. However, in phylogenetic examinations on Ramichloridium and allied genera Arzanlou et al. (2007) demonstrated that Zasmidium cellare (Pers.: Fr.) Fr., the type species of the older genus Zasmidium, clustered within the Mycosphaerellaceae together with former Stenella species. With regard to morphology and conidiogenesis, Zasmidium agrees well with Stenella species by foming verrucose, pigmented aerial hyphae, giving rise to solitary conidiophores and thickened, darkened conidiogenous loci. Most former Stenella species belong to the Mycosphaerellaceae as well, but Stenella arguata Syd., the type species, clusters within the *Teratosphaeriaceae*, i.e. this species is phylogenetically distinct. Furthermore, Stenella anamorphs of the Mycosphaerellaceae have conidiogenous loci and hila that are planate as in other cercosporoid genera, whereas the loci and hila in S. arguata, Teratosphaeriaceae, are pileate (see David 1993). There are several possible solutions of this generic issue, viz. the recognition of a single polyphyletic genus for Stenella-like hyphomycetes (either, following the rules of priority, under the oldest name Zasmidium or under Stenella, provided that the latte name would be conserved over Zasmidium) or two monophyletic genera, i.e. Zasmidium (Mycosphaerellaceae) and Stenella s. str. (Teratosphaeriaceae). In any case, the oldest name Zasmidium is available for the whole complex. Furthermore, there is a strong tendency to end up with monophyletic genera when possible. Therefore, Stenella s. str. should be confined to anamorphs of the Teratosphaeriaceae. All Stenella species have to be proven and reassessed, either by molecular methods or by detailed examinations of the scar structure. In this paper we introduce four new species of Zasmidium from Laos.

#### MATERIALS AND METHODS

#### Sample collection

Leaves of plants with leaf-spots or other lesions were collected during numerous field trips. Photos of the symptoms, including the fungal colonies or fruit bodies were taken. The specimens were collected in the Vientiane Capital and Bolikhamxay Province areas of Central Laos.

#### General information

- **Vientiane** is the capital city of Laos, situated in the Mekong Valley and it is located at 17°58' North, 102°36' East (17.9667, 102.6). Vientiane consists of an area 3,920 km<sup>2</sup>.
- **Bolikhamxay** (or Bolikhamsay) Province is located in central Laos in the narrow "neck", with moderately high mountains sloping Southwest into the Mekong River valley. Bolikhamxay can be reached by bus, 150 km or about 3 hours leaving from the Morning Market bus station. Bolikhamxay consists of an area 14,863 km<sup>2</sup>.

## **Examination of fungal structures**

Macroscopic characters were observed using a stereoscope to check (1) lesions/leaf spots (shape, size, colour, margin), and (2) colonies/caespituli (with details, e.g., amphigenous/epiphyllous, punctiform/pustulate/inconspicuous, effuse, loose, dense, brown/blackish, etc.).

#### Measurements

Where sufficient material was available, 30 measurements of mycelia (internal, external), hyphae (branched or not, width, septation, colour, wall thin/thick, smooth/verruculose), stromata (location, e.g., substomatal, intraepidermal; shape, size, colour; cells, angular or rounded in outline, size, wall thick/thin), conidiophores (formation, solitary/fasciculate/sporodochial, arising from internal/external hyphae/stromata, erumpent/through stomata; shape; size; septation; colour; wall, thin/thick, smooth/verrucuose), conidiogenous cells (integrated, terminal/intercalary; length, shape, e.g., cylindrical/geniculate/sinuous), conidiogenous loci [scars] (shape, size, thickened, darkened/pigmented or unthickend or inconspicuous, etc.), and conidia (formation, solitary/catenate; shape; size; septation; colour; wall, thin/thick, smooth/verruculose, apex; base; hila, size, thickened/unthickened, pigmented or not) have been carried out and the **standard variation** has been estimated by using the formula:

 $(\bar{x} = \times \mu m, n = y),$ Notes: x = is an average of the size of each components y = is a number of components

#### **Herbarium specimens**

Dried specimens were prepared and stored at the MFLU and the Biology Department, Faculty of Sciences, National University of Laos. Duplicates of collections and syntypes are preserved at the herbarium of the Institute of Biology, Geobotany and Botanical Garden, Halle (Saale), Germany (HAL) and MFLU.

# RESULTS

Five new Zasmidium species were identified:

Vientiane Capital Bolikhamxav Province Rank Fungi species MDDDUTMDDDUT1 Zasmidium aporosae sp. nov. 2 Zasmidium jasminicola sp. nov.  $\mathbf{x}$ Zasmidium pavettae sp. nov. Zasmidium meynae-laxiflorae comb. nov. X Zasmidium suregadae sp. nov.

Table 1. Zasmidium species recorded from Laos.

Note: MD = Mixed deciduous forest, DD = Dry dipterocarp forest, UT = Unstock forest.

#### **Taxonomy**

1. Zasmidium aporosae P. Phengsintham, K.D. Hyde & U. Braun, sp. nov.

(Figs 1-2)

MycoBank, MB 513397

Stenellae bischofiae-javanicae similis, sed conidiis in vivo angustioribus, 2-3 um latis, levibus vel subtiliter verruculosis.

Description: Leaf spots variable, typically deep brown to black, more or less irregularly orbicular, 1-15 mm in diam. Caespituli/colonies amphigenous, but chiefly hypophyllous. Mycelium: internal hyphae not observed; external hyphae often constricted at the septa, pale olivaceous-brown, almost smooth to verruculose, 1-7  $\mu$ m wide ( $\bar{x} = 2.57 \mu$ m, n = 30), distances between septa 5-29  $\mu$ m ( $\bar{x} = 11.67 \mu$ m, n = 30), thick-walled approximately 0.30-1  $\mu$ m ( $\bar{x} = 0.61 \mu$ m, n = 30). Stromata absent. Conidiophores borne on external mycelial hyphae, unbranched, septate, mid pale golden brown, smooth, 2-5-septate, thin-walled, 0.50-1  $\mu$ m ( $\bar{x} = 0.80 \mu$ m, n = 30), (6-)29-76(-83)  $\times$  3-4 µm ( $\bar{x}$  = 43.1  $\times$  3.2 µm, n = 30); conidiogenous cells intergrated, terminal or rarely intercalary, 7-15  $\times$  1-3  $\mu$ m ( $\bar{x} = 10.3 \times 2.8 \mu$ m, n = 30), conidiogenous loci forming minute, dark or refractive scars on lateral and terminal denticles, 1-2  $\mu$ m wide ( $\bar{x} = 1.72 \mu$ m, n = 30), planate, giving rise to branched conidial chains, occasionally terminally swollen. Conidia solitary or catenulate, pale olivaceous, small conidia ellipsoid-ovoid to subcylindrical, but most conidia longer and slightly obclavate to obclavate-subcylindrical, straight or slightly curved or sinuous, smooth or finely verruculose, thin-walled, rounded or subtruncate at the ends with thickened, planate hila, short obconically truncate at the base, variable in length and shape, occasionally with lateral branchlets (germ tubes), 0-3-septate,  $(5-)6-38(-39) \times 2-3 \mu m (\bar{x} = 17.47 \times 2.3 \mu m, n = 30).$ 

Known hosts: Aporosa villosa (Lindl.) H. Baill.

Known distribution: Laos.

Material examined: Laos, Vientiane Capital, Xaythany District, Nonh Saengchanh Village, on leaf of *Aporosa villosa* (Euphorbiaceae), 19 April 2006, P. Phengsintham, (NOUL P8, **holotype**); *ibid.*, 22 January 2007, (NOUL P201); *ibid.*, 25 May 2007 (NOUL P171); *ibid.*, Bolikhamxay Province, 5 May 2007 (NOUL P274).

Cultural characteristics: **Colonies** on PDA after three weeks at 25°C with spreading mycelium, surface ridged, black and wavy in the centre and grey margin, reaching 10-27 mm diam. **Hyphae** often constricted at the septa, distances between septa 4-20  $\mu$ m ( $\bar{x}=10.97~\mu$ m, n = 30), thin-walled approximately 0.5-1  $\mu$ m ( $\bar{x}=0.68~\mu$ m, n = 30), hyaline, smooth or verruculose, forming lateral and terminal minute, dark or refractive denticle-like scars, 1-2  $\mu$ m diam., giving rise to branched conidial chains, width of mycelial hyphae gradually decreasing from primary to secondary and any later colonies. **Conidia** solitary or catenulate, greenish, verruculose, more variable in length and shape than those from leaves, 17-66 × 3-4  $\mu$ m ( $\bar{x}=41.94\times3.3~\mu$ m, n = 18).

Remarks: The young conidia of Z. aporosae can be minutely verruculose, more evident than in adult conidia. Several species of the genus Stenella are known from hosts belonging to the Euphorbiaceae, but all of them are distinct from the new species Z. aporosae. Stenella bischofiae-javanicae R.K. Chauhary, Tripathi, P.N. Singh & S. Chaudhary (Chaudhary et al. 2001), described form India on Bischofia javanica, differs in having usually solitary conidia, up to 5  $\mu$ m wide, with 2-6 septa and a surface with loosely scattered coarse warts. S. brideliicola K. Srivast., A.K. Srivast. & Kamal on Bridelia stipularis in India (Srivastava et al. 1994) has much longer conidiophores, up to 310  $\times$  5-7  $\mu$ m, and broader conidia 4-7  $\mu$ m, formed singly. Zasmidium manihotis (U. Braun &

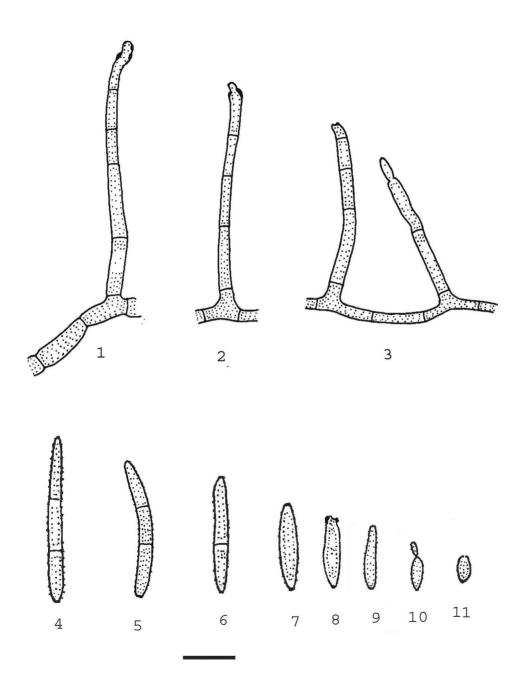


Fig. 1. 1-11. *Zasmidium aporosae* sp. nov. on *Aporosa villosa*: 1-3. External hyphae with attached conidiophores. 4-11. Conidia. Bar:  $1-11=10~\mu m$ .

F.O. Freire) U. Braun **comb. nov.** (Bas.: *Stenella manihotis* U. Braun & F.O. Freire, Cryptog. Mycol. 25: 240, 2004), MycoBank, MB 513397, known on *Manihot* sp. in Brazil, is characterized by having solitary as well as fasciculate conidiophores and much longer conidia,  $25-160 \times 3-4 \mu m$ , 2-16-septate, usually formed singly. *S. gorakhpurensis* (Kamal & P. Kumar) de Hoog (de Hoog *et al.* 1983) on *Glochidion multiloculare* in India is characterized by having colorless hyphae and smooth, very small, 1-3-septate conidia.

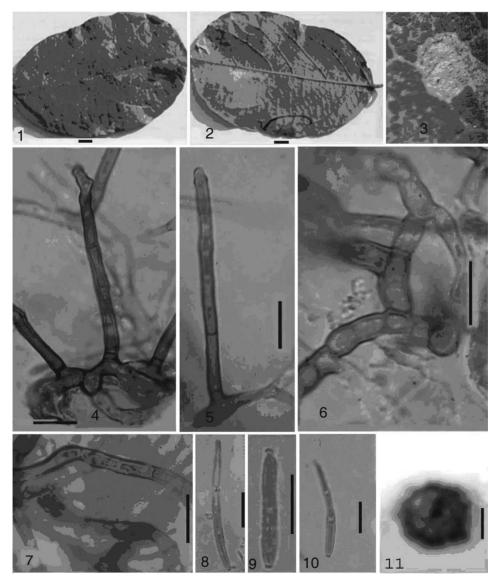


Fig. 2. 1-11. *Zasmidium aporosae* sp. nov. on *Aporosa villosa* from leaf spots/Lesions: 1-2. Lesions on host leaves (1. Upper surface, 2. Lower surface). 3. Caespituli. 4-5. Conidiophores. 6-7. External hyphae. 8-10. Conidia. 11. Culture. Bar: 1-2 = 10 mm. 4-10 = 10 µm. 11 = 10 mm.

# **2.** Zasmidium jasminicola P. Phengsintham, K.D. Hyde & U. Braun, sp. nov. (Figs 3-4)

MycoBank, MB 513398

Differt a Zasmidio pseudoramulario conidiophoris in vivo semper ex hyphis superficialibus oriundis et conidiis latioribus,  $(4-)5-42(-47) \times (2-)3-5(-6) \mu m$ , 0-6-septatis.

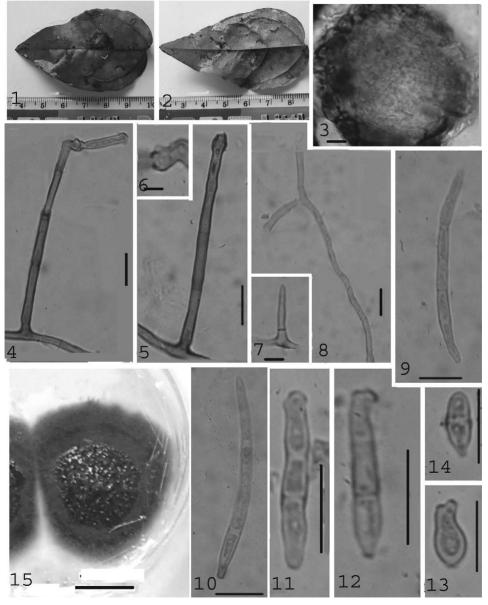


Fig. 3. 1-11. Zasmidium jasminicola sp. nov. on Jasminum undulatum: 1-4. Conidiophores. 5-11. Conidia. Bar: 1-11 =  $10~\mu m$ .

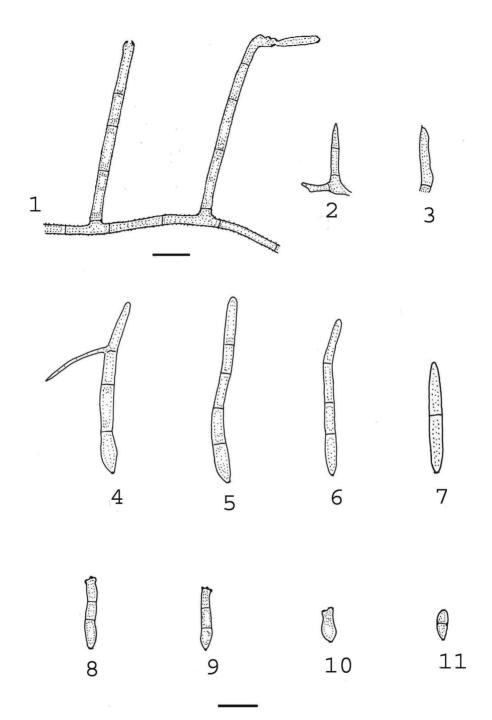


Fig. 4. 1-15. Zasmidium jasminicola sp. nov. on Jasminum undulatum from leaf spot: 1-2. Lesions on host leaves (1. Upper surface and 2. Lower surface). 3-4. Stromata. 5-8. Conidiophores. 9-14. Conidia. 15. Culture. Bar: 1-2=10 mm, 3-14=10  $\mu$ m, 15=10 mm.

Description: Leaf spots variable, typically deep brown, more or less irregularly orbicular, up to 10 mm. diam. Caespituli/colonies amphigenous. Mycelium: internal hyphae not observed; external hyphae verruculose, often constricted at the septa, pale olivaceous-brown, 1-2 µm wide, distances between septa 7-15 µm, thin-walled, approximately 0.50-0.80 µm. Stromata well-developed, brown, 6-40 µm diam. Conidiophores borne on external mycelial hyphae, unbranched, septate, mid pale golden brown, smooth, thinwalled, approximately 0.50-0.80  $\mu$ m, up to 3-septate, 10-46  $\times$  2-4  $\mu$ m ( $\bar{x} = 21.54$  $\times$  2.31  $\mu m$ , n = 13); conidiogenous cells intergrated, terminal or rarely intercalary, 7-15  $\times$  2-3  $\mu m$ , conidiogenous loci forming minute, dark or refractive scars on lateral and terminal denticles, 2-3 µm diam., planate, giving rise to branched conidial chains, occasionally terminally swollen. Conidia solitary or catenulate, pale olivaceous, ellipsoid-ovoid or subcylindrical but mostly slightly obclavate, straight or slightly curved or sinuous, smooth or finely verruculose, thin-walled, rounded or subtruncate at the ends, with thickened hila, 1-2 µm wide, planate, short obconically truncate at the base, about 0.5-1.5 µm wide, variable in length and shape, some conidia occasionally with a lateral branchlet (germ tube), 0-6-septate,  $(4-)5-42(-47) \times (2-)3-5(-6) \mu m$  $(\bar{x} = 13.98 \times 2.09 \ \mu \text{m}, \ n = 30).$ 

Known hosts: Jasminum undulatum Ker-Gawl. (this paper).

Known distribution: Laos (this paper).

Material examined: Laos, Vientiane Capital, Xaythany District, Xay Village, on leaf of *Jasminum undulatum* (Oleaceae), P. Phengsintham, 19 April 2006, (NOUL P 10, **holotype**).

Cultural characteristics: **Colonies** on PDA after three weeks at 25 °C with spreading mycelium, surface ridged, black and wavy in the centre and grey margin, reaching 10-27 mm diam. **Hyphae** often constricted at the septa, distances between septa (2-)6-27  $\mu$ m ( $\bar{x}=14.93~\mu$ m, n = 30), thin-walled, approximately 0.5  $\mu$ m, hyaline, smooth or verruculose, forming minute, dark or refractive scars on lateral and terminal denticles, 1.5-2  $\mu$ m diam., giving rise to branched conidial chains, width of mycelial hyphae gradually decreasing from primary to secondary and any later colonies, 1-5  $\mu$ m wide ( $\bar{x}=2.8~\mu$ m, n = 30). **Conidia** solitary or catenulate, greenish, verruculose, more variable in length and shape than those from leaves, 5-29 × 1.5-2.5  $\mu$ m ( $\bar{x}=10.5 \times 2.08~\mu$ m, n = 30).

Remarks: Zasmidium pseudoramularia (U. Braun) U. Braun **comb. nov.** (Bas.: Stenella pseudoramularia U. Braun, Nova Hedwigia 73: 431, 2001), MycoBank, MB 513398, the second species of Stenella on a host belonging to the Oleaceae, described from Indonesia on Nyctanthes arbor-tristis (Braun 2001), is distinguished from S. jasminicola by forming solitary or loosely aggregated (subfasciculate) conidiophores arising from immersed hyphae and much narrower (6-35  $\times$  1-3  $\mu$ m in vivo), 0-1(-2)-septate conidia The young conidia of S. jasminicola can be minutely verruculose, more evident than in adult conidia.

### 3. Zasmidium pavettae P. Phengsintham, K.D. Hyde & U. Braun, sp. nov.

(Figs 5-6)

MycoBank, MB 513399

Stenellae canthii similis, sed stromatibus formatibus, conidiophoris fasciculatis et solitariis, conidiis solitariis et catenatis, leniter brevioribus et angustioribus,  $(5-)6-59(-65) \times 2-4 \,\mu\text{m}$ . Differt a S. meynae-laxiflorae conidiophoris in fasciculis parvioribus, brevioribus, ad 34  $\mu$ m longis, conidiis brevioribus,  $(5-)6-59(-65) \,\mu\text{m}$  longis.

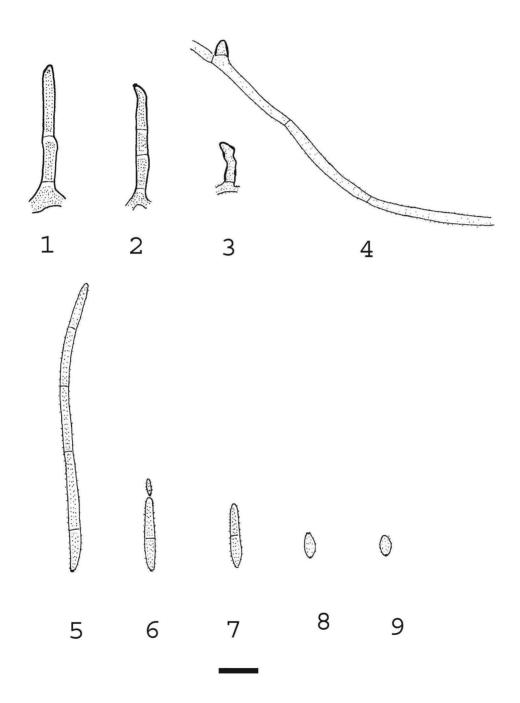


Fig. 5. 1-9. Zasmidium pavettae sp. nov. on Pavetta indica: 1-3. Conidiophores. 4. External mycelium with attached conidiophores. 6-9. Conidia. Bar:  $1-9=10~\mu m$ .

Description: **Leaf spots/lesions** circular to irregular, 3-8 mm in diam., grey-brown in the centre, and with greyish margin. **Caespituli/colonies** amphigenous, inconspicuous. **Mycelium** internal and external: **internal hyphae** inconspicuous; **external hyphae** branched, 2-3  $\mu$ m wide ( $\bar{x} = 2.8 \mu$ m, n = 17),

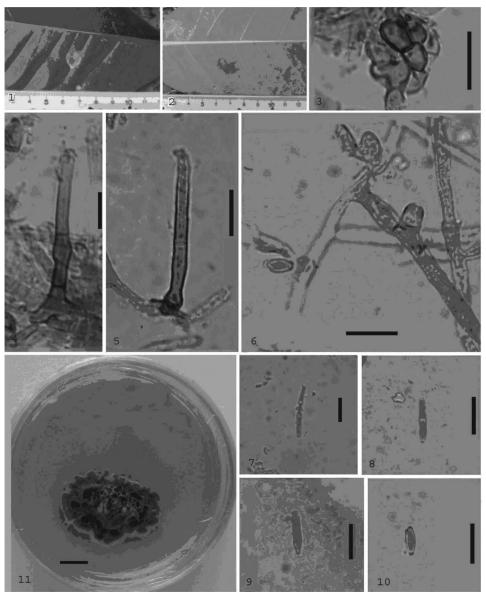


Fig. 6. 1-11. Zasmidium pavettae sp. nov. on Pavetta indica from leaf spot: 1-2 Lesions on host leaves (1. Upper surface and 2. Lower surface). 3. Stroma. 4-5. Conidiophores. 6. External mycelium with attached young conidiophores. 7-10. Conidia. 11. Culture. Bar: 1-2 = 10 mm,  $3-10 = 10 \mu m$ , 11 = 10 mm.

septate, constricted at the septa, distances between septa 9-24  $\mu$ m ( $\bar{x} = 15.5 \mu$ m, n = 17), pale olivaceous-brown, wall approximately 0.25-0.50 μm wide  $(\bar{x} = 0.44 \, \mu \text{m}, \, n = 17)$ , verruculose. **Stromata** not well-developed, subglobose, approximately 23 µm in diam., brown, stromatal cells oval, ellipsoidal to angular in outline, 3-5  $\mu$ m wide ( $\bar{x} = 3.75 \mu$ m, n = 8), brown to dark brown, wall approximately 0.50-0.80  $\mu m$  wide ( $\bar{x} = 0.54 \mu m$ , n = 8), smooth. Conidiophores fasciculate or solitary, arising from stromata and borne on external mycelial hyphae, unbranched, cylindrical,  $12-34 \times 3-4 \mu m$  ( $\bar{x} = 16.8 \times 3.5 \mu m$ , n = 5), 1-septate, brown, wall approximately 0.80 µm, smooth; conidiogenous cells integrated, terminal or intercalary,  $19-20 \times 3-4$  um, cylindrical, paler at the apex: conidiogenous loci forming minute, dark or refractive scars on lateral and terminal denticle-like protuberances, giving rise to branched conidial chains, 1-2 µm wide, planate, wall approximately 0.8-1 µm wide, thickened, darkened. Conidia solitary or catenulate, sometimes subcylindrical but mostly slightly obclavate, straight or slightly curved or sinuous,  $(5-)6-59(-65) \times 2-4 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu m \, (\bar{x} = 20 \times 10^{-5}) = 20 \, \mu \, (\bar{x} = 20 \times 10^{-5})$ 2.75  $\mu$ m, n = 20), 0-5-septate, pale olivaceous, wall approximately 0.25-0.50  $\mu$ m wide ( $\bar{x} = 0.36 \, \mu \text{m}$ , n = 20), smooth or finely verruculose, apex rounded or subtruncate, with a conspicuous hilum, base short obconically truncate, hila approximately 1 µm wide, wall approximately 0.6-0.8 µm thick.

Known host: Pavetta indica L.

Known distribution: Laos (this paper).

Material examined: Laos, Vientiane Capital, Xaythany District, Houay Den Meuang and Dong Mak Khai villages, on leaf of genus *Pavetta indica* (Rubiaceae), 26 April 2006, P. Phengsintham (NOUL P 24, **holotype**); ibid. 10 March 2007 (NUOL P 261, **paratype**).

Cultural characteristics: **Mycelial colonies** on PDA after three weeks at 25°C spreading surface ridged, black and brown in the centre, grey margin, reaching 10 mm in diam., hyphae 1-4  $\mu$ m wide ( $\bar{x}=2.6~\mu$ m, n = 30), septate, constricted at the septa, distances between septa 6-16  $\mu$ m ( $\bar{x}=11~\mu$ m, n = 30), brownish to subhyaline, wall approximately 0.25-0.50  $\mu$ m ( $\bar{x}=0.44~\mu$ m, n = 30), smooth or verruculose. **Conidiophores** and **conidia** not formed in the culture.

Remarks: Several Stenella species, all described from India, have been recorded on hosts belonging to the Rubiaceae. Stenella canthii J.M. Yen, A.K. Kar & B.K. Das (1982a), described from West Bengal on Canthium dedymum, is morphologically close to the fungus on Pavetta indica, but this species is distinguished by lacking stromata, consistently solitary conidiophores and somewhat longer, wider conidia, 18-112 × 3-4.5 µm. S. meynae-laxiflorae K. Srivast., A.K. Srivast. & Kamal (Srivastava et al. 1995) is another similar species, but it is distinct by its much longer conidiophores, up to about 90 µm in length, often formed in well-developed fascicles of 9-20 stalks. Most of the other Stenella species on Rubiaceae are characterized by having much longer pluriseptate conidia, viz., S. coffeae J.M. Yen, A.K. Kar & B.K. Das (conidia 33-200  $\times$  3-5(-6)  $\mu$ m, see Yen et al. 1982a), S. hyptiantherae S.K. Singh, Archana Singh & Kamal (conidia 18-177  $\times$  3-5  $\mu$ m, see Singh et al. 1997), S. plectroniae Ponnappa (conidia 40-210 × 2-3.5 μm, see Ellis 1976), S. vangueriae (Thirum. & Mishra) Deighton (conidia 30-258 × 3-4.5 µm, see Thirumalachar & Mishra 1953), and S. xeromphigena J.M. Yen, A.K. Kar & B.K. Das (conidia 20-156 × 3-4 µm, see Yen et al. 1982b). Stenella naucleae A.K. Das (Das 1990) differs in having larger, well-developed stromata with large fascicles composed of 7-55 conidiophores, and much longer, pluriseptate conidiophores,  $33-115 \times 3-5 \mu m$ .

**4.** Zasmidium meynae-laxiflorae (K. Srivast., A.K. Srivast. & Kamal)

P. Phengsintham, K.D. Hyde & U. Braun, comb. nov.

(Figs 7-8)

MycoBank, MB 513400

Bas.: Stenella mevnae-laxiflorae K. Srivast., A.K. Srivast. & Kamal, in Srivastava et al., Mycol. Res. 99: 235, 1995.

Description: **Leaf spots/lesions** circular to irregularly angular, 1-12 mm in diam., brown to dark brown in the centre and with yellowish margin. Caespituli/ colonies amphigenous, small, scattered, brown. Mycelium internal and external: internal hyphae inconspicuous; external hyphae branched, 2-3 µm wide ( $\bar{x} = 2.5 \mu m$ , n = 12), septate, constricted at the septa, distances between septa 5-18 μm  $(\bar{x} = 11.92 \mu m, n = 12)$ , pale olivaceous-brown, wall approximately 0.3-0.8  $\mu$ m wide  $(\bar{x} = 0.52 \text{ } \mu\text{m}, \text{ } n = 12), \text{ verruculose.}$  Stromata well-developed, subglobose, approximately 10-41 µm in diam., brown; stromatal cells oval, ellipsoidal to angular in outline, 3-9  $\mu$ m wide ( $\bar{x} = 5.67 \mu$ m, n = 30), brown to dark brown, wall approximately 0.5-1  $\mu$ m wide ( $\bar{x} = 0.74 \mu$ m, n = 30), smooth. Conidiophores fasciculate, arising from stromata (9-20 per fascicle) and solitary, borne on external mycelial hyphae, unbranched, cylindrical,  $(14-)15-93(-98) \times 3-4 \,\mu m$  ( $\bar{x} = 51.8 \times 10^{-9}$ 3.37 µm, n = 30), 0-7-septate, distances between septa 4-23 µm ( $\bar{x}$  = 11 µm, n = 30), brown to dark brown, wall approximately 0.5-1  $\mu m$  ( $\bar{x} = 0.63 \mu m$ , n = 30), smooth, 0-2-geniculate; **conidiogenous cells** polyblastic, integrated, terminal or intercalary, 9-23 × 3-4 µm, cylindrical, pale at the apex; conidiogenous loci forming minute, dark or refractive scars on lateral and terminal denticle-like protuberances giving rise to branched conidial chains, 1-1.5  $\mu$ m wide ( $\bar{x} = 1.68$   $\mu$ m, n = 13), wall approximately 0.5-1  $\mu$ m wide ( $\bar{x} = 0.81 \mu$ m, n = 13), thickened, darkened, with a minute central pore. Conidia solitary or catenulate, sometimes subcylindrical but mostly slightly obclavate, straight or slightly curved or sinuous,  $(4-)16-87 \times 2-4 \mu m$  $(\bar{x} = 30.57 \times 3.4 \, \mu \text{m}, \, \text{n} = 14), \, 0\text{-6-septate}, \, \text{pale olivaceous, wall approximately}$ 0.25-0.5 µm wide ( $\bar{x} = 0.3$  µm, n = 14), smooth or finely verruculose, apex rounded or subtruncate with a thickened hilum, base short obconically truncate, with a basal hilum, approximately 1-1.5  $\mu$ m wide ( $\bar{x} = 1.32 \mu$ m, n = 9), wall thickened, approximately 0.5-0.8  $\mu$ m wide ( $\bar{x} = 0.54 \mu$ m, n = 9).

Known hosts: Meyna laxiflora Robyns., M. pubescens (Kurz.) Robyns.

Known distribution: India, Laos (this paper).

Materials examined: Laos, Vientiane Capital, Xaythany District, Houay Den Meuang and Dong Mak Khai villages, on leaf of Meyna pubescens (Rubiaceae), 26 April 2006, P. Phengsintham, (NOUL P 29); ibid. 10 March. 2007, (NUOL P 295)

Cultural characteristics: Mycelial colonies on PDA after three weeks at 25°C spreading surface ridged, black and brown in the centre, grey margin, reaching 25 mm in diam. 1-8  $\mu$ m wide ( $\bar{x} = 3.38 \mu$ m, n = 30), septate, constricted at the septa, distances between septa 4-20  $\mu$ m ( $\bar{x} = 14.2 \mu$ m, n = 30), brownish to subhyaline, wall approximately 0.25-0.80  $\mu$ m ( $\bar{x} = 0.45 \mu$ m, n = 30), smooth or verruculose. Conidiophores and conidia not formed in the culture.

Remarks: The collection from Laos is very similar to the type collection from India which is, according to the original description, characterized as follows: Conidiophores superficial,  $16-52 \times 3-5.5 \mu m$ . Conidia cylindrical, obclavate, 12-92 × 2.5-4 μm, olivaceous-brown, verruculose. Meyna pubescens is a new host species of this fungus. The relation of Stenella meynae-laxiflorae and S. vangueriae (Thirum. & Mishra) Deighton (1979), both species described from India on Meyna laxiflora (= Vangueria spinosa), is not quite clear. The two taxa are similar, but the latter species was described with much longer and somewhat wider conidia, 30-258 × 3-4.5 µm, formed singly, sometimes with lateral branchlets.

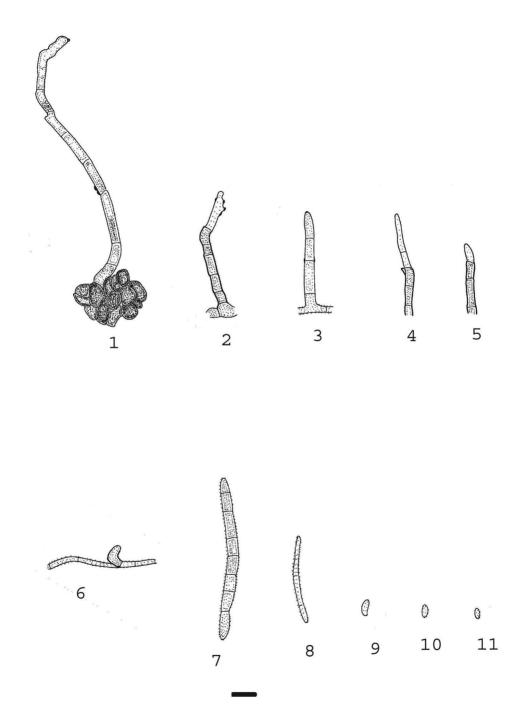


Fig. 7. 1-11. Zasmidium meynae-laxiflorae on Meyna pubescens: 1. Stroma with attached conidiophore. 2-3. External mycelia with attached conidiophores. 4-5. Conidiophores. 6. External mycelium with attached young conidiophore. 7-11. Conidia. Bar:  $1-11=10~\mu m$ .

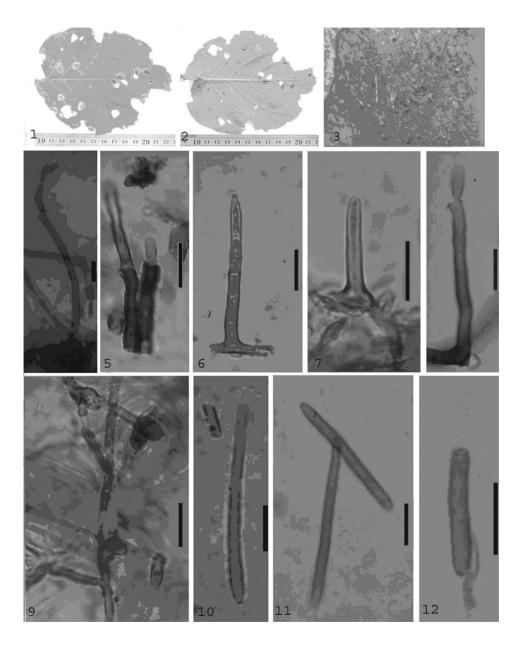


Fig 8. 1-12. Zasmidium meynae-laxiflorae on Meyna pubescens from leaf spot: 1-2 Lesions on host leaves (1. Upper surface and 2. Lower surface). 3. Caespituli. 4. Stroma with attached conidiophores. 5. Conidiophores. 6-7. External mycelia with attached conidiophores with attached young conidia. 9. External with attached young conidiophores. 10-12. Conidia. Bar:  $1-2=10~\mathrm{mm}$ ,  $4-12=10~\mathrm{\mu m}$ .

# 5. Zasmidium suregadae P. Phengsintham, K.D. Hyde & U. Braun sp. nov.

(Figs 9-10)

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Stenellae ateramnae similis, sed maculis formantibus et conidiis pallide olivaceis, brevioribus,  $(16-)17-128(-153) \times 2-4 \mu m$ , 1-10-septatis.

Description: **Leaf spots/lesions** circular to irregular, 1-3 mm diam., grey to grey-brown in the centre, and with yellow-green margin. Caespituli/colonies amphigenous, small, scattered, brown. **Mycelium** internal and external; **internal hyphae** branched, 2-4 µm wide ( $\bar{x} = 2.92 \mu m$ , n = 13), septate, constricted at the septa, distances between septa 4-19  $\mu$ m ( $\bar{x} = 10.08 \mu$ m, n = 13), pale olivaceousbrown, wall approximately 0.3-0.5  $\mu$ m wide ( $\bar{x} = 0.39 \mu$ m, n = 13), smooth; external hyphae superficial, branched, 1-4 µm wide ( $\bar{x} = 2.67$  µm, n = 30), septate, constricted at the septa, distances between septa 6-22  $\mu$ m ( $\bar{x} = 11.78 \mu$ m, n = 30), pale olivaceous-brown, wall approximately  $0.3-0.5 \mu m$  wide ( $\bar{x} = 0.44 \mu m$ , n = 30), almost smooth to verruculose. Stromata well-developed, subglobose, approximately 35-70 µm diam., brown, stroma cells oval, ellipsoidal to angular in outline, 3-10  $\mu$ m wide ( $\bar{x} = 6.37 \mu$ m, n = 30), brown to dark brown, wall approximately 0.5-1 µm wide ( $\bar{x} = 0.74$  µm, n = 30), smooth. Conidiophores fasciculate, arising from stromata (9-34 per fascicle) and solitary, borne on external mycelial hyphae, unbranched, cylindrical,  $(34-)40-86(-110) \times 3-4 \mu m$  ( $\bar{x} = 65.8 \times 3.3 \mu m$ , n = 30), 2-9septate, distances between septa 7-20  $\mu$ m ( $\bar{x} = 13.3 \mu$ m, n = 30), brown to dark brown, wall approximately 0.5-0.8  $\mu$ m ( $\bar{x} = 0.58 \mu$ m, n = 30), smooth, 0-2 times geniculate; conidiogenous cells polyblastic, integrated, terminal or intercalary, 8- $20 \times 2-3 \mu \text{m}$  ( $\bar{x} = 15.8 \times 3.14 \mu \text{m}$ , n = 30), cylindrical, pale at the apex; **conidiogenous loci** small, conspicuous, subplanate to planate, 1-1.5 µm wide  $(\bar{x} =$ 1.15 µm, n = 10), wall approximately 0.5-1 µm wide ( $\bar{x} = 0.76$  µm, n = 10), thickened, darkened. Conidia solitary or catenulate, sometimes subcylindrical, but mostly slightly obclavate, occasionally with lateral branches, straight or slightly curved to sinuous, (16-)17-128(-153)  $\times$  2-4 µm ( $\bar{x}$  = 74.13  $\times$  2.8 µm, n = 30), 1-10septate, pale olivaceous, wall approximately 0.3-0.5 µm wide ( $\bar{x} = 0.36$  µm,  $\bar{n} = 30$ ). smooth or finely verruculose, apex rounded or subtruncate, at the end of some conidia with a thickened hilum, base truncate, hila slightly thickened and darkened, approximately 1-1.5  $\mu$ m wide ( $\bar{x} = 1.04 \mu$ m, n = 13), wall approximately 0.5-0.8 µm wide ( $\bar{x} = 0.52$  µm, n = 13).

Known hosts: Suregada multiflora (Juss.) H. Baill.

Known distribution: Laos (this paper).

Material examined: Laos, Vientiane Capital, Xaythany District, Houay Den Meuang village, on leaf of *Suregada multiflora* (Euphorbiaceae), 8 May 2006, P. Phengsintham, (NOUL P 36, **holotype**).

Cultural characteristics: **Mycelial colonies** on PDA after three weeks at 25 °C spreading surface ridged, grey-brown in the centre, margin greenish black, reaching 11 mm diam., hyphae 1-4 µm wide ( $\bar{x}=2.8$  µm, n = 30), septate, constricted at the septa, distances between septa 6-18 µm ( $\bar{x}=11.9$  µm, n = 30), greenish to brownish, wall approximately 0.25-0.50 µm ( $\bar{x}=0.34$  µm, n = 30), smooth or verruculose. **Conidiophores** and **conidia** not formed in the culture.

Remarks: Five Stenella spp. have been recorded on other hosts of the family Euphorbiaceae, but there is no record from Suregada spp. Stenella bischofiae-javanicae R.K. Chaudhary et al. (Chaudhary et al. 2001), S. brideliicola K. Srivast. et al. (Srivastava et al. 1994), S. gorakhpurensis (Kamal & P. Kumar) de Hoog (Kamal & Kumar 1980, de Hoog et al. 1983) as well as Zasmidium aporosae, described above, are distinct from the new species by having

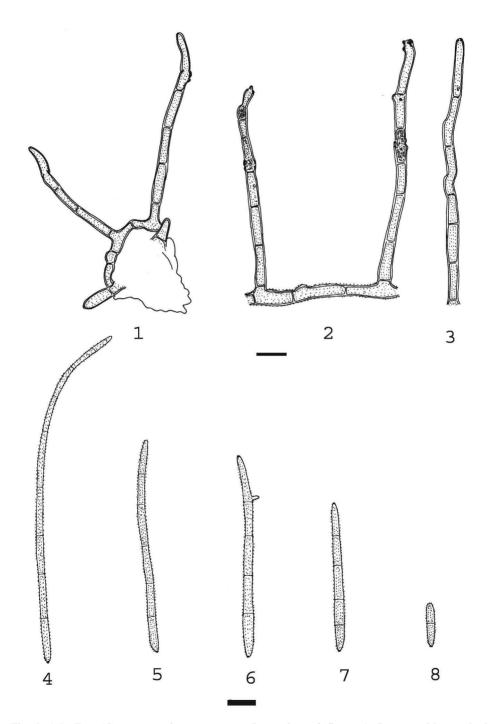


Fig. 9. 1-8. Zasmidium suregadae sp. nov on Suregada multiflorae: 1. Stroma with attached conidiophores. 2. External hypha with attached conidiophores. 3. Conidiophore. 4-8. Conidia. Bar:  $1\text{-}8 = 10~\mu\text{m}$ .

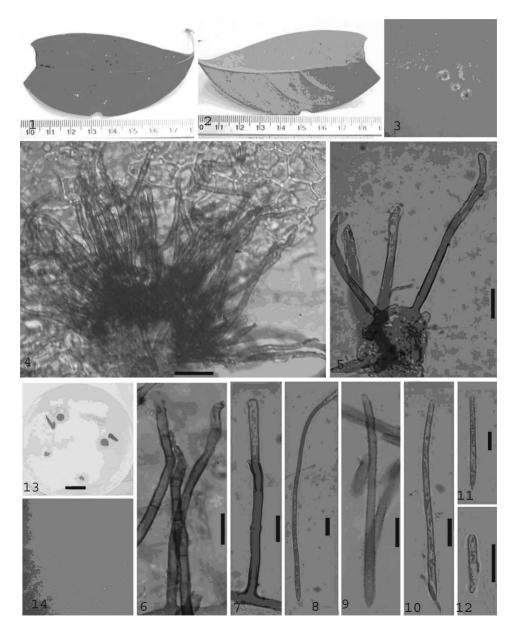


Fig. 10. 1-14. Zasmidium suregadae sp. nov. on Suregada multiflora from leaf spots: 1-2 Lesions on host leaves (1. Upper surface and 2. Lower surface). 3. Caespituli. 4-5. Stromata with attached conidiophores. 6. Conidiophores. 7. External hypha with attached conidiophore. 8-12. Conidia. 13. Culture. 14. Mycelia. Bar: 1-2=10 mm. 4-12=10 µm. 13=10 mm. 14.

conidiophores which are consistently formed singly, i.e. stromata and fasciculate conidiophores are lacking. Zasmidium manihotis ( $\equiv$  Stenella manihotis, Braun & Freire 2004), described from Brazil on Manihot sp., is a similar species with solitary as well as fasciculate conidiophores, but the stromata are much smaller, 10-25 µm diam., or even lacking, the conidiophores are formed in small fascicles, they are wider, 3-7 µm, and the conidia are also wider, 3-6 µm. S. ateramnae R.F. Castañeda & B. Kendr. (Castañeda & Kendrick 1991), described from Cuba on Ateramnus lucidus, is close to the new species by having large stromata, 50-80 × 25-45 µm, with numerous conidiophores in fascicles, but the brown conidia are much longer, up to 280 µm, with up to 20 septa. Furthermore, the latter species has been described from fallen leaves (lesions lacking) with usually internal mycelium (verruculose external hyphae not described).

**Acknowledgements.** We would like to thank Mushroom Research Foundation, Papae, Chiangmai, Thailand for support the project and the students of Mushroom Research Centre, Papae, Chiang Mai, Thailand for their assistance.

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