

FOUR SPECIES OF *LOPHODERMIIUM* ON *PINUS SYLVESTRIS*

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Four species of *Lophodermium* Chev. are distinguished on secondary needles of *Pinus sylvestris* L. by the manner in which their ascocarps are embedded in the host and their ability to produce stromatic lines of different sorts across the needle. The four species, *L. pinastri* (Schrad. ex Hook.) Chev., *L. conigenum* Hilitzer, *L. pini-excelsae* Ahmad and *L. seditiosum* sp.nov. are keyed out, described and figured.

In recent years several publications have suggested that *Lophodermium pinastri* (Schrad. ex Hook.) Chev. is an aggregation of distinct taxa (Millar & Watson, 1971; Staley, 1975; Staas-Ebregt & Gremmen, 1975). To investigate this we examined the morphology of *Lophodermium* Chev. on secondary needles of *Pinus sylvestris* L., i.e. what is usually identified as *L. pinastri*, using samples mainly from Great Britain and the Pacific North West of the United States, but also from Estonia, France, Germany, Greece, Yugoslavia, Australia, Japan and other parts of North America. Four species of *Lophodermium* can be recognized from these samples: *L. pinastri* sensu stricto; *L. conigenum* Hilitzer, a validly published but hitherto largely ignored species; *L. pini-excelsae* Ahmad, also validly published but hitherto largely ignored and known previously only from needles of haploxyton (five needle) pines, and *L. seditiosum* sp.nov.

The most useful characteristic for distinguishing these species is the manner of embedding of the ascocarp in the needle. When transverse sections taken from the mid-point of ascocarps of each species are examined, the position of the host epidermal cells in relation to the ascocarps differs. In *L. pinastri* and *L. pini-excelsae* more than five epidermal cells are 'down', usually grouped in a line on the ascocarp base. The remaining cells are 'up', above the ascocarp, and have never been separated from the cuticle. The 'down' cells are separated invariably from the cuticle covering the central part of the ascocarp's surface. In *L. conigenum* less than seven central epidermal cells are

'down', and although sometimes grouped, are more often separated and spread over the ascocarp base. The remaining cells on either side are 'up', and have never been separated from the cuticle. In *L. seditiosum* all the epidermal cells are 'up', and none is on the ascocarp floor.

Transverse sections taken from other points along the ascocarp reveal that *L. seditiosum* is totally subepidermal, whereas the other three species show variation depending on the site of the transverse section. Sections taken from near the extremities of these ascocarps show all the epidermal cells 'up', and between the extremities and the mid-point is a region where epidermal cells are neither 'up' nor 'down', but are lying in the hymenium. Serial transverse sections of this region show such epidermal cells to be transitional between the 'up' and 'down' positions. Ascocarps of these three species are thus partly subepidermal. The different positions of the epidermal cells are illustrated diagrammatically in Fig. 2.

The different positions of the epidermal cells have a direct effect on the external appearance of the dry ascocarps (wet ascocarps do not show this feature so well). Where the darkened clypeus is covered only by the cuticle the ascocarp surface appears black. Where it is covered by both cuticle and epidermis it appears grey. Ascocarps of *L. pinastri* and *L. pini-excelsae* therefore appear black for a large proportion of their surfaces because of the many epidermal cells in the 'down' position. *L. conigenum* appears black for a small proportion of the ascocarp surface because few epidermal cells are 'down'. *L. seditiosum*, however, appears totally

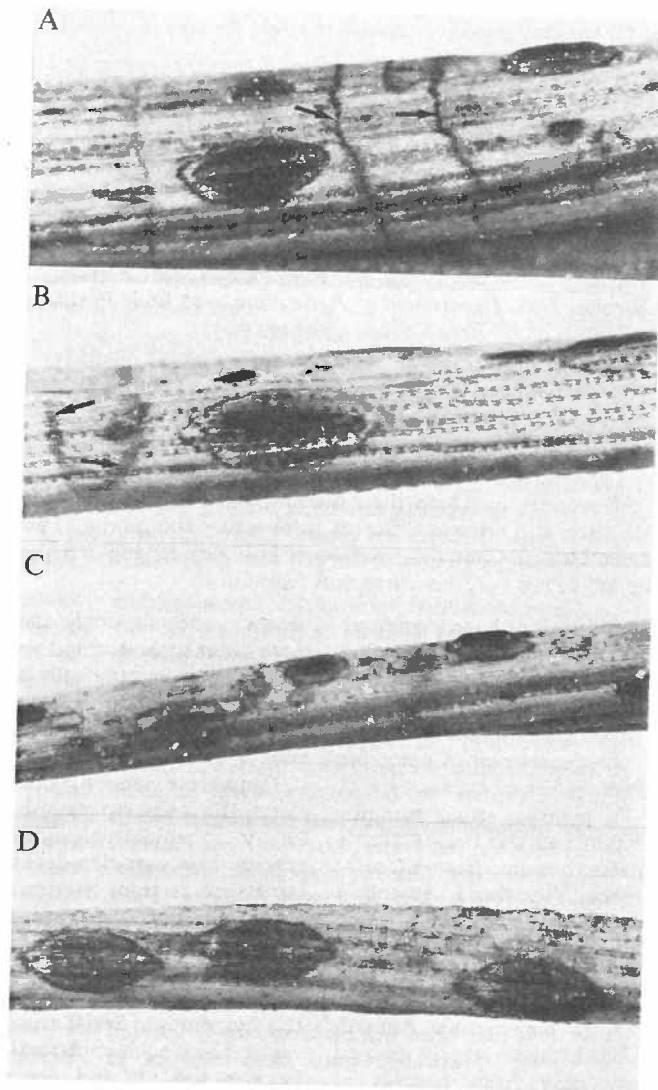


Fig. 1. Ascocarps of *Lophodermium* species. (A) *L. pinastri* (black stromatic lines arrowed). (B) *L. conigenum* (brown stromatic lines arrowed). (C) *L. pini-excelsae*. (D) *L. seditiosum*. All  $\times 20$ .

grey because all the epidermal cells are 'up'. With practice it is possible to distinguish by eye the epidermis layout from the external appearance and even use it as a field characteristic.

The species can also be divided into two groups according to the type of stromatic line produced across the needle. This, too, is a useful field characteristic. Stromatic lines are of two types, the first thin and black (associated with *L. pinastri* and *L. pini-excelsae*), and the second wider and brown (associated with *L. conigenum* and *L. seditiosum*). *Lophodermium pinastri* often produces as many

black lines as ascocarps, needles bearing ten or more such lines being not uncommon, whereas *L. pini-excelsae* produces fewer lines than ascocarps and sometimes scarcely any. *Lophodermium conigenum* and *L. seditiosum* produce very few brown lines as compared with ascocarps, needles bearing more than three brown lines being uncommon. No species produces more than one type of stromatic line. The key below is based largely on these two characteristics, the manner of embedding and the stromatic lines. A description of each species follows the key.

KEY TO *LOPHODERMIIUM* SPECIES ON SECONDARY NEEDLES OF *PINUS SYLVESTRIS*

- |   |   |  |
|---|---|--|
| 1 | Thin transverse black lines across needle<br>No lines or a few brown lines  | 2  |
| 2 | Many black lines; ascocarps more than 800 $\mu\text{m}$ long, usually with red lips<br>Few black lines; ascocarps less than 800 $\mu\text{m}$ long, always with grey lips | 3  |
| 3 | Ascocarps partly subepidermal<br>Ascocarps totally subepidermal   | <i>L. pinastri</i><br><i>L. pini-excelsae</i><br><i>L. conigenum</i><br><i>L. seditiosum</i> |

*LOPHODERMIIUM PINASTRI* (Schrad. ex Hook.) Chev., *Fl. env. Paris* 1, 436 (1826). (Figs. 1 A, 2 A, 3 A, 4).

*Hysterium pinastri* Schrad., *J. für Bot.* 2, 69 (1799).

*Hysterium pinastri* Schrad. ex Hook., *Fl. Scot.* 2, 8 (1821).

*Hypoderma pinastri* (Schrad.) DC. in Lamarck & De Candolle, *Fl. Fr.* 2, 305 (1805).

*Aporia obscura* Duby, *Mém. Soc. Phys. Hist. nat. Genève* 16, 63 (1861).

*Hysterium limitatum* Wiebel, *Primitiae Flora Werth.* 329 (1799).

*Schizothyrium obscurum* (Duby) Sacc., *Syll. fung.* 2, 725 (1883).

*Lophodermellina pinastri* (Schrad. ex Hook.) Höhn., *Annls Mycol.* 15, 311 (1917).

*Lophodermium pinicolum* Tehon, *Illinois biol. Monogr.* 13 (4), 55 (1935).

Ascocarps amphigenous on needles (more than 20% on adaxial side), when wet black, when dry black in the centre for more than half the total ascocarp surface, the remainder grey surrounded by a black line (Fig. 1 A). Lips hyaline, red, yellow, orange or rarely, when very wet, green. Ascocarps 600–1200  $\mu\text{m}$  long (fig. 4), covered by the host epidermis on either side, but above the epidermis in the centre (Figs. 2 A, 3 A). In mid-point trans-

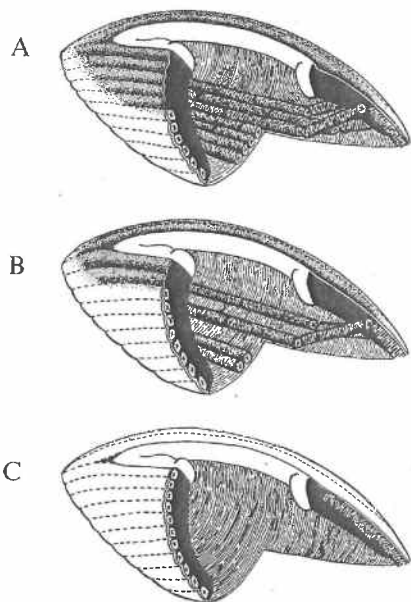


Fig. 2. Layout of host epidermal cells in ascocarps of *Lophodermium*. (A) *L. pinastri* and *L. pini-excelsae* (>5 cells down, grouped). (B) *L. conigenum* (<7 cells down, grouped). (C) *L. seditiosum* (0 cells down).

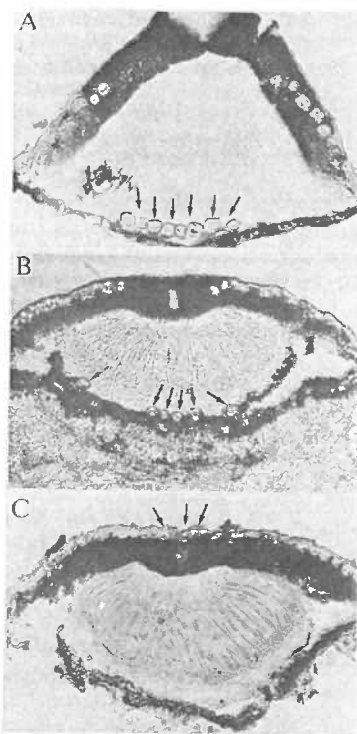


Fig. 3. Ascocarps of *Lophodermium* species in transverse vertical section. (A) *L. pinastri*. (B) *L. conigenum*. (C) *L. seditiosum*. All  $\times 250$ . (Arrows indicate host epidermal cells).

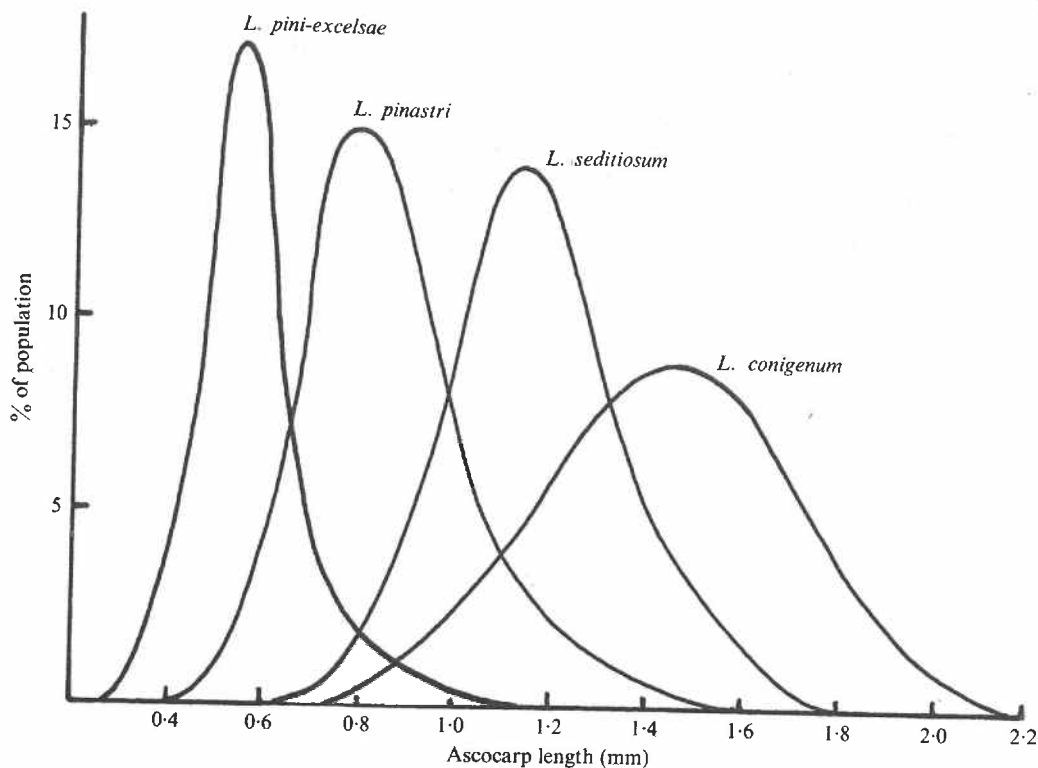


Fig. 4. Ascocarp length distributions of *Lophodermium* species.

verse section more than five epidermal cells can be seen in a group on the ascocarp base. Immature ascocarps appearing first as small, black and round, gradually becoming oblong, and developing the grey area and black surrounding line shortly before splitting open. Overmature ascocarps tending to lose the fungal lip cells, otherwise similar to mature ascocarps. Ascocarps accompanied by many thin black lines running across the needle (Fig. 1A). *Asci* cylindrical, 8-spored, 110–155  $\mu\text{m}$  long, 9.5–11.5  $\mu\text{m}$  wide. *Paraphyses* filiform, tips sometimes clavate usually straight and unswollen, as long as the asci. *Ascospores* filiform, sometimes coiled spirally at the upper end of the ascus, 70–110  $\mu\text{m}$  long, about 2  $\mu\text{m}$  wide, enveloped in a gelatinous sheath. *Conidiomata* pycnidial, sub-epidermal, rarely coalescing, 300–400  $\mu\text{m}$  long. *Conidia* bacillar, 4.5–6.25  $\mu\text{m}$  long.

*Habitat* mainly on detached needles in litter, also on cones.

Cultures on 2% Malt Agar slow-growing, rarely exceeding an increase in diam of 3 mm/day at room temperature, usually less. Growth invariably terminating before the edge of the Petri dish

(90 mm diam) is reached. Cultures at first white or creamy above and below, generally remaining so until shortly before radial growth stops, when black stromatic lines begin to appear round their periphery, usually a little behind the outermost hyphae. These black lines are visible above and below the cultures. Many cultures also produce black stromatic areas within the confines of the black line, and sometimes these occupy a large proportion of the culture's surface. Throughout the growth period the hyphae branch at a very short distance behind their tips thus giving the colony a compact appearance. Conidia produced frequently in vitro, the same size as those produced in vivo.

*Specimens examined*: Mougeot & Nestler No. 76 (lectotype) at University of California, Berkeley; isolectotypes at K and E; *Lophodermium pinastri* folder IMI including 184107 (Fontainebleu Forest, France), 225066 (Hokkaido, Japan), 225061 (Washington, U.S.A.), 225065 (Argyll, Scotland), 225060 (Culbin Forest, Morayshire, Scotland); *Lophodermium pinastri* folder, Forestry Dept, Aberdeen University (many collections on *Pinus sylvestris* from Great Britain and abroad).



Most collections identified as *L. pinastri* in herbaria are of this species. Schrader's (1799) illustration of *Hysterium pinastri* clearly shows many black lines. The exsiccatum, Mougeot & Nestler No. 76, which was cited by Hooker (1821) and Chevallier (1826) corresponds to this fungus. Terrier (1956) considered this exsiccatum had the best claim to be nomenclatural type and Staley (1975) designated as lectotype of *L. pinastri* the example of this exsiccatum at the University of California, Berkeley. Isolectotypes are available in many European herbaria. The following sub-specific taxa also correspond to this species: *L. pinastri* forma *pini-sylvestris* De Thümen, Mycotheca Universalis No. 282; *L. pinastri* forma *uncinatum* Roumeguère, Fungi Selecti Exsiccati No. 6941, fide Tehon (1935); *L. pinastri* biotype A, Millar & Watson (1971). Cultures correspond to Type 1 of Stephan (1973), and have been deposited as IMI 156241 and ATCC 28347.

LOPHODERMIIUM CONIGENUM Hilitzer, *Věd. Spisy čsl. Akad. zeměd.* 3, 76 (1929). (Figs. 1B, 2B, 3B, 4)

*Ascocarps* amphigenous on needles (less than 20% on adaxial side), when wet black, when dry black in the centre for less than a quarter of the total ascocarp surface, the remainder grey surrounded by a black line (Fig. 1B). Lips mostly hyaline or green, sometimes fawn. Ascocarps 900–2000  $\mu\text{m}$  long (Fig. 4), covered by the host epidermis on either side, but above the epidermis in the centre (Figs. 2B, 3B). In mid-point transverse section less than seven epidermal cells can be seen scattered over the ascocarp floor. Immature ascocarps appearing first as a thin black line, the grey area and black surrounding line appearing shortly before the ascocarp splits open. Overmature ascocarps tending to lose the fungal lip cells and the black surrounding line, thus becoming less easy to distinguish from the surrounding needle surface. Ascocarps accompanied by infrequent diffuse brown lines running across the needle (Fig. 1B). *Asci* cylindrical, 8-spored, 160–215  $\mu\text{m}$  long, 11.5–14  $\mu\text{m}$  wide. *Paraphyses* filiform, tips sometimes hooked, sometimes clavate, sometimes straight and unswollen, as long as the asci. *Ascospores* filiform, sometimes coiled spirally at the upper end of the ascus, 90–130  $\mu\text{m}$  long, about 2  $\mu\text{m}$  wide, enveloped in a gelatinous sheath. *Conidiomata* pycnidial, subepidermal, often coalescing, 350–450  $\mu\text{m}$  long. *Conidia* bacillar 5.25–7.5  $\mu\text{m}$  long.

*Habitat* mainly on needles attached to dead branches, also on cones.

Cultures on 2% Malt Agar fast growing, an increase in diam of 5 mm/day being common. Growth rarely terminating before the edge of the Petri dish (90 mm diam) is reached. Cultures at first white or creamy above and below, many remaining like this, some, however, showing areas of brown above and below, or yellowish patches above only, or a small circle of brown below only at the centre of the isolate. These colours usually appearing before, sometimes after growth has ceased. Some (less than 50%) cultures producing brown, sometimes dark brown stromatic lines at the edge of their growth, more diffuse than the black lines of *L. pinastri* cultures, these brown lines appearing a little behind the outermost hyphae. Throughout the growth period the hyphae branch much further behind their tips than in cultures of *L. pinastri*, giving the colony a more fluffy appearance. In some colonies when young the hyphae tend to grow in one particular direction giving the colony a swirling appearance reminiscent of pictures of galaxies. Conidia rarely, often not produced in vitro, the same size as those produced in vivo.

*Specimens examined* (on secondary needles only): *Lophodermium conigenum* folder IMI including 225121 (Tarland, Aberdeenshire, Scotland), 225122 (Strathdon, Aberdeenshire, Scotland), 225123 (Glenlivet, Banffshire, Scotland), 225124 (Feshie, Invernesshire, Scotland), 225126 (Raemoir, Aberdeenshire, Scotland), 225127 (Durriss, Aberdeenshire, Scotland), 225125 (Weeting Heath, Norfolk, England), 225128 (Michigan, U.S.A.): Fries Scl. Suec. No. 30 at E and K (sub *Lophodermium* (*Hysterium*) *pinastri*; *Lophodermium conigenum* folder, Forestry Dept, Aberdeen University (many collections on *Pinus sylvestris* from Great Britain and abroad).

Most collections of *L. conigenum* in herbaria are wrongly identified as *L. pinastri*. Hilitzer's original description is of a fungus on cones of *Pinus sylvestris* and *P. uliginosa* Neumann (now *Pinus uncinata* var. *rotundata* (Link) Antoine). Enquiries have revealed that this specimen is not present at Prague (PR) nor in Czechoslovakian regional museums and was probably destroyed during the last war. Examination of *Lophodermium* fruiting on cones of *Pinus sylvestris* collected from the type and many other areas shows that three of the four species which fruit on secondary needles can be found fruiting on cones, the exception being *L. pini-excelsae*. Moreover these three may be distinguished by the same criteria as on needles, i.e. manner of embedding in the host tissue and production of stromatic lines. *Lophodermium conigenum* is described by Hilitzer as being partly subepidermal (thus eliminating *L. seditiosum* which is wholly subepidermal) and as having ascocarps

1.5–2 mm long (thus eliminating *L. pinastri* whose ascocarps are much smaller). The question of selecting a lectotype is best resolved in a paper dealing in detail with the morphology of *Lophodermium* on cones. Cultural identity has been established between *L. conigenum* on cones and secondary needles. Cultures from secondary needles corresponding to Type 2 of Stephan (1973) have been deposited as IMI 156240 and ATCC 28346.

**LOPHODERMIIUM PINI-EXCELSAE** Ahmad, *Sydowia* 8, 172 (1954). (Figs. 1C, 2A, 4).

*Ascocarps* amphigenous on needles (more than 20% on adaxial side), when wet black, when dry black in the centre for more than half the total ascocarp surface, the remainder grey surrounded by a black line (Fig. 1C). Lips always grey. Ascocarps 350–800  $\mu\text{m}$  long (Fig. 4), covered by the host epidermis on either side, but above the epidermis in the centre (Fig. 2A). In mid-point transverse section more than five epidermal cells can be seen in a group on the ascocarp floor. Immature ascocarps similar in development to those of *L. pinastri*, overmature ascocarps tending to lose the fungal lips, otherwise similar to mature ascocarps. Ascocarps accompanied by few thin black lines running across the needle. *Asci* cylindrical, 8-spored, 80–130  $\mu\text{m}$  long, 10–12  $\mu\text{m}$  wide. *Paraphyses* filiform, tips neither swollen nor hooked, as long as the asci. *Ascospores* filiform, straight in the ascus, 50–75  $\mu\text{m}$  long, about 2  $\mu\text{m}$  wide, enveloped in a gelatinous sheath. *Conidiomata* pycnidial, subepidermal, indistinct, rarely coalescing, 150–300  $\mu\text{m}$  long. *Conidia* bacillar (5)–8–12  $\mu\text{m}$  long. Cultures indistinguishable visually from those of *L. pinastri*, but producing longer conidia which are the same as those produced in vivo.

*Specimens examined*: *Lophodermium pini-excelsae* folder IMI, on *Pinus excelsa*: 226109 (Chakar, Muza-ffarafal, Pakistan, det. Ahmad). On *P. sylvestris*: 225067 (Deskens Farm, Oregon, U.S.A.), 225063 (Strathdon, Aberdeenshire, Scotland), 225062 (Rae-moir, Aberdeenshire, Scotland), 224111 (Mamhead, Exeter, England). *L. pini-excelsae* folder FPF., 4497, 4437 (both from Lincoln Co., Oregon, U.S.A.).

Most collections of *L. pini-excelsae* in herbaria are wrongly identified as *L. pinastri*. Ahmad's original description is of a fungus on the needles of a *Haploxylon* (five needle) pine, with ascocarps far shorter than those of *L. pinastri*. This fungus is common and readily identifiable on needles of haploxylon pines and is shorter than *L. pinastri* which, when it fruits on haploxylon pines, retains its normal length. On *Pinus sylvestris*, *L. pini-*

*excelsae* is less common but can be recognized by the characteristic grey lips and very small size.

**Lophodermium seditiosum** sp. nov. (Figs. 1D, 2C, 3C, 4)

*Etymologia*: Epitheton 'seditiosum' duobus causis lectum est. Primum quod haec seditio arboribus solet esse species. Deinde ut fama celebretur Sed Sanders, indianus quidam ebriosus qui ex loco experimentalis (in quo primum scilicet inventa haec erat species) auctorem (J.M.S.) expellere temulenter conatus est.

*Ascocarps* in acubus amphigenis, 800–1600  $\mu\text{m}$  long., cellulis acuum epidermalibus omnino tectis, udo statu nigris, griseis autem statu sicco et lineola nonnumquam circumscriptis nigra. Labiis plerumque vel hyalinis vel viridibus, nonnumquam etiam caeruleis. Lineis stromaticis diffusis fuscis transversalibus infrequentius acus transeuntibus illas in quibus videantur ascocarpi. *Asci* cylindricis, octosporis, 140–170  $\mu\text{m}$  long., 11–13.5  $\mu\text{m}$  lat. *Paraphyses* filiformibus, apice saepe rectis et tenuibus, rare clavatis vel uncinatis, ascis aequilongis. *Ascospores* filiformibus, 90–120  $\mu\text{m}$  long., mucro involutis gelatinoso et saepe versus asci apicem convolutas ostendentibus spirales. *Conidiomata* pycnidialibus subepidermalibus saepe coalescentibus, 300–500  $\mu\text{m}$  long. *Conidiis* bacillaribus, 6–8  $\mu\text{m}$  long.

*Ascocarps* amphigenous on needles (less than 20% on adaxial side), when wet black, when dry grey and surrounded sometimes by a black line (Fig. 1D). Lips hyaline, green or sometimes blue. Ascocarps 800–1500  $\mu\text{m}$  long (Fig. 4), totally covered by the host epidermis (Figs. 2C, 3C). Immature ascocarps appearing gradually as a grey area on the needle, overmature ascocarps tending to lose the fungal lip cells, otherwise similar to mature ascocarps. Ascocarps sometimes accompanied by infrequent diffuse brown stromatic lines crossing the needle. *Asci* cylindrical, 8-spored, 140–170  $\mu\text{m}$  long, 11–13.5  $\mu\text{m}$  wide. *Paraphyses* filiform, tips usually straight and unswollen, occasionally swollen or hooked, as long as the asci. *Ascospores* filiform, 90–120  $\mu\text{m}$  long, wider than those of *L. pinastri*, sometimes coiled spirally at the upper end of the ascus, enveloped in a gelatinous sheath. *Conidiomata* pycnidial, subepidermal, often coalescing, 300–500  $\mu\text{m}$  long. *Conidia* bacillar 6–8  $\mu\text{m}$  long.

*Habitat* on needles attached to living or dead branches, also on cones.

Cultures on 2% Malt Agar initially fast-growing, an increase in diam of 4 mm/day being common, but eventually slowing down. Growth invariably terminating before the edge of the Petri dish (90 mm diam) is reached. Cultures indistinguishable from those of *L. conigenum* when young, later (as lateral growth terminates) browner

above and below, and less fluffy, often so brown that the brown diffuse stromatic lines round the culture cannot be distinguished from the centre. Occasionally cultures producing no brown occur, and these are indistinguishable from those of *L. conigenum*. Conidia sparsely produced in vitro, the same size as those produced in vivo. Collections of *L. seditiosum* can be found in many herbaria under the name *L. pinastri*. Cultures correspond to Type 3 of Stephan (1973) and have been deposited as IMI 156239 and ATCC 28345.

*Specimens examined: Lophodermium seditiosum* folder IMI including 225142 (Gray's Harbor Co., Washington, U.S.A., Sept. 1973, Millar & Staley) Type, 225140 (Rhum, Scotland), 225138 (Brimmond, Aberdeen, Scotland), 225139 (Brimmond, Aberdeen, Scotland), 225068 (Thurston Co., Washington, U.S.A.), 225070 (Lincoln Co., Oregon, U.S.A.), 225072 (Gray's Harbor Co., Washington, U.S.A.), 214290 (Dunwich Forest, Suffolk, England), 17514 (Woodbury Common, Devon, England); *Lophodermium seditiosum* folder, Forestry Dept, Aberdeen University (many collections on *Pinus sylvestris* from Great Britain and abroad). *Lophodermium seditiosum* folder FPF 4436 (isotype).

A paper describing the ecology and biology of *L. pinastri*, *L. conigenum* and *L. seditiosum* is in preparation. Part of the work was supported by a

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