PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 12, Part 4, pp. 463–468 (1985)

FIRST RECORD OF STEPHENSIA CROCEA QUÉL. IN THE NETHERLANDS

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A species of *Stephensia* with orange fruit-bodies, discovered in a garden at Drimmellen (prov. Noord-Brabant, Netherlands), August 1982, is described and classified as *S. crocea* Quél. This is a new addition to the mycoflora of the Netherlands. Pure cultures have been deposited in the collection of the 'Centraalbureau voor Schimmelcultures' at Baarn and registered as CBS 709.82. *Stephensia shanori* (Gilkey) Gilkey is regarded as a synonym.

The genus *Stephensia* Tul. emend. Gilkey was classified in the Eutuberaceae Fischer by Fischer (1896). Trappe (1979) placed it in the Pyronemataceae Corda sensu Korf and recognized six species.

In August 1982 Mrs. W. Sommer-Kenniphaas discovered a species of *Stephensia* with orange fruit-bodies in her garden at Drimmelen (prov. Noord-Brabant, Netherlands). Fruit-bodies were found over a distance of four to five meter on and at the side of an old, formerly gravelled path on heavy clay. The adjacent phanerogamic vegetation consisted of shrubs of *Weigelia, Deutzia*, and *Sorbus aucuparia* with a *Betula* tree a little further away.

In November 1982 Mrs. Sommer on request sent fresh specimens from the same locality to the author. The fungus resembled *Stephensia crocea* Quél. and *S. shanori* (Gilkey) Gilkey and differed from *S. bombycina* (Vitt.) Tul. in having smaller ascospores and orange instead of yellowish ascomata.

Pure cultures were made from a young specimen by means of tissue culture technique. These were compared with cultures of *S. bombycina* and *S. shanori.*

COLLECTIONS EXAMINED

The following collections of dried herbarium material were studied:

Stephensia bombycina: Netherlands, prov. Limburg, Slenaken, 4 Oct. 1968, *G. A. de Vries* (herb. de Vries 894). — German Democratic Republic, Kreis Weissenfels, Leissling, 21 Oct. 1968, *U. Nothnagel* (L 968.280.059).

Stephensia crocea: France, Charente Maritime, Rochefort, 1886 or earlier, (herb. P. Brunaud, PC). — Netherlands, prov. Noord-Brabant, Drimmelen, 15 Oct. 1982 and 18 Nov. 1982, *W. Sommer-Kenniphaas*, (herb. de Vries 1030).

Stephensia shanori: U.S.A., Illinois, Urbana, 14 June 1953, *L. Shanor* (type, Gilkey 764a, OSC) and Illinois, Urbana, Brownfield Woods, July 1960, *D. D. McClain* (OSC).

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Stephensia crocea Quél. — Figs. 1, 2

Ascomata up to 2 cm in diam., subglobse, often irregularly lobed, enclosing air in the tissues and lighter than water, smooth to tomentose, pale orange (5A5)[1] to orange (6C8), though brown (7E6) in the grooves. Herbarium specimens brownish orange (7C5) and light brown to brown (7D6 to 7E5). Peridium 400–700 μm, pale lemon yellow with a 40–45 μm thick, brown, pseudoparenchymatous cortex. Cortex consisting of several layers of irregularly arranged, thick-walled, brown, 10–20 μm wide cells which gradually change into a paler, plectenchymatous inner peridium of thin-walled, generally 2.5–4 μm thick hyphae. Ascoma covered with short, sometimes slightly capitate, hyaline to brown, septate, verrucose hyphae. Ostiolum either completely obturated with verrucose hyphae, rarely round or fissurate, especially in young ascomata. Gleba white to very pale lemon yellow, with narrow, winding cavities which are more or less filled with elongated paraphyses. Paraphyses branched, multiseptate, hyaline to pale brown, 2.5–5.0 μm thick, normally smooth, though rough when growing out above the asci in the vicinity of the ostiolum and then indistinguishable from the hyphae on the surface of the ascoma. Asci cylindrical to oblong, 120–200 × 18–23 μm, with narrow base, 4–8-spored, non-amyloid. Ascospores globose, hyaline, 10–17 μm, exceptionally up to 22 μm (average 13.0 ± 1.7 μm), with a c. 1 μm thick, smooth wall. Smell strong, pungent with iodine- or mustard-like component. Taste not investigated.

Cultural characters.—Malt extract agar (MEA) and Sabouraud's glucose agar (SGA) are excellent media for growth. Optimum temperature 24°C. Rate of growth 1.2– 2 mm/24 h. Colony lanose, pale orange (5A3 and 5A4), and orange (6C8) to greyish brown (5D6); Reverse on MEA yellowish brown (5F6), on SGA yellowish brown (5A7) to brown (6E8). Hyphae septate, sometimes anastomosing, branched, at first smooth-, later becoming rough-walled, hyaline to brown, sometimes with yellowish contents, 2– 5 μm thick, similar to those on the surface of the ascomata. No aleuroconidia observed.

Antibiotic action.—Slight inhibition of the growth of *Trichophyton mentagrophytes, T. rubrum, Microsporum canis*. No inhibition of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Nocardia asteroides, Candida albicans, Cryptococcus albidus, Absidia corymbifera, Aspergillus fumigatus*, and *Prototheca wickerhamii*.

Description based on.—*De Vries 1030* (herbarium material) and living culture (CBS 709.82).

The great scarcity of all *Stephensia* species, except *S. bombycina*, makes it very difficult to get a good idea of their intraspecific variability.

Gilkey (1961) accepted four species in her key which was based on the following characters: spore shape, number of spores per ascus, presence or absence of a peridial tomentum, and presence or absence of a central or basal cavity in the ascomata. Referring to *S. crocea*, she quoted Fischer (1938) who regarded as a variety of *S. bombycina*. The last mentioned author did not explain, why he reduced *S. crocea* to a varietal status.

Stephensia crocea was established in 1886 by Quélet with a very short latin diagnosis in which the sizes of spores and ascomata were not mentioned. In a more detailed French description of 1887 the same author described the ascomata as tomentose-velutinous, flesh-coloured orange, with brick-red spots at maturity, and the ascospores as hyaline, globose and 16 μm in diameter.

[1]Colour numbers refer to Kornerup & Wanscher (1978).

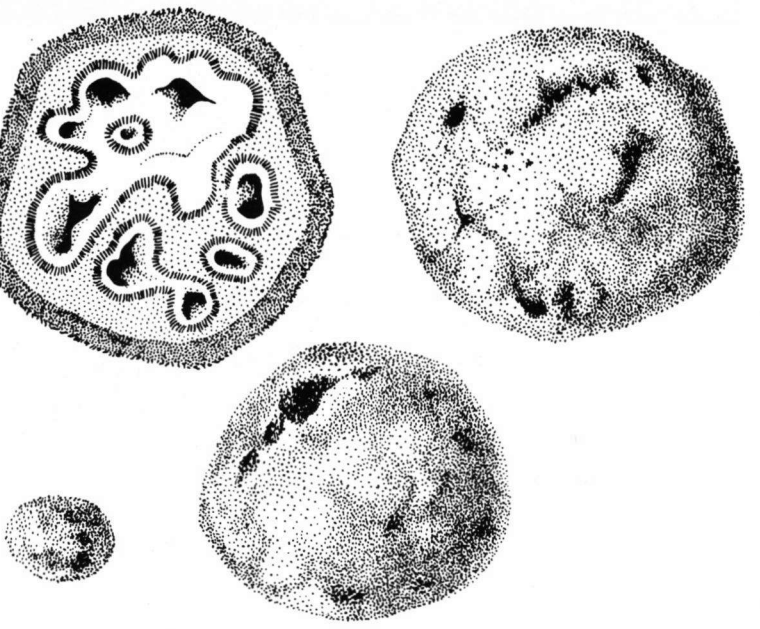

Fig. 1. *Stephensia crocea* (de Vries 1030). — Ascomata showing gleba in one specimen. (c. × 3).

The figure on Quélet's plate can be regarded as representing a section which did not pass through the centre of the ostiolar area. A later demonstration of an ostiolum or ostiolar area in the type material will be impossible, since only a thin carpophore slice remains. Difficulty in finding an ostiolum or ostiolar area was also experienced by the author during his study of the Drimmelen collection. Gilkey's (1954) establishment of the, now abandoned, genus *Densocarpa* and Uecker's (1967) opinion regarding angiocarpic development of *S. shanori* are further evidence for the fact that an ostiolum or ostiolar area is often concealed. Even in *S. bombycina*, where an ostiolum and a central or basal cavity are usually well developed, this character may become obsolete (Fischer, 1896). In the majority of the *S. crocea* specimens from Drimmelen no ostiolum was apparent. Some small ascomata, however, had a small but distinct ostiolum, the orientation of which could not be traced. Kers (1980) discovered that the ostiolum of *S. bombycina* could be apical, lateral or basal. Such a variable orientation may also be expected in *S. crocea*.

The colour of the ascomata is regarded as a good character for species differentiation. It is yellowish or yellowish brown in *S. bombycina* and orange or reddish in *S. crocea*.

The structure of the peridium of *S. crocea* is similar to that of *S. bombycina*. The pseudoparenchymatous cortex is made up of several layers of thick-walled, brown, isodiametric to elongated cells and gradually passes into the colourless to yellowish white, plectenchymatous innner peridium. A radial orientation of the cortical cells is of-

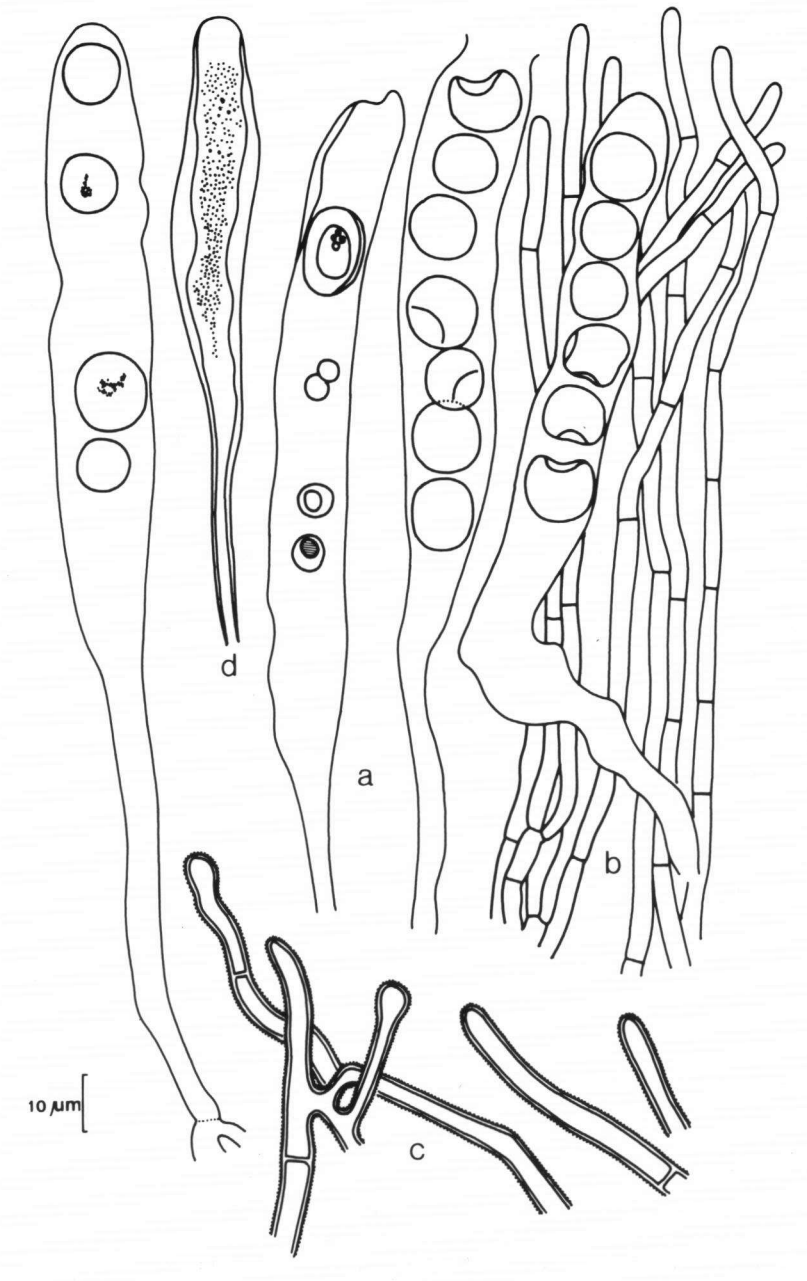

Fig. 2. *Stephensia crocea* (de Vries 1030). — a. Asci. — b. Paraphyses. — c. Hyphae of the peridial tomentum. — d. Ascus with thickened wall.

ten quite distinct in *S. bombycina*. It is less evident although not completely absent in the type material of *S. crocea* and *S. shanori*.

The velutinous ascoma of *S. bombycina* is covered with rather long hyphae. The hyphae on the cortex of *S. crocea* are shorter and often appressed to the surface of the ascoma. Ascomata of *S. crocea* are therefore smooth to tomentose. The tomentum is best developed and also persistent in the grooves. The hairiness of the cortex appears to be dependent on the age of the ascomata and the structure of the surrounding soil. Quélet (1887) described his *S. crocea* as tomentose-velutinous but pictured it as strictly velutinous. Gilkey (1961) supposed young ascomata of *S. shanori* to be tomentose. Uecker could not confirm this.

The ascus wall is usually rather thin. In some cases, however, it is more or less thickened and composed of several layers. This wall thickening, which should not be confused with folding of the ascus wall, was first reported by Uecker. It was observed by the present author in the type material of *S. crocea* and in the specimens collected at Drimmelen, and is regarded as an abnormality. Another abnormality, also observed by Uecker, is the occasional occurrence of a ring near the tip of the ascus. This last mentioned phenomenon was observed only once in a specimen from Drimmelen.

The occurrence of less than eight spores per ascus appears to be quite common. Quélet (1887) reported *S. crocea* as having eight spores per ascus. Examination of the type material, however, showed that the number of spores per ascus was often less than eight. Gilkey (1954) described *S. shanori* as having eight spores at first and one to four at maturity. Fischer (1896) did not record a reduction of the spore number of *S. bombycina*, although he shows several asci with less than eight spores in his figure 11 D.

The spore size is regarded by the present author as a good taxonomical character. There is, however, a considerable variation partly depending on the number of spores per ascus and partly depending on other factors involved in cases where a slight variation in asci with eight spores is observed (cf. Uecker, 1967, fig. 24). Occasionally a very large *S. crocea* spore is seen, the size of which overlaps the lower range of the *S. bombycina* spore size. The averages of the spore sizes of *S. crocea* and *S. bombycina* are distinctly different (Table I).

Uecker observed the production of conidia in his cultures. These conidia can be regarded as aleuroconidia. In 1983 production of conidia was also observed in *S. bombycina* (A. Fontana, pers. comm). They were not seen in the cultures obtained from the ascomata collected at Drimmelen. It is, however, not unlikely that they will be discovered when more pure cultures can be examined.

Whether *S. crocea* forms mycorrhiza is unknown. As several cultures are already available and the species is very easily obtained in pure culture, it is possible to try to establish the synthesis of mycorrhiza under experimental conditions. Fontana & Giovannetti (1980/81) cultured *S. bombycina* and *Salix* and *Quercus* together without obtaining mycorrhizae.

The smaller ascospores and the orange colour of the tomentose, usually rather compact ascomata without a distinct central or basal cavity, are regarded by the author to provide sufficient reason for maintaining *S. crocea* as a separate species, distinct from

S. bombycina. *Stephensia shanori* strongly resembles *S. crocea* and is regarded as conspecific.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. C. Bas, (Rijksherbarium, Leiden), Dr. J. Mouchacca (Laboratoire de Cryptogamie, Muséum d'Histoire Naturelle, Paris) and Prof. Dr. J. M. Trappe (Forestry Sciences Laboratory, Corvallis, Oregon, U.S.A.) for placing herbarium specimens at his disposal, Mrs. W. Sommer-Kenniphaas for the fresh specimens, Dr. A. Fontana (Centro di Studio per la Micologia dell'terreno, Ist. Bot. dell'Universita, Valentino-Torino, Italia) and Dr. F. A. Uecker (Northeastern Region Beltsville Agricultural Research Center, Beltsville, Maryland, U.S.A.) for sending cultures, and Mrs. C. J. Verwoerd-Kuyt for her technical assistance.

Table I. Spore sizes with averages and standard deviations of six *Stephensia* collections.

S. crocea	Type	13.0–15.6 av. 14.0 ± 0.8 µm
S. crocea	Drimmelen	10.0–17.0 av. 13.0 ± 1.7 µm
S. shanori	Type	12.5–15.0 av. 14.1 ± 0.7 µm
S. shanori	Brownfield	11.5–19.2 av. 14.2 ± 2.2 µm
S. bombycina	Slenaken	16.0–24.0 av. 19.6 ± 2.2 µm
S. bombycina	Weissenfels	15.5–26.5 av. 19.2 ± 2.7 µm

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