

Zornitsa Stoyanova¹
Dimitar Stoykov²

Records of *Naemacyclus* and *Stictis* (Ascomycota) from Bulgaria

Authors' addresses:

¹Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Correspondence:

Dimitar Stoykov

²Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113, Sofia, Bulgaria
Phone: +359 29793715
e-mail: stoykovdimitar@abv.bg

Article info:

Received: 11 February 2022

Accepted: 12 July 2022

ABSTRACT

Naemacyclus fimbriatus is reported for the second time from the Balkan Peninsula, observed on old pine cones from collections in the Vitosha region (Vitosha and Plana Mts) and Rila Mts. *Stictis radiata* is recorded in Bulgaria on the bark of a living *Platanus* tree in Belasitsa Mts. The materials, examined under light microscopes, are presented with brief descriptions and colour illustrations. Notes on the known distribution and ecology are included.

Key words: Bulgarian mycota, Lecanoromycetes, Ostropomycetidae, *Platanus*, Scots pine

Introduction

Members of order *Ostropales* s.l. are a large group of ascomycetes with microscopic fruitbodies, and unite both lichenized and non-lichenized fungi (Wedin et al., 2004, 2006; Thiagaraja et al., 2021; Wei et al., 2021). Family *Stictidaceae* was named after the genus *Stictis* Pers. This genus was at first described with 'immersed globose or subglobose apothecia, having annular excipulum, friable below, heterogenous, and surrounded by an asterisk-like margin, ascigerous, later globoid, gelatinous-waxy and fluffy coagulated, on deciduous wood' (Fries, 1849). Species of *Stictis* have been confirmed as members of *Stictidaceae* family by using combining updated data for two molecular markers, continuous nuclear ITS and LSU rDNA, and mtSSU rDNA alignments of genes (Wedin et al., 2006).

The first report of *Stictis* from Bulgaria can be found in Barzakov (1931), with the collection of *Stictis mollis* Pers. on *Corylus avellana* L. Genus *Naemacyclus* Fuckel is known with more than six species worldwide (Kirk et al., 2008). *Naemacyclus fimbriatus* was originally described from Philadelphia by Schweinitz (1834) as *Stictis fimbriata*, with white to gray apothecial discs formed in groups on the inner side of the strobili scales of Jersey Pine (*Pinus inops*). From the Balkans, *N. fimbriatus* was known in Turkey on squamules of fallen female cones from *Pinus nigra* (Erdoğan et al., 2017).

The aim of the present work is to study and illustrate two species from the genera *Naemacyclus* and *Stictis*, both known from the materials collected in Bulgaria

Materials and Methods

The following work is based on the examination of specimens on dead pine cone scales, housed at the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (SOMF), and on a single collection of pieces of bark from living *Platanus orientalis* tree, found in April 2013 from Belasitsa Mts. The macro- and microphotographs were made 'ex-situ', with the help of digital cameras Canon PS A1400 HD and Canon IXUS 175 HD on Boeco B-3500 dissecting stereomicroscope, Boeco BM-180 T/SP LM, and Olympus BX-41 LM, on dry materials, from water mounts, or mounted in aqueous lactophenol; the reaction of the subhymenial layer was tested in iodine (IKI solution) – recipes after Kirk et al. (2008). The ascomata discs were cross-sectioned with a razor blade and were studied in water. The determination of the materials was justified generally after the studies of Sherwood (1977), Dennis (1978), DiCosmo et al. (1984), Breitenbach & Kränzlin (1984), Medardi (2012), and Konoreva et al. (2016). Measurements of the discs, asci or ascospores were made from jpeg image files with the help of specialized software for digital images Carnoy 2.0 © Peter Schols 2001 (Schols & al.,

2002). The resultant data are presented in the descriptions of the fungal characters in the form: of minimum–maximum values [mean \pm SD], n, where ‘n’ - denotes the number of measurements taken.

Results and Discussion

Lecanoromycetes

Ostropomycetidae

Ostropales

Stictidaceae

Naemacyclus fimbriatus (Schwein.) DiCosmo, Peredo & Minter, Eur. J. For. Path., 13(4): 207 (1983) (Figures 1-6)

Stictis fimbriata Schwein., Trans. Amer. Philos. Soc., n. s. 4(2): 179 (1834).

Apothecia in the form of small discs, at first immersed in plant tissues in the form of a pore, and rising the overlying host tissues, later cracking the host periderm, up to 300–500 (–600) μm in diameter, \pm globose, single, usually scattered or united in small groups, black, dark gray, with whitish margins. **Subhymenium** hyaline. **The hymenium** is about 100–120 μm thick. **Pseudoparaphyses** 1–1.5 μm wide, elongate, hyaline. **Asci** 60–80 \times 8–10 μm [$67.2 \pm 11.4 \times 9.1 \pm 0.5$], $n_1=10$, clavate to oblong fusiform, sometimes with a short stipe, not blueing in iodine (IKI solution). **Ascospores** (45–) 55–60 (–70) \times 2–2.5 (–3) [$58.0 \pm 5.8 \times 2.35 \pm 0.23$] μm , $n_1=15$; (45–) 55–60 (–70) \times 2–2.5 [$61.1 \pm 7.7 \times 2.13 \pm 0.17$] μm , $n_2=15$, hyaline, phragmo-scleosporous, 7-septate, straight or slightly curved.

Specimens examined: On the inner side of scales from fallen female cones from *Pinus sylvestris* L. – Vitosha region: Mt Plana, 28 Oct. 1966, B. Alexandrov, SOMF 6045, sub. *Stictis fimbriata* Schwein.; idem., 25 Nov. 1965, Cv. Hinkova, B. Alexandrov, SOMF 5296, sub. *S. fimbriata* Schwein.; idem., alt. 1060 m, 15 Nov. 1967, B. Alexandrov, SOMF 6598, sub. *S. fimbriata* Schwein.; Mt Vitosha, near the village of Chujpetlovo, alt. 1250 m, 28 Apr. 1969, B. Alexandrov, SOMF 6979, sub. *S. fimbriata* Schwein.; Rila Mts, Chaira, alt. 1300 m, 11 May 1954, Cv. Hinkova, B. Alexandrov, SOMF 5098, sub. *Stictis fimbriata* Schwein.

Ecology and distribution. *Naemacyclus fimbriatus* has been reported on needles from *Pinus maritima* Lam. (= *P. pinaster* Aiton), *P. pinaster*, and on female cones and needles of *Pinus brutia* Ten., *P. halepensis* Mill., *P. nigra* Arnold, *P. resinosa* Aiton, *P. rigida* Mill., *P. sylvestris*, *Pinus* sp. (DiCosmo et al., 1983, 1984; Dennis, 1978; Vujanovic et al., 1998; Erdođdu et al., 2017, etc.), with a known area of distribution in Europe (Austria, Germany, Sweden, Turkey), Asia (China), and North America (Canada, USA).

Notes. Dimitrova (1999) gives a thorough list of discomycetous fungi found on needles, cones, and twigs of

coniferous trees in Bulgaria, mentioning also *Nemacyclus* genus without additional description. DiCosmo et al. (1984) reported usually 7-septate ascospores with a size of 75–90 \times 2–2.5 μm . Vujanovic et al. (1998) also described *N. fimbriatus* with 7-septate spores, sized 71–91 \times 2–2.5 μm , a width very closer to the data, received after the examination of the old material (SOMF 6979). A relatively recent find, reported from Turkey by Erdođdu et al. (2017), was described with dark-brown to blackish apothecia, 8-spored, cylindrical and short-stalked asci, sized 85–95 \times 8.5–9.5 μm , and hyaline, slightly curved ascospores with (0-) 3–5-septa, sized (50-) 54–80 \times 2–2.2 μm . However, the records of *N. fimbriatus* from Asia are known to have thinner spores, about 1–1.5 μm wide.

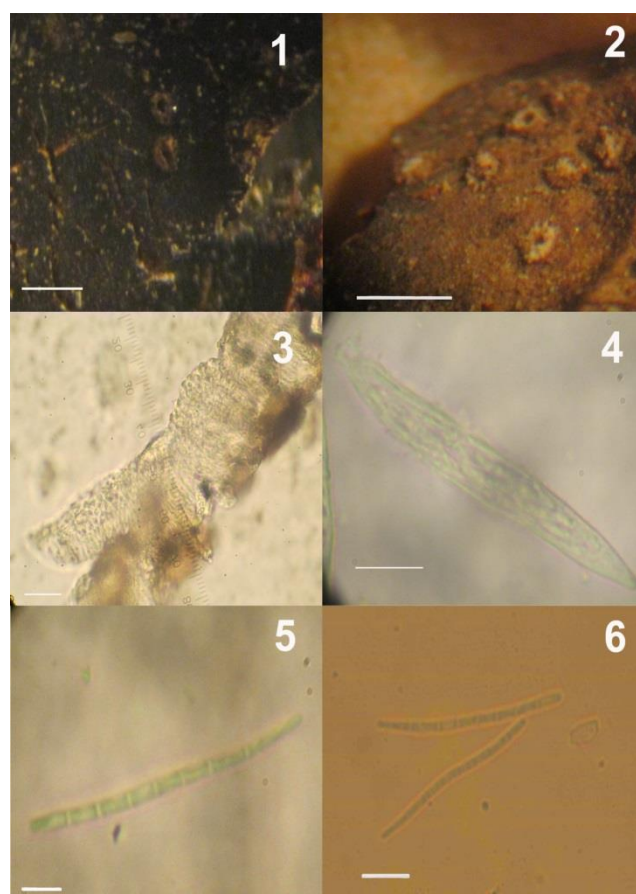


Figure 1-6. *Naemacyclus fimbriatus*:

1. Two apothecia immersed on the inner side of a cone scale of fallen female cone by *Pinus sylvestris*, in close view, SOMF 5098, scale bar = 1 mm;
2. Apothecia with whitish margins, immersed on the inner side of fallen female cone scale of *P. sylvestris*, in close view, SOMF 6979, scale bar = 1 mm;
3. Part of cross section from apothecium, showing details of hymenium and subhymenium, in water, SOMF 5098, scale bar = 100 μm ;
4. Ascus with parallel hyaline spores, in water, SOMF 5296, scale bar = 10 μm ;
5. Ascospore, in water, SOMF 5296, scale bar = 10 μm ;
6. Ascospores, in lactophenol, SOMF 5098, scale bar = 15 μm .

Stictis radiata (L. : Fr.) Pers., Obs. Mycol. 2: 73, 1799 (1800), Syst. Mycol. 2: 194, 1822 (1823) (Figures 7-10)

Schmitzomia radiata (L.) W. Phillips, A manual of the British Discomycetes, p. 379, 1887 (1893); *Schmitzomia radiata* (Pers.) Fr., Summa Veg. Scand., Sect. Post., p. 364 (1849); *Stictis annulata* Cooke & W. Phillips, Grevillea, 9: 8 (1880).

Apothecial disc 375–600 × 500–700 µm in diameter [517 ± 7 × 595 ± 6 µm], $n_1=20$; 185–505 × 195–600 µm in diameter [375 ± 80 × 430 ± 100 µm], $n_2=25$, irregularly globose, with thick white apothecial margin, slightly rising above the substrate surface and subdivided in the form of aster. **Hymenium** immersed in plant tissues, yellowish to ochraceous in living state. **Asci** 150–165 × 6.5–7.5 µm, $n=5$, 8-spored, with parallel colourless spores, in the cluster. **Paraphyses** filiform, about 1.5 µm wide. **Ascospores** 145–150 × 1.5–2 µm, $n=10$, hyaline, filiform, smooth, septate.

Material examined: On a piece of bark from *Platanus orientalis* L. – Belasitsa Mts Blagoevgrad distr., Petrich Municipality, the village of Skrat, N 41°21'33.8", E 22°59'57.1", alt. ca 568 m, 6 Apr. 2013, D. Stoykov.

Note. The lectotype is described on the bark of *Pinus abies* L. (= *Picea abies* (L.) Karst.) from an unknown locality (Wedin et al., 2006), in Germany (Konoreva et al., 2016), while Sánchez Flores et al. (2020) mentioned the type on rotten spruce wood in Sweden.

Known distribution and ecology. Europe (Denmark, Great Britain, Italy, Netherlands, Northern Ireland, Sweden), Asia (India, Japan, Russia), South Africa, Australia, New Zealand, North America (Canada, Mexico, USA), South America (Argentina, Brasil), all data on the distribution followed Phillips (1893), Dennis (1978), Wedin et al. (2006), Medardi (2012), Konoreva et al. (2016), Popov et al. (2020), Sánchez Flores et al. (2020), and the map generated with GBIF.org software about the occurrence of *S. radiata* s.l. worldwide. *Stictis radiata* is recorded in Scandinavia on dead, often decorticated twigs of *Picea abies*, *Populus tremula*, *Salix caprea*, and *Ulmus* or other trees, on wood (Wedin et al. 2006; Konoreva et al., 2016; Popov et al. 2020). Until recently, *Stictis radiata* was considered a rare species, known on the territory of Russia on dead branches from *Picea abies*, *P. excelsa* Link, *P. obovata* Ledeb., and *Populus tremula* with six localities (Konoreva et al., 2016 - a map). However, Popov et al. (2020) expanded the known information on the species ecology and distribution, and considered it as rather common, publishing numerous records on bark and branches of *Populus suaveolens* Fisch., *Picea jezoensis* (Siebold & Zucc.) Carrière subsp. *ajanensis*, *P. orientalis* (L.) Peterm., *Picea* sp., *Pinus koraiensis* Siebold et Zucc., *Populus maximowiczii* Henry, *Salix udensis* Trautv., *Salix* sp., *Sorbus commixta* Hedl., *Sorbus* sp., and *Ulmus laciniata* Trautv. In Scandinavia, *S.*

radiata is known on the bark of *Picea abies*, *Populus tremula* and *Salix caprea* (Wedin et al., 2006).

Comments. The genus *Stictis* was described with apothecial discs having distant periphysoidal layers, without part-spores or thick epithecium in the ascomata (Wedin et al. 2006). It is a cosmopolitan genus, predominantly saprophytic,

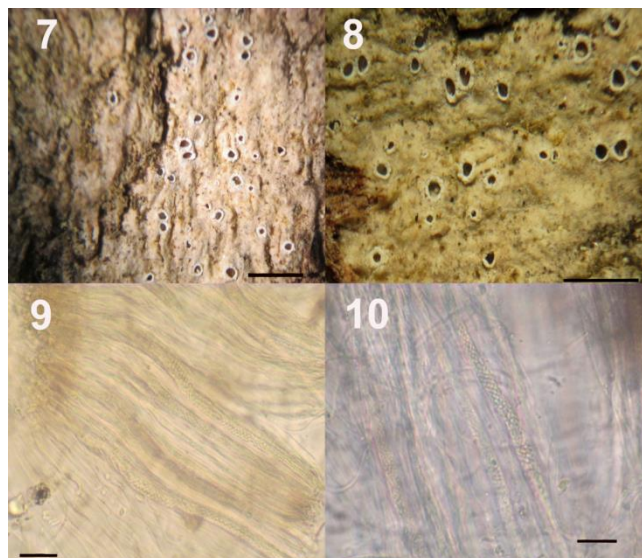


Figure 7-10. *Stictis radiata*:

7, 8. Apothecia immersed in piece of bark from *Platanus orientalis* (ex situ), scale bars = 2 mm;

9. Cross section of hymenium, showing asci with paraphyses, in water, scale bar = 15 µm;

10. Asci and paraphyses, in water, scale bar = 15 µm.

known with more than 68 species and recorded on many substrates like decaying leaves, wood, bark, the stem of herbaceous plants, culms of grasses, and rachises of ferns (Sherwood, 1977; Whitton, 1999; Kirk et al., 2008; Konoreva et al., 2016; Popov et al., 2020). Whitton (1999) included three species of *Stictis*, growing on *Pandanaceae* collected from Asia, Australia, New Zealand, and the Philippines, with *S. pandani* Whitton et al. having 3-septate ascospores. The subhymenium of the members of the genus is often described as consisting of pseudo tissue blueing or not blueing in iodine (Whitton, 1999; Wedin et al., 2006). Wedin et al. (2004) showed that the species of *Stictis* have peculiarities in their lifestyle, where the individuals of the same species have the option to develop as lichen, or as a non-lichenized fungus, depending on the substrate bark type, with most of the observed cases connected to the bark of *Populus tremula* L.

Stictis radiata is known on other twigs and bark, including *Viburnum opulus*, or on leaves and was characterized with asci usually (150–) 160–180 (–200) × 6–10 µm, and hymenium blueing in iodine, ascospores (140–) 150–160 (–220) × (1.5–) 2–2.5 (–3) µm (Rehm, 1896; Schröter, 1908; Breitenbach & Krantzlin, 1984; Wedin et al. 2006; Medardi 2012). Sánchez

Flores et al. (2020) described *S. radiata* with ‘scattered apothecia immersed in the plant tissue, curved white margin and orange-yellow or testaceous hymenium, asci 150–180 × 10–16 µm, ascospores 140–160 × 1.5–2 µm, hyaline, filiform, and multiseptate’.

The only known Bulgarian record of *Stictis mollis* Pers. was based on material from June 8, 1930, and was collected by A. Popnikolov in Mt Ljulin on hazel (Barzakov, 1931), but the corresponding specimen was not conserved in SOMF. *Stictis mollis* and its morphologically adjacent *S. brunescens* Gilenstam et al. are shown to occur on wood as saprotrophs, with larger apothecia, and with smaller ascomata, as lichens on bark, on trees and shrubs mainly from *Populus tremula* and *Salix caprea* L. (Wedin et al., 2006; Popov et al., 2020). Their hymenium is observed with intense blueing in iodine. *Stictis stellata* Wallr., very similar in close view to our find, has apothecial discs about 300–600 µm in diameter and longer asci, occurs basically on stems of herbaceous plants, and can be distinguished by having ascospores nearly 1–1.5 µm, and by the negative reaction of the hymenium in iodine (Rehm, 1896; Dennis, 1978; Breitenbach & Kränzlin, 1984).

Conclusions

In conclusion, a nomenclature revision of the specimens of *Stictis fimbriata* Schwein., collected by Boris Alexandrov and Cvetana Hinkova, housed at the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF) is made. *Naemacyclus fimbriatus* and *Stictis radiata* are described and illustrated from Bulgaria, based on the examined materials of ascomata from fallen female pine cones and on pieces of bark from a living tree.

Acknowledgements

This study was conducted within the framework of ‘Phylogeny, distribution and sustainable use of fungi’ project.

References

- Barzakov B. 1931. New fungi for Bulgaria. *Izv. Bulg. Bot. Druz.*, 4: 44-47. (In Bulgarian)
- Breitenbach J, Kränzlin F. 1984. *Champignons de Suisse*. Tome 1. Les Ascomycètes. – Edition Mycologica, Lucern.
- Dennis RWG. 1978. *British Ascomycetes*. Second Ed. – J. Cramer, Vaduz.
- DiCosmo F, Peredo H, Minter DW. 1983. *Cyclaneusma* gen. nov., *Naemacyclus* and *Lasiosstictis*, a nomenclatural problem resolved. *Eur. J. For. Path.*, 13: 206-212.
- DiCosmo, F, Nag Raj, TR, Kendrick, WB. 1984. A revision of the *Phacidiaceae* and related anamorphs. *Mycotaxon*, 21: 1-234.
- Dimitrova ED. 1999. Discomycetous fungi found in Bulgaria on needles, cones and twigs of conifers. *Phytol. Balcan.*, 5(1): 137-144.
- Erdođdu ME, Dođan G, Hüseyin E, Suludere Z. 2017. A contribution to the study of *Helotiales* and *Rhytismatales* in Turkey. *Mycotaxon*, 132: 885-893.
- Fries EM. 1849. *Summa Vegetabilium Scandinaviae*. Vol. 2. – A. Bonnier. Excudebant Vypograph, Upsaliae, p. 261-572.
- Kirk PM, Cannon PF, David JC, Stalpers JA (eds). 2008. *Dictionary of the fungi*. Ninth Ed. – CAB International, Oxon, UK.
- Konoreva LA, Chesnokov S, Davydov EA. 2016. *Stictis* and *Schizoxylon* (Stictidaceae, Ostropales) in Russia. *Herzogia*, 29(2): 706-711.
- Medardi G. 2012. *Atlante fotografico degli Ascomiceti d’Italia*. – A.M.B. Fondazione Centro Studi Micologici, Vicenza.
- Phillips W. 1893. *A Manual of the British Discomycetes with descriptions of all the species of fungi hitherto found in Britain included in the family and illustrations of the genera*. Second Ed. – Paul Kegan, Trench, Trubner & Co, London.
- Popov ES, Chesnokov SV, Konoreva LA, Ezhkin AK, Stepanchikova IS, Kuznetsova ES, Himelbrant DE, Galanina IA, Tchabanenk SI. 2020. *Botanica Pacifica*, 9(2): 1-8.
- Sánchez Flores M, Valenzuela R, Hernández-Muñoz MA, García Jiménez J, Martínez-Pineda M, Raymundo T. 2020. Ascomicetos del bosque mesófilo de montaña de Honey, Puebla de los Angeles, México. *Acta Bot. Mexicana*, 127: no. e1719.
- Rehm H. 1896. *Die Pilze Deutschland, Oesterreich und der Schweiz*. III. Abt. Ascomyceten: Hysteriaceen und Discomyceten. – In: Winter, G. & Rehm, H. (eds), *Rabenhorst’s Kryptog.-Fl. von Deutschland, Oesterreich und der Schweiz*, Vol. 2. Second Edn, Verlag E. Kummer, Leipzig, p. 175-181.
- Schols P, Dessein S, D’hondt C, Huysmann S, Smets E. 2002. Carnoy: a new digital measurement tool for palynology. *Grana*, 41: 124-126.
- Schröter J. 1908. *Die Pilze Schlesiens*. – In: Cohn, F. (ed.), *Kryptog.-Fl. Schles. Part 2*. J.U. Kern’s Verlag, Breslau, p. 156-157.
- Sherwood MA. 1977. The Ostropalean fungi. *Mycotaxon*, 5(1): 1-277.
- Schweinitz LD. 1834. *Synopsis Fungorum in America Boreali media degentium. Secundum Observationes Ludvici Davidis de Schweinitz*. – Trans. Am. Philos. Soc., n.s., vol. 4. Am. Philos. Soc., James Kay Jun. & Co. Philadelphia, p. 141-316.
- Thiyagaraja V, Lücking R, Ertz D, Karunarathna CS, Wanasinghe DN, Lumyong S, Hyde KD. 2021. The evolution of life modes in *Stictidaceae*, with three novel taxa. *J. Fungi*, 7(2): no. 105.
- Wei D-P, Wanasinghe DN, Gentekaki E, Thiyagaraja V, Lücking R, Lumyong S, Hyde KD. 2021. Morphological and phylogenetic appraisal of novel and extant taxa of *Stictidaceae* from Northern Thailand. *J. Fungi*, 7(10): no. 880.
- Wedin M, Döring H, Gilenstam G. 2004. Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the *Stictis-Conotrema* complex. *New Phytologist*, 164: 459-465.
- Wedin M, Döring H, Gilenstam G. 2006. *Stictis* s. lat. (*Ostropales, Ascomycota*) in northern Scandinavia, with a key and notes on morphological variation in relation to lifestyle. *Mycol. Res.*, 110: 773-789.
- Whitton SR. 1999. *Microfungi on Pandanaceae*. A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Hong Kong. Department of Ecology and Biodiversity, University of Hong Kong, 625 p. [pdf file accessed by request, 25.10.2022, <https://hub.hku.hk>]
- Vujanovic V, St-Arnaud M, Neumann P. 1998. First report of *Naemacyclus fimbriatus* infecting Pitch Pine (*Pinus rigida*). *Plant Disease*, 82(8), p. 959.