

1 **Four rhytismataceous ascomycetes on needles of pine from China**

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3 by

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11 With 22 figures

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14 Received, accepted in revised form ...

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26 **Abstract:** Three species of Rhytismataceae are described from needles of pines from China.  
27 *Davisomycella intermedia* is a new species growing on *Pinus yunnanensis* var. *tenuifolia*.  
28 *Ploioderma pini-armandii* and *Soleella pinicola* on *Pinus armandii* have been described  
29 previously only in Chinese with a short Latin diagnosis and therefore are presented here in  
30 detail. *Naemacyclus fimbriatus*, formerly regarded as belonging to the Rhytismatales, now to  
31 Helotiales, is reported for the first time for China, described, and illustrated.

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33 Key words: Ascomycota, needle cast, Rhytismataceae, Pinaceae.  
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## Introduction

Many fungi of the Rhytismataceae are associated with needle cast of pines, including species of *Davisomycella*, *Elytroderma*, *Lophodermella*, *Lophodermium*, and *Ploioderma*. Their diversity, ecology, biology, and pathogenicity are well studied in Europe, North America, and the Indian subcontinent (Cannon & Minter 1986, Darker 1932, Minter 1981, 1985, 1995, Minter & Millar 1980, Minter et al. 1978). For China, many new taxa of Rhytismataceae on needles of pines have been reported recently (He et al. 1986, Hou & Liu 1992, 1993, Hou et al. 1996, Lin & Tang 1988, Lin & Ren 1992, Lin et al. 1993, 1995a, 1995b, Liu & Qiu 1995). Almost all species, however, are described only in Chinese with few drawings and short Latin diagnoses. Therefore, in the present paper, in addition to the new species, several known species are described and illustrated in detail.

## Materials and methods

Ascomata were sectioned by hand to different thickness using razor blades. Microscopic preparations were made in water, Melzer's reagent, 5 % KOH, or 0.1 % (w/v) cotton blue in lactic acid and observed with a Zeiss Standard Microscope. Vertical sections of ascomata were mounted in lactic acid or cotton blue with pre-treatment in water. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water, or cotton blue in lactic acid. Ascospore contents are drawn based on observations in water. Measurements were made using material mounted in 5 % KOH or Melzer's reagent. For each specimen 20 ascospores and asci were measured. The drawings were made free-hand at a fixed scale.

## Results

***Davisomycella intermedia*** C.-L. Hou, J. Gao & M. Piepenbr., sp. nov. MycoBank No. xxx

Figs 1-5

ETYMOLOGY: This species is morphologically intermediate between species of *Davisomycella* and *Lophodermella*.

Ascomata 700-3000 x 220-300  $\mu$ m, elliptica vel elongate elliptica, subepidermalia vel partim subhypoepidermalia; paraphyses filiformes, simplices, apicibus ad 2-3  $\mu$ m incrassatis; asci 50-90(-100) x 10-15  $\mu$ m, cylindrici; ascosporae 47-80 x 1.5-3  $\mu$ m, quasi filiformes, in tunica gelatinosa inclusae.

HOLOTYPE: On *Pinus yunnanensis* var. *tenuifolia* Cheng & Law (Pinaceae), China, Yunnan province, Lijiang, Tiejiaohan, ca 2800 m alt., 31 July 2001, C.-L. Hou, M. Piepenbring, R. Kirschner, and Z.-L. Yang 159 (AAUF).

Ascomata on both sides of the needles. Ascomata in surface view 700-3000 x 220-300  $\mu$ m, elliptical to elongate-elliptical, slightly raising above the surface of the substratum, opening by a single longitudinal split extending over about 2/3 of the length of the ascomata, the entire surface of the ascomata dark brown. Lips absent. Perimeter line absent. In median vertical section ascomata subepidermal near the centre of the ascomata, but lower part of the covering stroma near the basal layer lined by two layers of hypodermal cells, 170-200  $\mu$ m deep. Covering stroma up to 40-50  $\mu$ m thick near the centre of the ascoma, the black fungal tissue limited to the central parts of an ascoma not extending to the basal layer, consisting of thick-walled textura angularis with cells of 3-5  $\mu$ m diam. Basal stroma absent, the cells underneath the subhymenium slightly brown. Subhymenium 7-10  $\mu$ m thick, hyaline, composed of 2-3 layers of pseudoparenchymatous cells. Paraphyses 90-115 x 1-1.5  $\mu$ m, filiform, unbranched, usually septate, swollen to 2-3  $\mu$ m at the apex, covered by gelatinous sheaths. Asci ripening sequentially, 50-90(-100) x 10-15  $\mu$ m, cylindrical, thin-walled, J-, slightly rostrate at the apex, without circumapical thickening, 8-spored. Ascospores fasciculate, 47-80 x 1.5-3  $\mu$ m, almost filiform, looking like nematodes, slightly swollen at about one third of the length from the tip,

1 slightly tapering towards the tip and strongly tapering towards the base, hyaline, aseptate,  
2 with a 2-3  $\mu\text{m}$  thick gelatinous sheath.

3 Conidiomata not seen.

4 Zone lines not observed.

5 DISTRIBUTION: Only one specimen has been collected. Repeated observation of this species  
6 by the senior author (without collecting) showed that it is widely distributed in Lijiang,  
7 Yunnan province.

8 HABITAT: *D. intermedia* forms fruiting-bodies on dead parts of living needles or on dead  
9 needles still attached to twigs.

10 NOTES: Ascoma shape, depth of ascomata in the host tissue, lack of lips and basal stromatic  
11 tissue, as well as the aggressive nature show that the present species is closely related to  
12 species of *Lophodermella* and *Davisomycella* (Darker 1967). *Lophodermella* spp. have  
13 subhypodermal, more or less concolorous ascomata, while *Davisomycella* spp. have  
14 subepidermal, dark ascomata (Darker 1967). In the present species, ascomata are totally  
15 subepidermal near the opening, but present a lower part of the covering stroma near the basal  
16 layer lined by two layers of hypodermal cells. Therefore, it is morphologically intermediate  
17 between the genera *Lophodermella* and *Davisomycella*. The colour of the stromata is more  
18 similar to the colour of species of *Davisomycella* than to species of *Lophodermella*. Although  
19 Darker's system is artificial and unsatisfactory, no suitable alternative has yet been proposed  
20 (Cannon & Minter 1986, Hou et al. 2004). Therefore, we place the new taxon in the genus  
21 *Davisomycella* based on the color of the ascomata.

22 The new species differs from all known species of *Davisomycella* by the combination of  
23 depth of embedding of the ascomata and nematode-like ascospores. The generic position of  
24 the new species is preliminary and has to be confirmed by molecular data.

25 This species is an aggressive parasite and causes serious needle cast of *Pinus yunnanensis* var.  
26 *tenuifolia* in young pine plantations in the Yunnan province.

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28 ***Naemacyclus fimbriatus*** (Schw.) DiCosmo, Peredo & Minter, European J. For. Pathol. **13**:  
29 207, 1983. Figs 6-11

30 TYPE. On cones of *Pinus* sp. For further data see DiCosmo et al. (1983).

31 Ascomata on both sides of the needles. Ascomata in surface view scattered, circular, 250-400  
32  $\mu\text{m}$  diam., concolorous with the surface of the needles while immature, later strongly raising  
33 above the needle surface and forming swellings, then rupturing the overlying host tissue and  
34 centrally exposing the dark brown fungal tissue, opening first along the line of stomata, then  
35 forming a cross. In median vertical section ascomata deeply immersed in the host tissue, 175-  
36 230  $\mu\text{m}$  deep. Covering stroma up to 40-60  $\mu\text{m}$  thick near the centre of the ascoma, thinner  
37 towards the edges, composed of an outer layer of host tissue including one layer of epidermal  
38 cells, two layers of hypodermal cells, one layer of incomplete cells of the mesophyll, and an  
39 inner layer of fungal tissue, which is composed of one layer of dark brown textura globulosa  
40 adjacent to the host tissue and an innermost layer of extremely thick-walled, pale brown to  
41 dark brown hyphal strands. Hyphal strands 15-30  $\times$  4-8  $\mu\text{m}$ , usually with 2-4 septa, pale  
42 brown to hyaline at the apex. In immature ascomata, the hyphal strands of the covering layer  
43 are vertically oriented. Basal stroma absent. Subhymenium 11-14  $\mu\text{m}$  thick, hyaline,  
44 composed of hyaline cells of 1-2 diam. Paraphyses 90-120  $\times$  1  $\mu\text{m}$ , filiform, not branched,  
45 septate, not swollen at the apex, covered by a thin gelatinous sheath. Asci ripening  
46 sequentially, 80-105  $\times$  6-9  $\mu\text{m}$ , cylindrical, short-stalked, thin-walled, apex strongly rostrate,  
47 slightly blue in iodine after treatment with 5 % KOH, the blue color disappearing after 15-25  
48 min, without circumapical thickening, discharging spores through a small apical hole, 8-

1 spored. Ascospores fasciculate, 65-95 x 1.0-1.5  $\mu\text{m}$ , filiform, slightly tapering towards the  
 2 base, rounded at the apex, hyaline, usually with (2-)4-6 septa, rarely aseptate, with a 1-2(-3)  
 3  $\mu\text{m}$  thick gelatinous sheath.

4 Conidiomata not seen.

5 Zone lines not observed.

6 SPECIMENS EXAMINED: China, Anhui province, Yuexi, Miaodaoshan National Forest Park, ca 1070 m alt., 10  
 7 June 1995, C.-L. Hou 260 (AAUF); China, Anhui province, Yuexi, Miaodaoshan National Forest Park, ca 1070  
 8 m alt., 2 June 1996, C.-L. Hou 260b (AAUF). ADDITIONAL SPECIMENS EXAMINED: On cones of *Pinus* sp.,  
 9 Germany, Oberfranken, Weißmain, am Kordigast, June 1909, A. Ade s. n. (M 80713). On *Pinus* sp., Germany,  
 10 Königstein a. E., May 1893, W. Krieger (M 80712).

11 HOST SPECIES: *Pinus taiwanensis* Hayata (Pinaceae), *Pinus* spp. (DiCosmo et al. 1983).

12 KNOWN DISTRIBUTION: This species is widely distributed in Europe and North America  
 13 (DiCosmo et al. 1983). In China, it is only known from Yuexi, Anhui Province.

14 HABITAT: *N. fimbriatus* was collected on fallen needles in litter or on almost decayed needles  
 15 attached to dead twigs which were harmed by insects or snow. Needles with ascomata are  
 16 usually strongly bleached and fragile.

17 NOTES: Sherwood (1974) pointed out that the apex of the ascus of *Naemacyclus fimbriatus* is  
 18 faintly blue in iodine. Other mycologists, however, did not observe this phenomenon  
 19 (DiCosmo et al. 1983, Minter 1985). We mounted asci in Melzer's reagent with pretreatment  
 20 in 5 % KOH, and the apex of ascus showed a slight blue tint that disappeared after 10-25 min.  
 21 In the two European specimens we checked, however, this faint amyloid color was not  
 22 observed. This might be due to the fact that the specimens were rather old.

23 While nomenclatural problems of *N. fimbriatus* have been resolved by DiCosmo et al. (1983),  
 24 the systematic position of *N. fimbriatus* is still unclear. It was placed into the Phacidiaceae in  
 25 the past, but DiCosmo (1979) suggested that it is more closely related to Rhytismataceae  
 26 rather than the Phacidiaceae because of J- asci and ascospores covered by a gelatinous sheath  
 27 (comp. also Minter 1985, 1995, Hawksworth et al. 1995, Kirk et al. 2001). More recently, the  
 28 analysis of partial small subunit rDNA showed that *N. fimbriatus* is closely related to species  
 29 of the Helotiales, not to the Rhytismatales.

30 The specimen of *N. fimbriatus* collected in China is morphologically similar to specimens  
 31 described in literature (DiCosmo et al. 1983, Sherwood 1974). Some aspects, however, are  
 32 slightly different. Ascospores in the specimen from China usually have 4-6 septa rather than 7  
 33 septa, and are slightly narrower, and conspicuous hair-like periphysoids near the opening are  
 34 lacking. In addition, specimens from Europe and North America are mostly observed on  
 35 cones (Minter 1985, DiCosmo et al. 1984, Sherwood 1974) while in China this species has so  
 36 far never been observed on cones. We do not know whether these differences are intraspecific  
 37 or interspecific, or result from different host species, substrata, geography, or other factors.

38 Molecular data are needed to determine the delimitation of this species.

39 *N. fimbriatus* has rarely been collected in China. Based on the information available, *N.*  
 40 *fimbriatus* appears to be saprotrophic.

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42 ***Ploiderma pini-armandii*** C.-L. Hou & S.-Q. Liu, Acta Mycol. Sinica **12**: 99, 1993.

43

Figs 12-17

44 TYPE. On *Pinus armandii* Franch., China, Shanxi, Xinjiashan, Xihe, June 1986, W.-H. Li 0158 (AAUF 90003).

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46 Ascomata on dead parts of living needles, usually 1-3 needles in a group of 5 needles infected,  
 47 ascomata only on the abaxial sides of the needles, scattered. Ascomata in surface view  
 48 entirely black and shiny, without perimeter, with a light surrounding area when young,  
 49 elliptical, 800-1400 x (400-)450-620  $\mu\text{m}$ , slightly raising above the surface of the needles,  
 50 opening by a single longitudinal split extending along the entire length of the ascoma. Lips  
 51 absent. In median vertical section ascomata deeply embedded in the host tissue, subcuticular

1 near the opening, subhypodermal in the middle part of the covering layers and deeply  
 2 embedded in host tissue near the base, 400-550  $\mu\text{m}$  deep. Some epidermal cells in the  
 3 covering layer separated from the cuticle and filled with brown fungal cells. Covering layer  
 4 up to 45-65  $\mu\text{m}$  thick near the centre of the ascoma, fungal tissue not extending to the basal  
 5 layer, composed of an outer layer of host tissue, a layer of thick-walled dark brown textura  
 6 angularis with cells of 3-5  $\mu\text{m}$  diam., some brown, thick-walled hyphae, and an innermost  
 7 layer with one row of hyaline cells which often disappear in older ascomata. Basal stroma  
 8 absent. Resin canals filled with hyaline hyphae. Subhymenium 10-20  $\mu\text{m}$  thick, composed of  
 9 hyaline, thin-walled cells of variable size and shape. Paraphyses 230-270 x 1  $\mu\text{m}$ , filiform, not  
 10 branched, multi-septate, strongly twisted at the apex and the entire paraphyses embedded in a  
 11 gelatinous sheath. Asci ripening sequentially, (120-)170-250 x 24-30  $\mu\text{m}$ , cylindrical, thin-  
 12 walled, J-, without circumapical thickening, discharging spores through a small apical hole or  
 13 an irregular split, 8-spored. Ascospores 38-50 x 4-6  $\mu\text{m}$ , fusiform-cylindrical, slightly tapering  
 14 towards the base, hyaline, aseptate, with a 4-6  $\mu\text{m}$  thick gelatinous sheath. In water, the  
 15 gelatinous sheaths usually appearing bilayered, occasionally the outer sheath lacking.  
 16 Conidiomata on abaxial sides. Conidiomata in surface view variable in shape and size,  
 17 sometimes as wide as the needles, concolorous with the substrate or slightly lighter than the  
 18 surface of the needle, opening by ostioles or slits at the sides (?). In vertical section,  
 19 conidiomata subepidermal, 35-45  $\mu\text{m}$  deep. Conidia not observed.

20 Zone lines not seen.

21 SPECIMEN EXAMINED: China, Yunnan province, Lijiang, Tiejieshan, ca 2800 m alt., 31 July 2001, C.-L. Hou, M.  
 22 Piepenbring, R. Kirschner, and Z.-L. Yang 161 (AAUF).

23 HOST SPECIES: *Pinus armandii* Franch. (Pinaceae).

24 KNOWN DISTRIBUTION: This species is known from the Shanxi province (Hou & Liu 1993)  
 25 and it is reported here for the first time for the Yunnan province. Only one specimen has been  
 26 collected. However, it is widely distributed in the plantations of *P. armandii* in Yunnan  
 27 province based on observation of the senior author (without collecting).

28 HABITAT: *P. pini-armandii* was collected from needles whose basal parts were still green.

29 NOTES: *P. pini-armandii* is morphologically somewhat intermediate between species of  
 30 *Meloderma* and *Ploioderma*. Its fusiform-cylindrical ascospores are similar to those of species  
 31 of *Meloderma*, while the deeply embedded ascomata, lack of lip cells, and aggressive nature  
 32 indicate a close relationship to species of *Ploioderma*. This species causes serious needle  
 33 blister on *Pinus armandii* in plantations in Yunnan. It usually infects needles of about 10-  
 34 year-old trees and one to three of a bundle of five needles are infected. The infected needles  
 35 become yellow and die in late fall but the basal parts of infected needles are still green.

36 *P. pini-armandii* was only collected in plantation and has not been found in natural forests in  
 37 the Yunnan province.

38 ***Soleella pinicola*** Y.-R. Lin & W. Ren, Acta Mycol. Sinica **11**: 210, 1995. Figs 18-23

39 TYPE. On *Pinus armandii* (Pinaceae), China, Yunnan, Kunming, Yuzhusi, 11 July 1957, J.-W. Chen 16346  
 40 (AAUF 66454).

41 Ascomata on dead needles still attached to twigs. Ascomata on the abaxial side of needles,  
 42 scattered. Ascomata in surface view black in the centre for more than the half of the surface,  
 43 with a light surrounding area, without or with an inconspicuous perimeter line, elliptical, 600-  
 44 1050 x (400-)300-450  $\mu\text{m}$  (including the light surrounding area), usually raising above the  
 45 surface of the substratum in the central part of the ascomata, opening by a single longitudinal  
 46 split. Lips present. Ascomata in median vertical section partly subepidermal, 210-280  $\mu\text{m}$   
 47 deep. Some epidermal cells in the covering layer separated from the cuticle and embedded in  
 48 the covering stroma. Covering stroma up to 35-50  $\mu\text{m}$  thick near the centre of the ascoma,  
 49 fungal tissue extending sometimes to the basal layer, which is composed of an outer layer of  
 50 host tissue; a layer of thick-walled, dark brown textura angularis with cells of 3-5  $\mu\text{m}$  diam.

1 and almost hyaline textura angularis near the basal layer; 4 or more epidermal cells separated  
 2 from the epidermis and located on the basal layer. Basal stroma absent or poorly developed.  
 3 Subhymenium 8-12  $\mu\text{m}$  thick, composed of hyaline, thin-walled, small cells. Paraphyses 230-  
 4 270  $\times$  1  $\mu\text{m}$ , filiform, not branched, septate, slightly swollen or not at the apex and embedded  
 5 in a thin gelatinous sheath. Asci ripening sequentially, 16-24  $\times$  (55-)70-120(-140)  $\mu\text{m}$ ,  
 6 cylindrical, thin-walled, J-, without circumapical thickening, discharging spores through a  
 7 small apical hole, 8-spored. Ascospores 18-32  $\times$  (3-)4-6(-7)  $\mu\text{m}$ , bifusiform or slightly  
 8 cylindrical-ellipsoidal, hyaline, aseptate, with a 4-8  $\mu\text{m}$  thick gelatinous sheath.  
 9 Conidiomata not seen.

10 Zone lines not seen.

11 SPECIMEN EXAMINED: China, Yunnan province, Kunming, Xishan, ca 2300 m alt., 17 Aug. 2001, C.-L. Hou, M.  
 12 Piepenbring, R. Kirschner, and Z.-L. Yang 203 (AAUF).

13 HOST SPECIES: *Pinus armandii* Franch. (Pinaceae).

14 KNOWN DISTRIBUTION: *S. pinicola* is only known from Yunnan, China.

15 HABITAT: *S. pinicola* was collected on needles which were attached to or hanging down from  
 16 twigs.

17 NOTES: *S. chinensis* Y.-R. Lin & W. Ren, a species occurring on *Pinus taiwanensis* Hayata, is  
 18 similar to *S. pinicola* but differs by partly hypodermal ascomata, shorter and wider asci and  
 19 ascospores, as well as the lack of conidiomata (Lin et al. 1995b). *S. pinicola* often occurs  
 20 together with *Lophodermium pini-excelsae* on the same needles. *S. pinicola* seems to be a  
 21 weak parasite, not causing disease in Yunnan province.

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 27 National Natural Science Foundation of China.

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### 34 Figure Legends

35

36 **Figs 1-5.** *Davisomycella intermedia* on *Pinus yunnanensis* var. *tenuifolia*.. 1. Needles  
37 bearing ascomata. Scale bar = 1 cm. 2. Ascomata as seen under a dissecting microscope.  
38 Scale bar = 1 mm. 3. Ascoma in vertical section. Scale bar = 100  $\mu$ m. 4. Detail of an  
39 ascoma in vertical section. Scale bar = 50  $\mu$ m. 5. Paraphyses, a young ascus, mature asci  
40 with ascospores, an ascus after the liberation of the ascospores, and liberated ascospores  
41 with gelatinous sheaths. Scale bar = 10  $\mu$ m.

42

43 **Figs 6-11.** *Naemacyclus fimbriatus* on *Pinus taiwanensis*. 6. Needles bearing ascomata.  
44 Scale bar = 1 cm. 7. Ascomata as seen under a dissecting microscope. Scale bar = 500  $\mu$ m.  
45 8. Young ascoma in vertical section. Scale bar = 50  $\mu$ m. 9. Ascoma in vertical section.  
46 Scale bar = 50  $\mu$ m. 10. Detail of the inner part of the covering stroma. Scale bar = 10  $\mu$ m.  
47 11. Paraphyses, a young ascus, mature asci with ascospores, an ascus after the liberation of  
48 the ascospores, and liberated ascospores with a gelatinous sheath. Scale bar = 10  $\mu$ m.

49

1 **Figs 12-17.** *Ploioderma pini-armandii* on *Pinus armandii*. 12. Needles bearing ascomata.  
2 Scale bar = 1 cm. 13. Ascomata and conidiomata as seen under a dissecting microscope.  
3 Scale bar = 1 mm. 14. Ascoma in vertical section. Scale bar = 500  $\mu\text{m}$ . 15. Conidioma in  
4 vertical section. Scale bar = 500  $\mu\text{m}$ . 16. Detail of an ascoma in vertical section. Scale bar =  
5 100  $\mu\text{m}$ . 17. Young asci, mature asci with ascospores, two asci after liberation of ascospores,  
6 paraphyses, and liberated ascospores with thin gelatinous sheaths. Scale bar = 20  $\mu\text{m}$ .

7  
8 **Figs 18-22.** *Soleella pinicola* on *Pinus armandii*. 18. Needles bearing ascomata. Scale bar =  
9 1 cm. 19. Ascomata and conidiomata as seen under a dissecting microscope. Scale bar =  
10 500  $\mu\text{m}$ . 20. Ascoma in vertical section. Scale bar = 50  $\mu\text{m}$ . 21. Detail of an ascoma in  
11 vertical section. Scale bar = 50  $\mu\text{m}$ . 22. Young ascus, mature asci with ascospores, an ascus  
12 after liberation of ascospores, paraphyses, and liberated ascospores with a thin gelatinous  
13 sheath. Scale bar = 20  $\mu\text{m}$ .