

## Three new *Stirtonia* from Everglades National Park with a key to neotropical species

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**Abstract:** *Stirtonia byssoidea*, *S. coei* and *S. latispora*, all from coastal hammocks within Everglades National Park, are described as new to science. *Stirtonia byssoidea* is characterized by a felty byssoid thallus, conspicuous immersed white pruinose-like lirelliform ascigerous zones, small ascospores and the presence of perlatolic acid. It is closest to *S. alba* but has a different thallus type and much smaller ascospores. *Stirtonia coei* is recognized by its lirellate ascigerous zones raised well above a greyish crystallate thallus, large ascospores and a lack of chemistry. It is closest to *S. curvata* but differs by its I+ blue thallus, strongly raised ascigerous zones and by having no lichen substances. *Stirtonia latispora* can be identified by its white lirelliform ascigerous areas immersed in a greyish green thallus, large, broad ascospores with a conspicuously enlarged mid cell and lack of substances. It would also key out close to *S. curvata* but differs by the aforementioned ascigerous zones, an I+ blue thallus, wider ascospores with a different locular configuration and its lack of chemistry. A key to the six species known from the Neotropics is also provided.

**Key words:** Fakahatchee, Florida, Florida Bay, lichens, taxonomy

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### Introduction

In our experience, collectors often pass over lichens where a conspicuous fruiting structure is absent, possibly believing a reliable identification to be difficult. This view is shared in papers by other authors, especially those concerning genera such as *Cryptothecia* Stirton, *Stirtonia* A. L. Smith and some species of *Herpothallon* Tobler where asci are either absent, dispersed indiscriminately across the thallus or often in cryptic ascigerous zones (Makhija & Patwardhan 1998; Cáceres 2007; Wolseley & Aptroot 2009). In the case of *Stirtonia*, prior to the publication of a world key (Aptroot 2009), there had been a paucity of publications in existence that recorded *Stirtonia* species (Aptroot 2009; Wolseley & Aptroot 2009), possibly for the above reason. The publication of a world key to any given genus frequently precipitates a

flow of new species into the genus (Aptroot *et al.* 2013; Seavey & Seavey 2014). However, in the case of *Stirtonia*, only one new addition to the genus has been described over a span of five years (Kalb *et al.* 2012). Although this may indicate an intrinsic rarity of species within the genus, it seems more likely to us that the genus is a victim of undercollecting due to the cryptic nature of its fruiting characteristics and/or thallus. This argument is strengthened by the finding of three new additions to the genus within a four hectare forest (this paper), when only 14 species are known worldwide.

After the transfer of described foliicolous *Stirtonia* to different genera (Lücking 2008), the genus was believed to be restricted to the Asian tropics (Makhija & Patwardhan 1998). However, in more recent times *S. neotropica* Aptroot and *S. schummii* Aptroot have been described from the Netherland Antilles and the Seychelles, respectively (Aptroot 2009), and two previously named species were discovered in south Florida, USA (Lücking *et al.* 2011). These, coupled with the three

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new species described from Everglades National Park in this paper, suggest the genus can be considered pantropical.

All three of the new species described herein were found in coastal forested habitat and, with one exception, within less than 200 m from the shore of Florida Bay. While *Stirtonia byssoidea* has also been collected from other locations and habitats, *S. coei* and *S. latispora* are currently known only from multiple collections at the site given above. By Florida standards, where periodic hurricanes rarely allow tree species to attain their full stature, the site has a moderately large mature arboreal flora but without a closed canopy which allows a large degree of filtered sunlight to penetrate. Understorey shrubs and forbs are essentially absent. The bark of *Krugiodendron ferreum* (Vahl) Urban (*Rhamnaceae*) was the favoured substratum (8 out of 27 aggregate *Stirtonia* collections), although 23 other arboreal species were present. That tree has smooth to furrowed bark which has a neutral or very slightly basic pH (F. Seavey & J. Seavey, unpublished data).

### Materials and Methods

All collections were examined using standard stereoscopic and light microscope techniques. A Leica DFC295 compound microscope and a Leica S8APO inspection microscope were used to view hand-cut sections and thalline superficial structures, respectively. All macroscopic and microscopic images were captured via computer using Leica Application Suite V4.2.0 28 software. Ascigerous zone dimensions were determined from computer images using the software's measuring program. Measurements of internal structures were obtained from untreated water mounts. The software's automatic setting was employed and may have enhanced some of the images. No additional enhancement was used unless otherwise noted. Thin-layer chromatography (TLC) was carried out in accordance with Orange *et al.* (2001), using solvents A and C. Controls used were atranorin (*Lecanora leprosa* Fée), norstictic acid (*Graphis librata* C. Knight) and perlatolic acid [*Canoparmelia caroliniana* (Nyl.) Elix & Hale]. Spot test abbreviations used are C (sodium hypochlorite), K (potassium hydroxide), I (Lugol's solution 1%) and P (*para*-phenylenediamine). All collections were made by the authors within Everglades National Park and will be curated at the South Florida Collections Management Center (FNPS). They are corticolous unless otherwise noted.

### The New Species

Individual colour images of the new species are available at: <http://www.seaveyfieldguides.com/Lichens/>

#### *Stirtonia byssoidea* F. Seavey & J. Seavey sp. nov.

Mycobank No.: 809720

Similar to *Stirtonia alba* but differing by its byssoid, thicker thallus and smaller ascospores.

Type: USA, Florida, Everglades National Park, Monroe County, Key Largo Ranger Station, 25°05'N, 80°27'W, corticolous on *Guapira discolor* in coastal hammock forest, 8 April 2014, F. Seavey & J. Seavey 12934E (FNPS—holotype; FLAS—isotype).

(Fig. 1A–C)

*Thallus* corticolous, ecorticate, endophloeodal, byssoid, continuous, up to 8 cm diam., greenish white to greyish green, 100–200 µm thick, interspersed with calcium oxalate crystals 15–20 µm diam., I+ blue, often appearing pruinose due to projecting greyish hyphae, usually with a distinct white prothallus. *Photobiont* *Trentepohlia*.

*Ascigerous zones* indicated by white pruinose-like lines level or slightly raised above the thallus surface, branched or unbranched, 0.5–4.0 × 0.08–0.35 mm, I+ blue. *Asci* subglobose to globose, fissitunicate, 8-spored, not visible in surface view. *Ascospores* obovoid, 22–28 × 10–12 µm, 5–6-celled, upper cell often larger, occasionally subdividing into 6–7 cells.

*Chemistry*. K–, KC–, C–, P–. UV– but white ascigerous zones UV+ white. Perlatolic acid by TLC.

*Etymology*. The specific epithet refers to the distinct byssoid thalline surface.

*Habitat and distribution*. *Stirtonia byssoidea* is widespread in the south-east quadrant of Everglades National Park especially close to water, including Florida Bay. It is common and better developed in the coastal hammock discussed above, appearing on the bark of at least 11 different tree species. No host specificity was observed and the bark of the 11 species ranged from smooth to rough and somewhat furrowed.

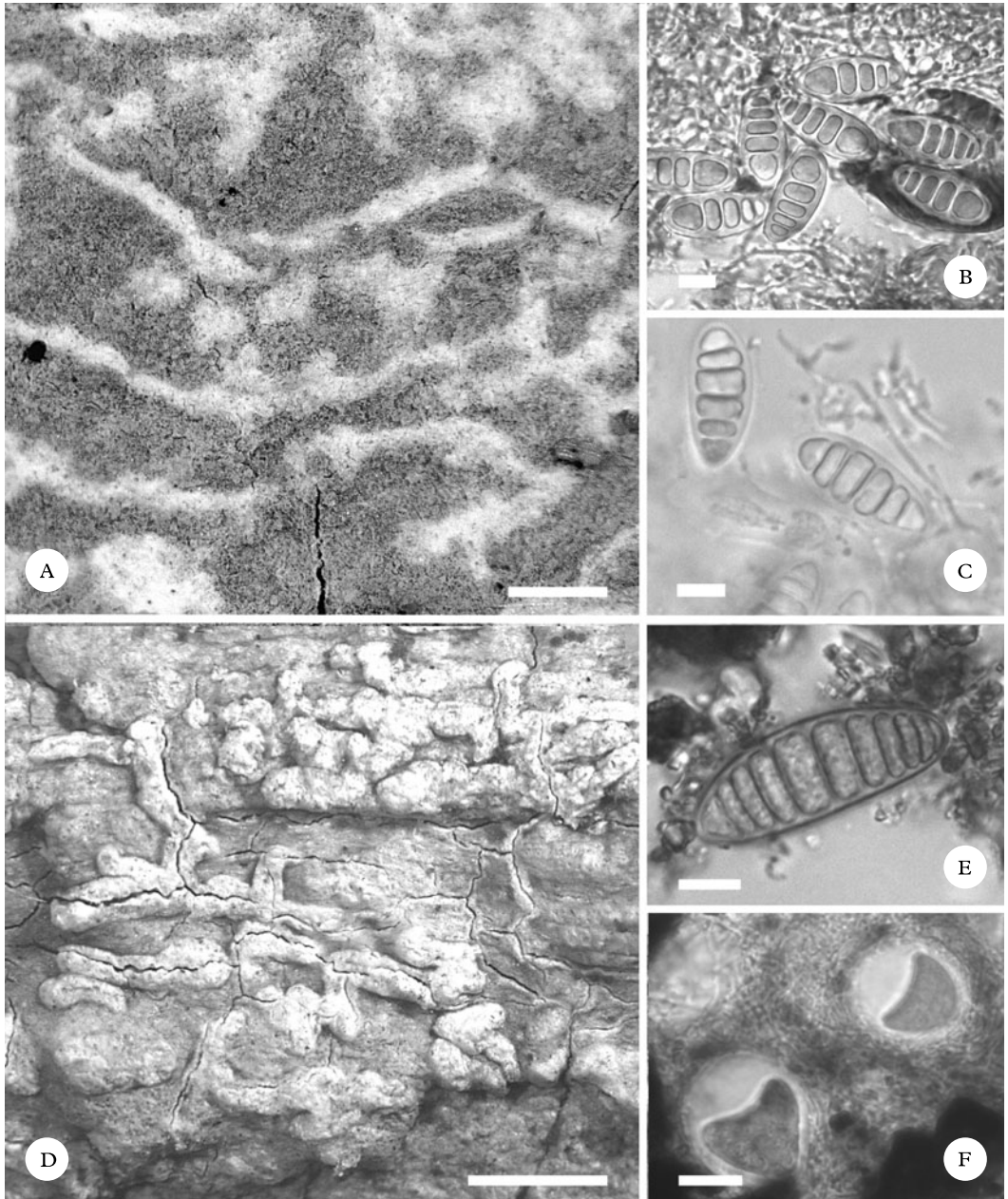


FIG. 1. A–C, *Stirtonia byssoidea*; A, byssoid nature of the thallus and white ascigerous zones; B, megacephalic ascospores; C, rarely observed ascospores where the enlarged upper cell subdivides. D–F, *S. coei*. D, thallus with ascigerous zones raised significantly above the thallus surface; E, typical ascospore stained with Lugol's; F, asci showing the more or less globose configuration stained with Lugol's. Scales: A & D = 1 mm; B, C & E = 10  $\mu$ m; F = 100  $\mu$ m.

**Discussion.** In the field, *Stirtonia byssoidea* can be recognized by its distinct greenish grey byssoid thallus usually appearing wispy with a hand lens. The white ascigerous zones may be barely discernible or encompass over one half of the thallus in larger well-developed specimens. Nearly all of the ascospores obtained from 19 collections of this taxon were 5-celled, with an enlarged upper cell in apparent full maturity (Fig. 1B). However, in one collection we observed the upper cell subdividing, creating 6–7 cells of roughly equal size (Fig. 1C). Although this seems a rare event, the key below includes both configurations. *Stirtonia alba* Groenh. ex Makhija & Patw. also contains perlatolic acid and has ascospores with enlarged upper cells, but the latter are much larger ( $38\text{--}60 \times 14\text{--}22 \mu\text{m}$ ) while its thallus lacks the distinct byssoid nature. *Stirtonia dubia* A. L. Sm. has similar size ascospores but also lacks the byssoid thallus and contains no substances.

*Selected additional specimens examined.* **USA:** Florida: Miami-Dade Co., Goodrich Hammock, on *Salix caroliniana*, 2013, 6351; south of Old Tamiami Trail, on *Salix caroliniana*, 2005, 5961; Monroe Co., Key Largo Hammock, on bark of dead tree, 2014, 6347; Key Largo Hammock, on *Gymnanthes lucida*, 2014, 6348; Key Largo Hammock, on *Sideroxylon salicifolium*, 2014, 6349; Key Largo Hammock, on *Sideroxylon celastrinum*, 2014, 6350.

***Stirtonia coei* F. Seavey & J. Seavey  
sp. nov.**

Mycobank No.: 809721

Similar to *Stirtonia neotropica* and *S. curvata* Aptroot but differing from both by containing no substances, lacking a more or less shiny thalline surface, strongly raised ascigerous zones and having an I+ blue reacting thallus.

Type: USA, Florida, Everglades National Park, Monroe County, Key Largo Ranger Station,  $25^{\circ}05'N$ ,  $80^{\circ}27'W$ , corticolous on *Krugiodendron ferreum* in coastal hammock forest, 8 April 2014, F. Seavey & J. Seavey 12915E (FNPS—holotype; FLAS—istotype).

(Fig. 1D–F)

**Thallus** corticolous, ecorticate, thin, endophloeodal, matt to somewhat shiny, continuous, 8–12 cm diam., grey to greenish grey, 30–60  $\mu\text{m}$  thick, interspersed with small calcium oxalate crystals 10–15  $\mu\text{m}$  wide, I+

blue, with brownish white prothallus. *Photobiont* *Trentepohlia*.

**Ascigerous zones** indicated by white thick pruinose-like lines strongly raised above the thallus surface, branched or unbranched,  $0.60\text{--}3.60 \times 0.25\text{--}0.65 \text{ mm}$ , I+ blue. *Asci* subglobose to globose, fissitunicate, 8-spored, not visible in surface view. *Ascospores*  $40\text{--}45 \times 15\text{--}16 \mu\text{m}$ , 10–12-celled, cells isolocular or mid cells slightly larger.

**Chemistry.** K–, KC–, C–, P–. UV–. No substances by TLC.

**Etymology.** The name commemorates the untiring efforts of Ernest F. ‘Tom’ Coe, who first advocated the creation of Everglades National Park in 1928 and then fought valiantly until its fruition in 1947. He is recognized as the single most important driving force for the park’s establishment.

**Habitat and distribution.** *Stirtonia coei* is common in the coastal hammock mentioned above. It occurs in sunnier locations than the above species and well back from the shoreline. Although locally common, it is currently known only from this location but the same comments given for *S. latispora* (below) could apply here also. Its position in the hammock might indicate an intolerance of even the finest salt spray.

**Discussion.** *Stirtonia coei* is an easily overlooked species as the grey thallus blends in cryptically with its substratum. In many cases it appears as a grey stain on grey bark and, unless the white ascigerous lines are abundant, it will likely be missed. In the Aptroot key it would key out at couplet 12 that includes *S. neotropica* and *S. curvata*. It differs from the latter by its I+ blue thallus, wider, more strongly raised ascigerous zones, and lack of substances. *Stirtonia neotropica* has smaller ascospores, an I– ecystallate thallus lacking raised ascigerous zones and contains gyrophoric acid. *Stirtonia latispora*, described below, also contains no substances but has larger and much wider ascospores, immersed white ascigerous zones and a distinct green epiphloeodal thallus containing

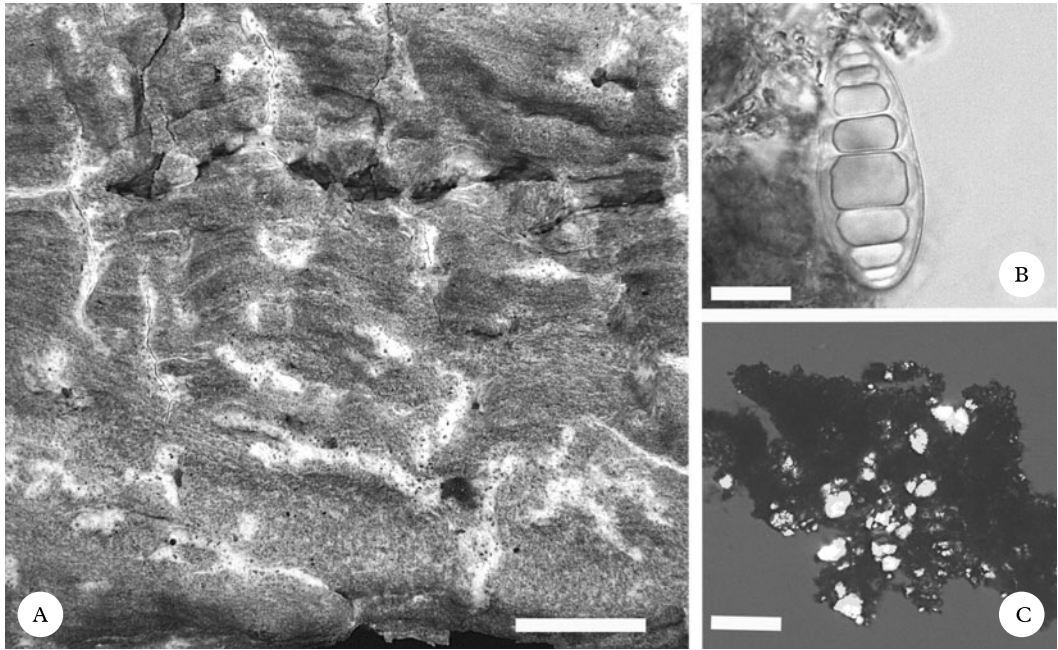


FIG. 2. A–C, *Stirtonia latispora*; A, white ascigerous zones contrasting sharply with bright green thallus; B, ascospore showing the large middle cell; C, large calcium oxalate crystals restricted to non-ascigerous parts of the thallus. Scales: A = 1 mm; B = 20  $\mu$ m; C = 100  $\mu$ m.

larger calcium oxalate crystals. *Stirtonia ramosa* Makhija & Patw., described from the Andaman Islands, lacks thalline oxalate crystals, has ascospores with fewer septa and differs further by containing 2'-*O*-methyl-norsuperphyllinic and 4'-*O*-demethylsuperconfluent acids.

*Selected additional specimens examined.* **USA:** Florida: Monroe Co., Key Largo Hammock, on *Krugiodendron ferreum*, 2014, 6343E; Key Largo Hammock, on *Reynoldsia septentrionalis*, 2014, 6344E.

***Stirtonia latispora* F. Seavey & J. Seavey sp. nov.**

Mycobank No.: 809722

Similar to *Stirtonia curvata* but differs by its larger ascospores with an enlarged mid cell, an I+ blue thallus and lack of perlatolic acid.

Type: USA, Florida, Everglades National Park, Monroe County, Key Largo Ranger Station, 25°05'N, 80°27'W, corticolous on *Krugiodendron ferreum* in coastal hammock forest, 25 April 2014, F. Seavey & J. Seavey 12933E (FNPS—holotype).

(Fig. 2A–C)

*Thallus* corticolous, ecorticate, epiphloeodal, matt to somewhat shiny, continuous, 2.5–3.5 cm diam., greyish green, 50–80  $\mu$ m thick, interspersed with large calcium oxalate crystals 25–30  $\mu$ m diam., I+ blue. Prothallus absent. *Photobiont* *Trentepohlia*.

*Ascigerous zones* in the form of white pruinose-like lines immersed or level with the thallus surface, irregularly branched, 0.65–2.75  $\times$  0.15–0.30 mm, I+ blue. *Asci* globose, fissitunicate, 8-spored, but rarely more than 2–4 maturing; often visible in surface view as dark spots. *Ascospores* ellipsoid, 52–55  $\times$  20–23  $\mu$ m, 10–12-celled, one mid cell much larger.

*Chemistry.* K–, KC–, C–, P–. UV–. No substances by TLC.

*Etymology.* The specific epithet refers to the broad ascospores of this species.

*Habitat and distribution.* Currently known from three corticolous collections on *Krugiodendron ferreum*, *Reynosia septentrionalis* Urb. and *Piscidia piscipula* (L.) Sarg., all from the coastal hammock mentioned above. Several other similar coastal forests exist in the park, also within a few metres of the bay, but they are remote and difficult to access either by foot or boat. These may well be fertile areas for other populations of this species.

*Discussion.* This species is quite easy to recognize in the field as the thin bright white lines of the ascigerous areas stand out in sharp contrast to the greyish green thallus. The white of the ascigerous zones is a mix of asci, hyphae surrounding the asci and crystalline structures. It is only the hyphae which react I+ blue leaving the non-hyphal interspaces I-. The ascospore ontogeny of this species appears to us to be taxonomically significant. Multiple sections encountering several dozen ascospores show that the septation begins with a mid-septum followed rapidly by two additional septa subdividing each half leaving four more or less equal cells.

Septation then continues from the ends toward the middle. Curiously, however, septation ceases in one of the mid cells while the other continues subdividing until as many as 12(13) cells are produced. Even post-mature ascospores retain the enlarged mid cell. As we observed no deviation from this pattern, the trait is included in both the protologue above and the key below. In the worldwide key of Aptroot, *Stirtonia latispora* would key out at couplet 12 along with *S. curvata* and *S. neotropica*. However, the former contains perlatolic acid and has ascospores that are shorter, narrower and isolocular. The latter contains gyrophoric acid and much smaller, narrower ascospores. The recently described *S. rhizophorae* Kalb & Mongkolsuk (Kalb *et al.* 2012) is also somewhat similar but has much larger ascospores with a larger number of septa and contains confluent acid.

*Selected additional specimens examined. USA: Florida:* Monroe Co., Key Largo Hammock, on *Piscidia piscipula*, 2014, 6540E; Key Largo Hammock, on *Krugiodendron ferreum*, 2014, 12935E.

### Key to *Stirtonia* species known from the Neotropics

- |      |   |   |
|------|---|---|
| 1    | Thallus without secondary metabolites . . . . .   | 2   |
|      | Thallus containing perlatolic, gyrophoric or 2'-O-methylperlatolic acids . . . . .                  | 4   |
| 2(1) | Ascospores < 35 µm long, with 8 cells or less . . . . .   | <b>S. dubia</b> A. L. Sm.                 |
|      | Ascospores > 40 µm long, with 10 cells or more . . . . .  | 3   |
| 3(2) | Ascospores 40–45 × 15–16 µm, cells isolocular, ascigerous zones strongly raised . . . . .           | <b>S. coei</b> F. Seavey & J. Seavey      |
|      | Ascospores 52–55 × 20–23 µm, mid cell distinctly enlarged, ascigerous zones immersed . . . . .      | <b>S. latispora</b> F. Seavey & J. Seavey |
| 4(1) | Ascospores 22–28 × 10–12 µm, with enlarged upper cell, thallus containing perlatolic acid . . . . . | <b>S. byssoidea</b> F. Seavey & J. Seavey |
|      | Ascospores without enlarged upper cell . . . . .  | 5   |
| 5(4) | Ascospores 22–28 × 10–12 µm, 6–7-celled, thallus containing perlatolic acid . . . . .               | <b>S. byssoidea</b> F. Seavey & J. Seavey |
|      | Ascospores > 30 µm long, perlatolic acid absent . . . . .   | 6   |
| 6(5) | Thallus containing 2'-O-methylperlatolic acid, ascospores 50–110 µm long, 12–17-celled . . . . .    | <b>S. macrocarpa</b> Makhija & Patw.      |
|      | Thallus containing gyrophoric acid, ascospores 35–38 µm long, 11–12-celled . . . . .                | <b>S. neotropica</b> Aptroot              |

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