

## *Sphinctrina paramerae*, a new Mediterranean lichenicolous species with non-septate spores

D. MUÑIZ, E. LLOP and N. L. HLADUN

**Abstract:** The new species, *Sphinctrina paramerae*, is proposed. Characteristics of ascospores (size, morphology and ornamentation), apothecia (morphology, size and chemical reactions) and ecological features revealed significant differences between *S. paramerae* and the currently known species of *Sphinctrina* with non-septate ascospores. The new species grows on *Pertusaria paramerae*, epiphytic on the bark of *Juniperus thurifera*, which occurs on well-exposed areas of parameras in the Iberian high plateau and other montane Mediterranean areas.

**Key words:** calicioid fungi, lichenicolous fungi, *Mycocaliciales*, *Pertusaria*, *Sphinctrinaceae*

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### Introduction

*Sphinctrina* Fr. was described by E. Fries (1825, 1828) and placed among the fungi. Later, Th. M. Fries (1861) relocated the genus, including it with other genera of *Caliciales* s. lat. such as *Calicium* Ach. or *Chaenotheca* (Th. Fr.) Th. Fr. Currently, *Sphinctrina* is placed in *Sphinctrinaceae*, together with *Pyrgidium*, according to phylogenetic analyses using molecular markers (Wedin & Tibell 1997; Tibell & Wedin 2000; Tibell & Vinuesa 2005).

*Sphinctrina* was characterized by the shape of the capitulum, size of asci and spores, and the lack of a well-developed thallus. Löfgren & Tibell (1979) recognized five species, four of them with non-septate spores [*S. anglica* Nyl., *S. leucopoda* Nyl., *S. tubiformis* A. Massal. and *S. turbinata* (Pers. ex Fr.) de Not.] and one with 1-septate spores (*S. prorrectula* Nyl.). Recently, three additional species of *Sphinctrina* with 1-septate spores have been recognized: *S. ophioparmae* Kalb, *S. benmargana* Selva, and *S. palidella* (Willey) Selva (Kalb 2001; Selva 2004).

The four species of *Sphinctrina* hitherto known from the Iberian Peninsula have non-septate spores (Hladun & Llimona 2007). However, several specimens previously identified as *S. tubiformis* or *S. turbinata* differ in terms of ecology and morphology. In addition to Iberian samples, similar specimens from other Mediterranean areas do not fit into currently described species of *Sphinctrina*. Therefore, a new species, *Sphinctrina paramerae*, is proposed to include all these samples.

### Materials and Methods

The material of *Sphinctrina* examined includes Mediterranean and non-Mediterranean specimens. Fresh material was collected in several localities from the Iberian Peninsula, and is kept in the herbarium BCN. Dry material from several European herbaria (GZU, LEB, MACB, MA-LICH, MU, SALAF, SANT, UPS and VAB-LICH) was examined.

Thin and ultrathin sections of apothecia were examined using light microscopy. Thin sections of apothecia in calcium alginate were obtained using a freezing microtome. The inclusion in calcium alginate facilitates handling and orientation of the apothecia. Some sections were stained with lactic blue and cleaned with distilled water. Sections were dehydrated and fixed in DPX. Ultrathin sections were prepared at the Serveis Científicotècnics, Parc Científic, Universitat de Barcelona. Sections were stained with a 1% solution of methylene blue and 1% solution of sodium tetraborate and then heated for 30–60 s at 90°C.

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Scanning electron microscope (SEM) examinations were carried out after fixing the samplers on a glue surface and coating with gold.

Chemical tests of apothecia were made on squash preparations mounted in water, applying a 10% aqueous solution of potassium hydroxide (K). Other reacting agents (nitric acid or bleach) were also used, but no reactions were obtained.

Measurements of apothecium height, capitulum width, stalk width, ascus length and width, and ascospore length and width follow Löfgren & Tibell (1979): (absolute minimum)  $a-b$  (absolute maximum), where  $a$  represents arithmetic mean minus one standard deviation, and  $b$  represents arithmetic mean plus the standard deviation. Extreme values, arithmetic mean ( $\chi$ ), standard deviation ( $\sigma$ ) and number of measurements ( $n$ ) are given within parentheses.

Measurements of height and width of apothecia were made under a binocular lens on dry material. At least ten measurements of each sample were recorded when the material allowed.

Ascospore length, width, and length-breadth ratio were recorded from 38 specimens of *S. turbinata*, 9 of *S. anglica*, 10 of *S. leucopoda*, 12 of *S. tubiformis*, and 8 of *S. paramerae*, material permitting. All measurements were made with a  $\times 100$  (1.80 NA) oil immersion lens on water-mounted squash material. At least twenty spores of each specimen were drawn with the aid of a drawing tube, avoiding irregular morphology and immature spores with a thick gelatinous coat. All the measurements were made using the ImageJ program (Abramoff *et al.* 2004). The mean of these measurements were used in all subsequent statistics to represent the specimens. Data did not fulfil requirements of normality and homogeneity of variance. A Kruskal-Wallis test including all species, as implemented in SPSS for Windows (version 14, SPSS Inc., Chicago, IL), was performed for each three characters to check the differences between species. As the Kruskal-Wallis tests show differences between the species for each character, a pairwise Mann-Whitney test was carried out for the three variables between species. It was assumed that individual specimens had been classified to species *a priori*. This classification was achieved using excipular structure, K reaction, presence or absence of a stalk, habitat, spore ornamentation and spore shape, among other characteristics. Graphical representations were carried out using R (R Development Core Team 2004).

### The Species

#### *Sphinctrina paramerae* Muñiz & Hladun *sp. nov.*

MycoBank No: MB 564705

Lichen parasiticum insidens *Pertusaria paramerae*. Apothecia exigua, 0.15–0.50 mm alta. Stipia non manifesta. Capitula sphaeroidea, parum depressa, nitida nigra praeter orbis margino albo. Excipulum spadiceum vel

hyalinum cum margino xerampelino. Hypothecium brunneum. Asci (57.6) 63.4–74.2(80.6)  $\times$  (7.2)8.1–9.5(10.1)  $\mu\text{m}$  lati, cylindrici. Sporae non septatae, fuscae, sphaeroideae vel ellipsoideae, (7.9)8.9–11.7(14.4)  $\times$  (6.5)7.8–9.3(10.7)  $\mu\text{m}$ , ornamiento parum rugoso, involucri gelatinoso parum manifesto.

Typus: Spain, Catalonia, Tarragona, Terra Alta, Serra dels Ports, Arnes, Barranc del Grevolar, 31TBF7125, 800–900 m, on *Pertusaria paramerae* on *Juniperus phoenicea*, 20 October 1990, M. Boqueras (holotypus—BCC-Lich 6174).

(Fig. 1)

*Apothecia* sessile, (0.15)0.21–0.42(0.50) mm high, black or seldom dark brown. *Capitulum* (0.21)0.32–0.36(0.41) mm broad, spherical to vertically compressed, shining black, usually with a colourless excipular rim caused by loosely interwoven hyaline hyphae protruding beyond the coloured tissues. Mazedium black, well-developed. *Excipulum* with an outer scleroplectenchymatous layer (Yoshimura & Shinada 1980), consisting of isodiametric to ovoid cells with thick gelatinous walls reddish brown to pale brown, almost hyaline inside; inner layer prosoplectenchymatous of periclinally arranged hyaline hyphae, 6–10  $\mu\text{m}$  wide. *Hypothecium* medium to pale brown in section, formed by an upper layer of periclinally arranged thin hyphae and paraplectenchymatous below, cells with thin walls. *Asci* cylindrical, 8-spored, (57.6)63.4–74.2(80.6)  $\times$  (7.2)8.1–9.5(10.1)  $\mu\text{m}$ . *Sporae* uniseriate, non-septate, dark brown, elliptical to subspherical, (7.9)8.9–11.7(14.4)  $\times$  (6.5)7.8–9.3(10.7)  $\mu\text{m}$ , (length:  $\chi = 10.02$ ,  $\sigma = 1.05$ ,  $n = 98$ ; width:  $\chi = 8.57$ ,  $\sigma = 0.76$ ,  $n = 98$ ). The distal and proximal ascospores have an ovoid shape. *Ornamentation* indistinct under the light microscope, slightly rugose in SEM. *Spore coat* very thin, seldom distinct in water.

*Pycnidia* not observed.

*Chemistry.* All reddish pigmented structures react with K intensifying the reddish brown colour. The pigment dissolves and stains the adjacent hyaline structures with a yellowish tinge.

*Etymology.* The name *paramerae* refers to the host, *Pertusaria paramerae* Crespo & Vězda.

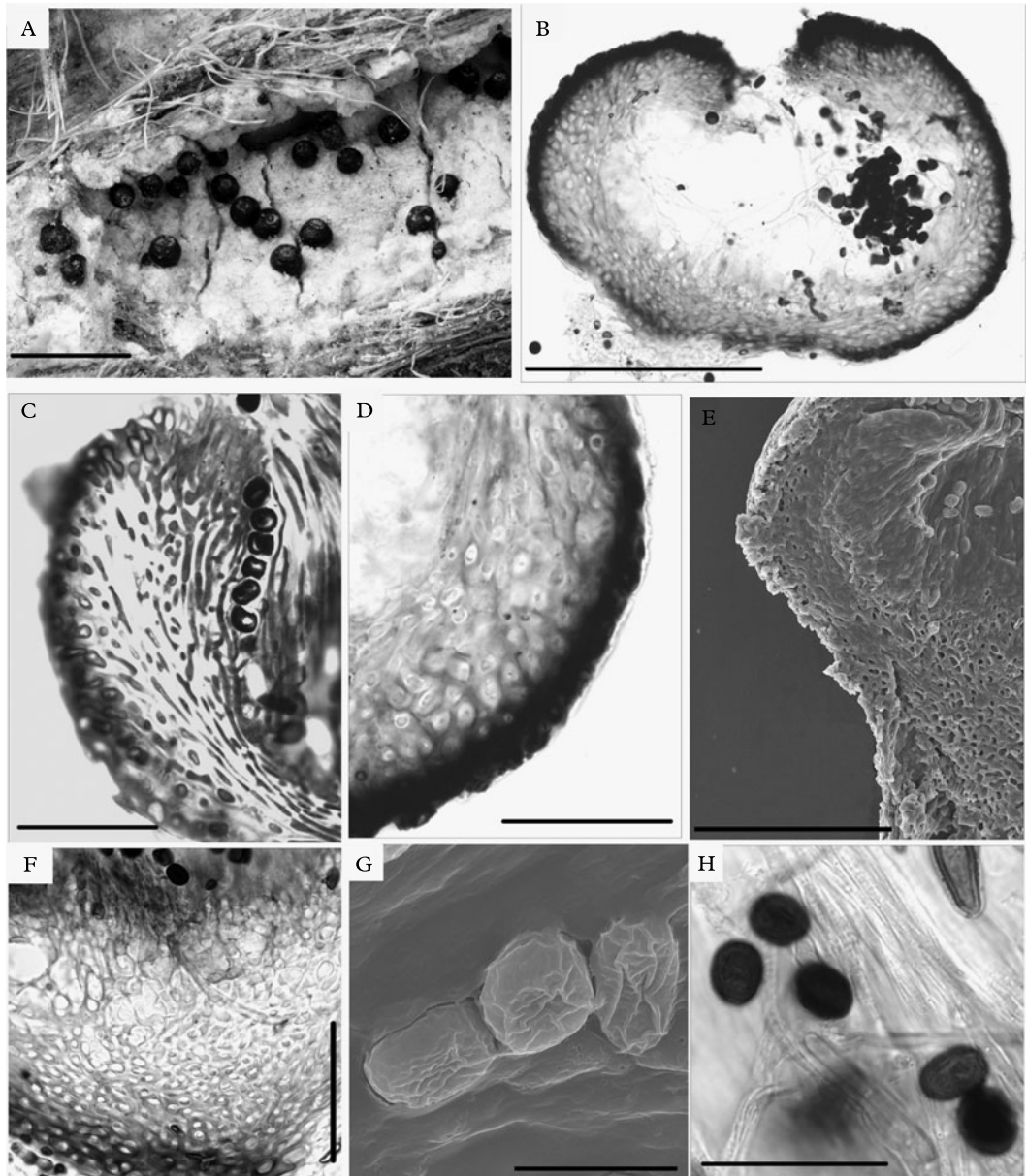


FIG. 1. *Sphinctrina paramerae*. A, habitus (BCN 2933); B, section of apothecium (UPS 41694); C, ultrathin section of exciple stained with methylene blue (MA 11266); D, detail of excipulum (UPS 41694); E, section of apothecium (SEM) (BCN 6174, holotype); F, detail of hypothecium (UPS 143231); G, spore ornamentation (SEM) (BCN 6174, holotype); H, spore ornamentation (light microscope) (BCN 6174, holotype). Scales: A = 1 mm; B, E = 100  $\mu$ m; C, D & F = 50  $\mu$ m; G = 10  $\mu$ m; H = 20  $\mu$ m.

**Ecology and habitat.** *Sphinctrina paramerae* grows on *Pertusaria paramerae*. The host grows as an epiphyte on *Juniperus thurifera* in the most exposed areas of juniper forests of the Iberian high plateau (paramera). These

habitats are characterized by a high environmental summer humidity and winter drought, with temperatures between 8–17°C at altitudes ranging between 800–1300 m. *Pertusaria paramerae* also occurs on *J. phoeni-*

cea and *f. oxycedrus* at lower altitudes (Crespo & Vězda 1985).

**Distribution.** *Sphinctrina paramerae* ranges from the temperate mesomediterranean to cold supramediterranean belts of the Iberian Peninsula, Morocco, Libya, Turkey and the FYROM.

**Additional specimens examined.** **Libya:** *Cyrenaica:* 65 km WSW of Derna, 24 km SW of Lamludah, on stems of *Juniperus phoenicea* in open *f. phoenicea* forest, 13 iii 1983, *G. Thor* [BCN 2933, UPS 41694 sub. *Sphinctrina turbinata* (Pers. Fr.) de Not.].—**Morocco:** Subiendo El Tigri-Mighkit, on *Pertusaria* sp., 11 iv 1987, *A. Gómez-Bolea* (BCN).—**Spain:** *Cuenca:* Serranía de Cuenca, Cotillas, 30TWK8738, 1200 m, on *Pertusaria paramerae* on *Juniperus thurifera*, 14 iii 1998, *G. Aragón & I. Martínez* (MA-Lichen 11249 sub. *Sphinctrina tubiformis* A. Massal.); Hoya de la Madre de las Latas, 30TWK9238, 1350 m, on *Pertusaria paramerae* on *Juniperus thurifera*, 14 iii 1998, *G. Aragón & I. Martínez* (MA-Lichen 11266 sub. *Sphinctrina tubiformis* A. Massal.). *Tarragona:* Terra Alta, Serra dels Ports, Arnes, Barranc del Grevolar, 31TBF7125, 800–900 m, on *Pertusaria paramerae* on *Juniperus phoenicea*, 20 x 1990, *M. Boqueras* [BCN 6174 sub. *Sphinctrina turbinata* (Pers.) De Not.].—**Turkey:** *Antalya:* Kaşa, transitus Sinekibeli Geçidi in montibus Akdaglari, alt. 1500 m s.m., ad lignum trunci *Juniperus* sp. in thallo *Pertusariae* sp. vicens, 11 iv 1996, *A. Vězda* (UPS 143231).—**FYROM:** *Makedonien:* Galičica, Paß zw. Oteševo und Trpejca, Paßhöhe, um 1600 m, W-exponierte, auf *Juniperus* sp., 26 viii 1976, *H. Mayrhofer* (GZU sub. *Sphinctrina cf. turbinata*).

## Discussion

*Sphinctrina paramerae* differs from other species of *Sphinctrina* with non-septate ascospores by having non-stalked apothecia. In addition, morphological features of ascospores supply good diagnostic characters for distinguishing between Mediterranean species of *Sphinctrina* (Table 1). *Sphinctrina paramerae* externally resembles *S. turbinata* and *S. tubiformis* (Fig. 1). However, *S. paramerae* can be easily separated from these species by anatomical characteristics of the apothecia, spore shape and spore size (Table 1). The main feature distinguishing *S. paramerae* from *S. tubiformis* is spore shape, the latter having almond-shaped verrucose ascospores. Many of the specimens of *S. paramerae* were previously misidentified as *S. turbinata*. Both species have a reddish pigment in the exciple that reacts with KOH; however, it turns red-

dish brown in *S. paramerae* and purple in *S. turbinata*. *Sphinctrina paramerae* has elliptical to subspherical spores, longer and wider than in *S. turbinata* (Fig. 2, Table 2). *Sphinctrina paramerae* also differs in the lack of a well-developed outer gelatinous spore coat which is present in *S. turbinata*. In addition, ascospores of *S. turbinata* have a distinct verrucose ornamentation, while *S. paramerae* has an irregularly ridged ornamentation.

*Sphinctrina paramerae* could also be mistaken for *S. anglica* since spore dimensions are rather similar, although they are significantly longer in *S. paramerae* (Fig. 2, Table 2). The presence of a dotted ornamentation in the spores of *S. anglica* also distinguishes this species from *S. paramerae*. Additionally, *S. paramerae* differs from *S. anglica* in that the latter has stalked apothecia with a very different anatomy (Table 1). *Sphinctrina paramerae* differs from *S. leucopoda* in all the considered characters except the ornamentation of the spore, which consists of irregular ridges as seen in SEM and is almost smooth under light microscopy in both species.

Features on the morphology of ascospores of the species of *Sphinctrina* are useful as diagnostic characters. The presence or absence of a septum distinguishes two main groups of species. Among the species with non-septate spores, ascospore shape distinguishes *S. tubiformis*, with almond-shape ascospores from the species with subspherical to ellipsoidal ascospores: *S. anglica*, *S. leucopoda*, *S. turbinata* and the new species *S. paramerae* (Table 1). The comparison of spore size features of species with non-septate ascospores showed significant differences in terms of length and width (Table 2), the ascospores of *S. paramerae* being largest among the species with subspherical to ellipsoidal ascospores (Fig. 2). Among the species of *Sphinctrina* with non-septate ascospores, *S. leucopoda* and *S. turbinata* are the closest in terms of ascospore size (Fig. 2, Table 2). Both species can be separated by the colour of the apothecium. *Sphinctrina turbinata* has an unidentified crimson pigment reacting strikingly purple with K.

TABLE 1. Comparison of main morphological characters of non-septate *Sphinctrina* species.

	<i>S. anglica</i>	<i>S. leucopoda</i>	<i>S. tubiformis</i>	<i>S. turbinata</i>	<i>S. paramerae</i>
Apothecium height	(0.21) 0.27–0.37 (0.46) mm	(0.08) 0.20–0.41 (0.38) mm	(0.18) 0.20–0.29 (0.33) mm	(0.49) 0.37–0.23 (0.13) mm	(0.15) 0.21–0.42 (0.31) mm
Stalk structure	Dark to medium brown periclinally arranged long hyphae with thick gelatinous coat	Medium to pale brown periclinally arranged hyphae with thick gelatinous coat	Irregular interwoven arranged reddish brown hyphae	Anticlinally arranged pale crimson hyphae ending in isodiametric cells	–
Capitulum width	(0.10) 0.14–0.21 (0.31) mm	(0.08) 0.16–0.32 (0.38) mm	(0.10) 0.15–0.20 (0.23) mm	(0.33) 0.28–0.18 (0.10) mm	(0.21) 0.32–0.36 (0.41) mm
Excipulum structure	Periclinal layers of highly sclerotized dark brown isodiametric cells	An inner dark brown layer of sclerotized periclinal hyphae and an external layer of isodiametric cells	An inner layer of poorly sclerotized, dark to reddish brown periclinal hyphae and outer layer of anticlinally arranged hyphae	Highly sclerotized crimson wall and inner layer of periclinally arranged reddish hyphae	An inner layer of brown to hyaline periclinally arranged hyphae and an outer layer of reddish brown isodiametric to ovoid cells.
Hypothecium colour	Medium to pale brown	Hyaline	Hyaline	Pale brown	Medium to pale brown
Spore shape	Subspherical	Subspherical	Almond-shape	Subspherical	Subspherical to elliptical
Spore size	(7.4)8.3–10.1(12.9) × (7.0)7.8–8.9(10.0) μm	(4.7)5.4–6.2(7.2) × (4.5)5.0–5.8(6.3) μm	(8.7)10.9–14.0(19.1) × (4.5)6.2–7.8(9.2) μm	(5.3)6.2–7.2(8.8) × (4.6)5.7–6.6(7.4) μm	(7.8)8.9–11.1(12.9) × (7.1)7.8–9.3(10.7) μm
Spore ornamentation	Dotted	Irregularly ridged	Striated	Verrucose	Irregularly ridged
Spore coat	Hard to distinguish in water	Well-developed	Hard to distinguish in water	Well-developed	Hard to distinguish in water
KOH reaction	–	–	–	+ (striking purple)	+ (slightly reddish brown)

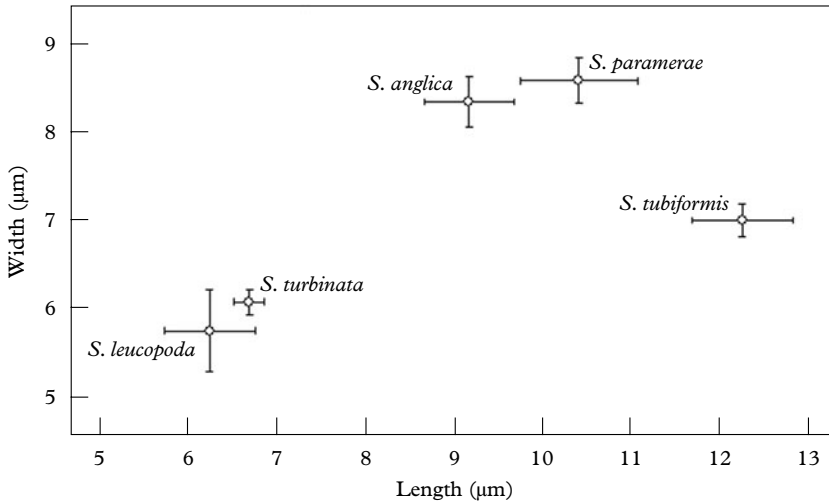


FIG. 2. Comparison of spore length and width of *Sphinctrina* species with non-septate spores, represented as the mean value (circle) and 95% of confidence for each variable (whiskers).

TABLE 2. Pairwise comparison of the species included in the non-septate group of *Sphinctrina* based on ascospore characters, using a Mann-Whitney test.

		Length	Width	Length-breadth ratio
<i>S. anglica</i>	<i>S. leucopoda</i>	*	*	ns
	<i>S. paramerae</i>	*	ns	*
	<i>S. tubiformis</i>	*	*	*
	<i>S. turbinata</i>	*	*	ns
<i>S. leucopoda</i>	<i>S. paramerae</i>	*	*	*
	<i>S. tubiformis</i>	*	*	*
	<i>S. turbinata</i>	ns	ns	ns
<i>S. paramerae</i>	<i>S. tubiformis</i>	*	*	*
	<i>S. turbinata</i>	*	*	*
<i>S. tubiformis</i>	<i>S. turbinata</i>	*	*	*

\* significant at  $P < 0.05$ ; ns indicates no significant differences.

**Key to the Mediterranean non-septate species of *Sphinctrina***

- 1 Ascospores almond-shaped . . . . . **S. tubiformis**
- Ascospores subspherical to ellipsoid . . . . . 2
- 2(1) Ascospores 4.0–6.5 µm in length . . . . . 3
- Ascospores 7–12 µm in length . . . . . 4
- 3(2) Apothecia K+ purplish . . . . . **S. turbinata**
- Apothecia K- . . . . . **S. leucopoda**
- 4(2) Apothecia stalked, ascospores with a well-developed dotted ornamentation; exciple brown to dark brown in section, highly sclerotized . . . . . **S. anglica**
- Apothecia sessile; ascospores smooth or with irregularly ridged ornamentation; exciple colourless to pale brown in section, not sclerotized . . . . . **S. paramerae**

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