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New species in *Helicogloea* and *Spiculogloea*, including a type study of *H. graminicola* (Bres.) G.E. Baker (Basidiomycota, Pucciniomycotina)

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Abstract – Two new heterobasidiomycetes species belonging to the genera *Helicogloea* and *Spiculogloea* (Pucciniomycotina) are described from Belgium. *Helicogloea jozefii* sp. nov. and *Spiculogloea inaequalis* sp. nov. are proposed with descriptions and illustrations of macro- and microscopical features. The latter species grows as an intrahymenial parasite in *Sistotrema* spp. Identification keys to the new species are provided for each genus. The newly described species are compared to morphologically similar species and their ecology is briefly discussed. A type study of *H. graminicola* is included.

Heterobasidiomycetes / intrahymenial / morphology / parasite / *Sistotrema* / stichobasidia / taxonomy

INTRODUCTION

The Heterobasidiomycetes s.l. are a polyphyletic group of Basidiomycota characterised by septate basidia and/or repetitive spores and the capability of most species' basidiocarps to rehydrate and swell up after a period of drought when in contact with water. A considerable number of species/genera grow inside the hymenial tissues of other fungi, mainly in corticioids or in other heterobasidiomycetes. Most of them do not form basidiocarps, but only form some scattered hyphae, basidia and sometimes haustorial cells and/or cystidia between those of the host species. Most heterobasidiomycetes are saprotrophs, while some well-known groups are known to be parasitic or mycorrhizal.

Nowadays, thanks to molecular phylogenies, we know this artificial form group is highly polyphyletic and its representatives are distributed over all subphyla of the Basidiomycota (Ustilaginomycotina, Pucciniomycotina & Agaricomycotina), suggesting these characteristics are the result of convergent evolution, having evolved several times independently. Both *Helicogloea* and *Spiculogloea* species possess transversally septate basidia, known as auricularioid or stichobasidia. These genera belong to the Pucciniomycotina (Wang *et al.* 2015a, 2015b).

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During the winter of 2015, the grandfather of the first author (Jozef Schoutteten) collected an unknown *Helicogloea* species. After microscopical investigation the collection could not be assigned to any described species but seemed to be very similar to three other previously collected specimens (in 2003, 2010 and 2011). The first collection, dating from 2003 and found at the same location as the holotype, was investigated by Karel Van De Put and Peter Roberts and found to represent a new species by both. Consequently a provisional description of *Helicogloea* sp. 'Schoutteten 03/29' was published by Van De Put (2004). In this paper, we propose the new species *Helicogloea jozefii* sp. nov. for these collections. *Helicogloea graminicola* (Bres.) G.E. Baker, described in 1936, is a morphologically similar species, for which we compared the holotype collection with our new species.

During an inventory programme of the forest reserve Sint-Pietersbos in southern Flanders in the early spring of 2017, the first author collected a new *Spiculogloea* species (Schoutteten, 2017). This species grows as an intrahymenial parasite in *Sistotrema* sp. and is here proposed as *Spiculogloea inaequalis* sp. nov.

MATERIAL AND METHODS

Macroscopic characters are all based on fresh material, except for the type of *H. graminicola*. Dried material was rehydrated for several hours prior to examination. Permanent slides were made from all collections. Microscopic features were studied in a Congo-red L4 solution and a drop of GSM was added to each slide to preserve the material (Clemençon, 2009). Linedrawings were made by N. Schoutteten and A. Verbeken, with the aid of a drawing tube at magnification 1500 × for all elements. Basidia length excludes sterigmata length. Spores were measured in side view and measurements are given as [AVa-2*SD] - *AVa* - [AVa + 2*SD], in which AVa = mean value for the measured collection and SD = standard deviation. Q stands for "quotient length/width" and is given as MINQ - *AvQ* – MAXQ, in which AvQ stands for the mean quotient for the measured spores. Twenty spores were measured per collection, except for *Spiculogloea*, where the spores were rarely observed (5 spores measured).

RESULTS

Helicogloea jozefii Schoutteten & Verbeken **sp. nov.** **Figs 1-2**

Mycobank: MB825550

Etymology: named after Jozef Schoutteten, expert in Corticiaceae and heterobasidiomycetes and grandfather of the first author.

Diagnosis: differs from all other described *Helicogloea* species in the combination of clamps and slender ellipsoid, lacrymoid spores.

Holotypus: Belgium. Prov. Oost-Vlaanderen, Brakel, Brusselsestraat, coordinates: N50.807935, E3.784405, on dead bark of a deciduous branch, 07/12/2015, J. Schoutteten 15/044 (GENT).

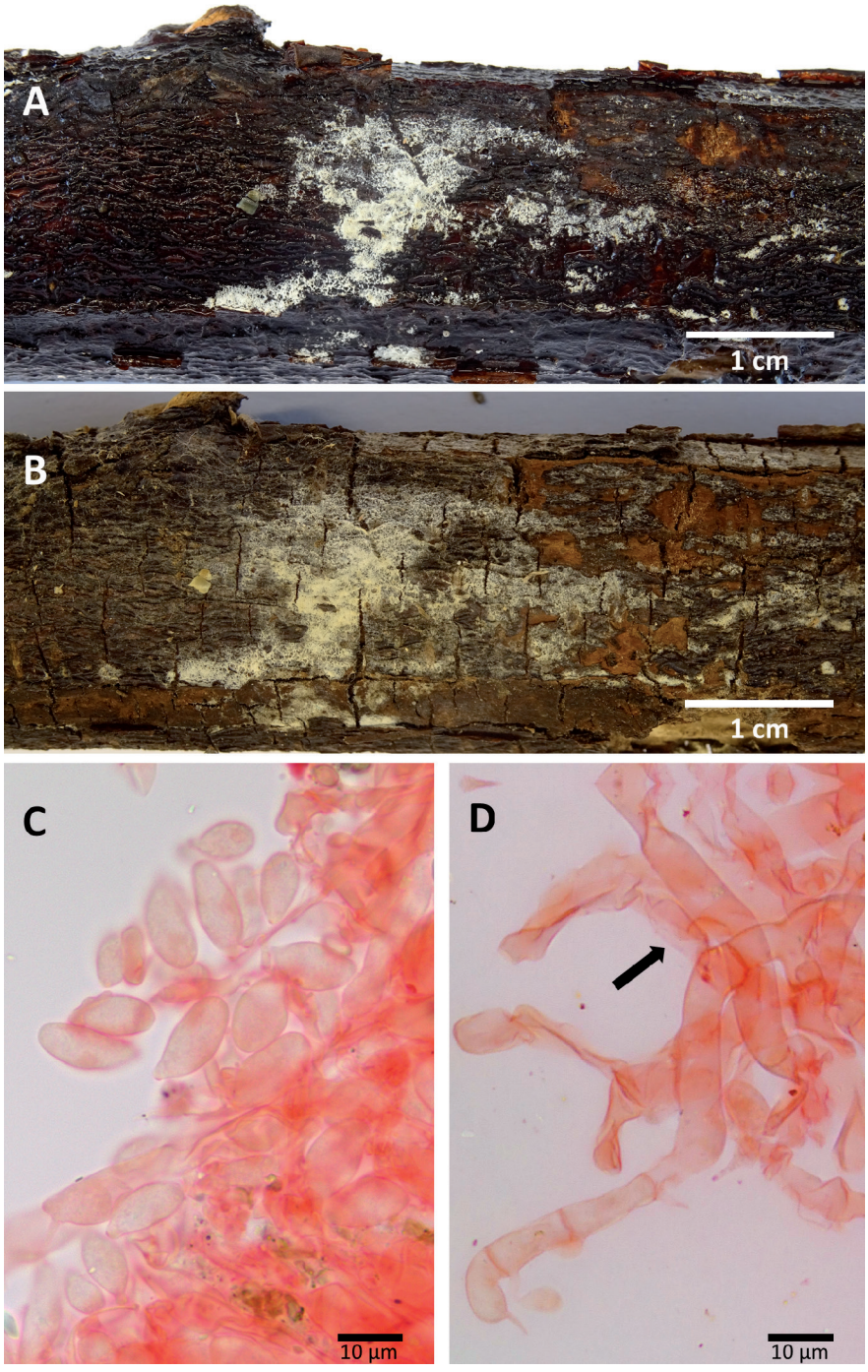

Fig. 1. *Helicogloea jozefii* (holotypus). **a.** Rehydrated basidiocarp. **b.** Dried basidiocarp. **c.** Spores. **d.** Basidium, black arrow points to probasidial sac.

Basidiocarp resupinate, flat, white to whitish grey and membranaceous when dry, turning more distinctly whitish and arachnoid to fibrillose, floccose when moistened.

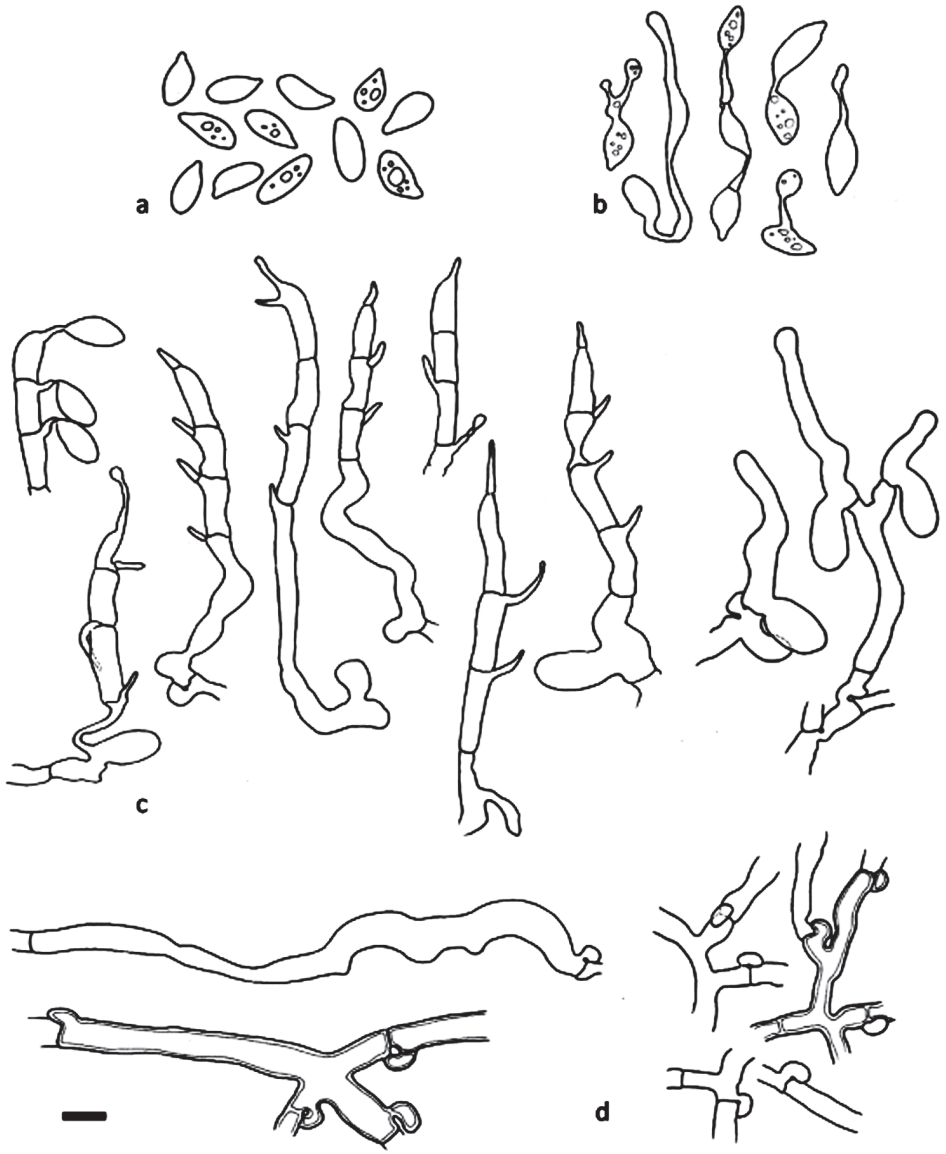

Fig. 2. *Helicogloea jozefii* (holotypus). **a.** Spores. **b.** Germinating spores and secondary spore formation. **c.** Basidia **d.** Hyphae. Scalebar = 10 μm.

Subhymenial hyphae thin-walled, hyaline, 4-5 µm diam. **Basal hyphae** slightly thick-walled, hyaline, 5-8 µm diam. **Probasidia** sacculiform, 11-19 x 5.5-8 µm, laterally attached at the base of the basidia. **Basidia** stichic, transversally septate (auricularioid), triseptate, 70-100 x 4-6 µm; sterigmata short, mostly curved, up to 11 µm long, laterally inserted; upper sterigmen apically positioned. **Basidiospores** smooth, thin-walled, 9.3-*12.6*-17.2 (21) x 4.6-*6.1*-7.7 µm (n = 20), Q = 1.57-*2.08*-2.61, slender and elongate ellipsoid to lacrymoid, some slightly curved, non-amyloid, producing secondary spores. **Cystidia** absent. **Clamps** present.

Additional studied material: BELGIUM. Prov. Oost-Vlaanderen, Brakel, Brusselsestraat, coordinates: N 50.807935, E 3.784405, on dead bark of a rhododendron branch, 28/03/2003, J. Schoutteten 03/029 (K 137226); Ronse, Ninoofsesteenweg, coordinates: N 50.752310, E 3.641849, on dead bark of a deciduous branch, 18/01/2010, W. Termonia 10/05 (GENT); ibid., 02/12/2011, W. Termonia 11/088 (GENT).

Helicogloea graminicola (Bres.) G.E. Baker, Annals of the Missouri Botanical Garden 23: 90. 1936 **Figs 3-4**

Basidiocarp resupinate, effused, margin whitish, centre of the basidiocarp more cream-coloured to ochraceous, hypochnoid to fibrillose when dry, when moistened becoming muco-gelatinous, with an easily removable pellicular texture, consisting of loosely interwoven hyphae, having a hyaline to yellowish / brownish context and some white to cream-coloured pruinose patches at the surface.

Subhymenial hyphae thin-walled, hyaline, up to 5 µm diam. **Basal hyphae** slightly thick-walled, hyaline, 7-9 µm diam.; thickened walls up to 1-1.5 µm. **Probasidia** sacculiform, 10-18 x 6.5-9 µm, laterally attached at the base of the basidia. **Basidia** stichic, transversally septate (auricularioid), triseptate, 60-70 x 5-6.5 µm; sterigmata straight to curved, up to 7 µm long, laterally inserted; upper sterigmen apically positioned. **Basidiospores** smooth, thin-walled, 8.7-*10.9*-12.8 (13.1) x 5.5-*7.1*-8.7 µm (n = 20), Q = 1.22-*1.54*-1.73, broadly ellipsoid to ellipsoid, very rarely elongate, some lacrymoid, non-amyloid, producing secondary spores. **Cystidia** absent. **Clamps** present.

Studied material: POLAND. No locality, 1903, Eichler s.n. (Holotypus, FH 01142894)

Commentary: The type specimen of *H. graminicola* at the Farlow Herbarium is in rather poor condition. Some old dried scales of insects were found in-between the material but enough was still present for morphological studies. The specimen was not only growing on old grasses but also on soil. A single grass stem covered with fungal material was rehydrated in a Petri dish with water. The specimen still contained a lot of spores, some even showing secondary spore formation. Spore measurements resulted in spore sizes very close to those of Bresadola in his original description (Bresadola, 1903) as already indicated by Baker (1936) who investigated this specimen but did not give any measurements besides some drawings. As Baker (1936) already stated, the adjective ‘tomentose’ used by Bresadola in his original description is not justifiable. However, both authors use the colour ‘vinaceous-buff’. The basidiocarp is now rather cream-coloured to ochraceous (because of the age of the specimen?), having a hyaline to brownish basal muco-gelatinous layer.

Helicogloea was published by Patouillard (1892) with *H. lagerheimii* from Ecuador as type species. Möller (1895) published the microscopically similar genus *Saccoblastia*, containing two species from Brazil, *S. ovispora* and *S. sphaerospora*. Subsequent authors either distinguished these two genera on macromorphological

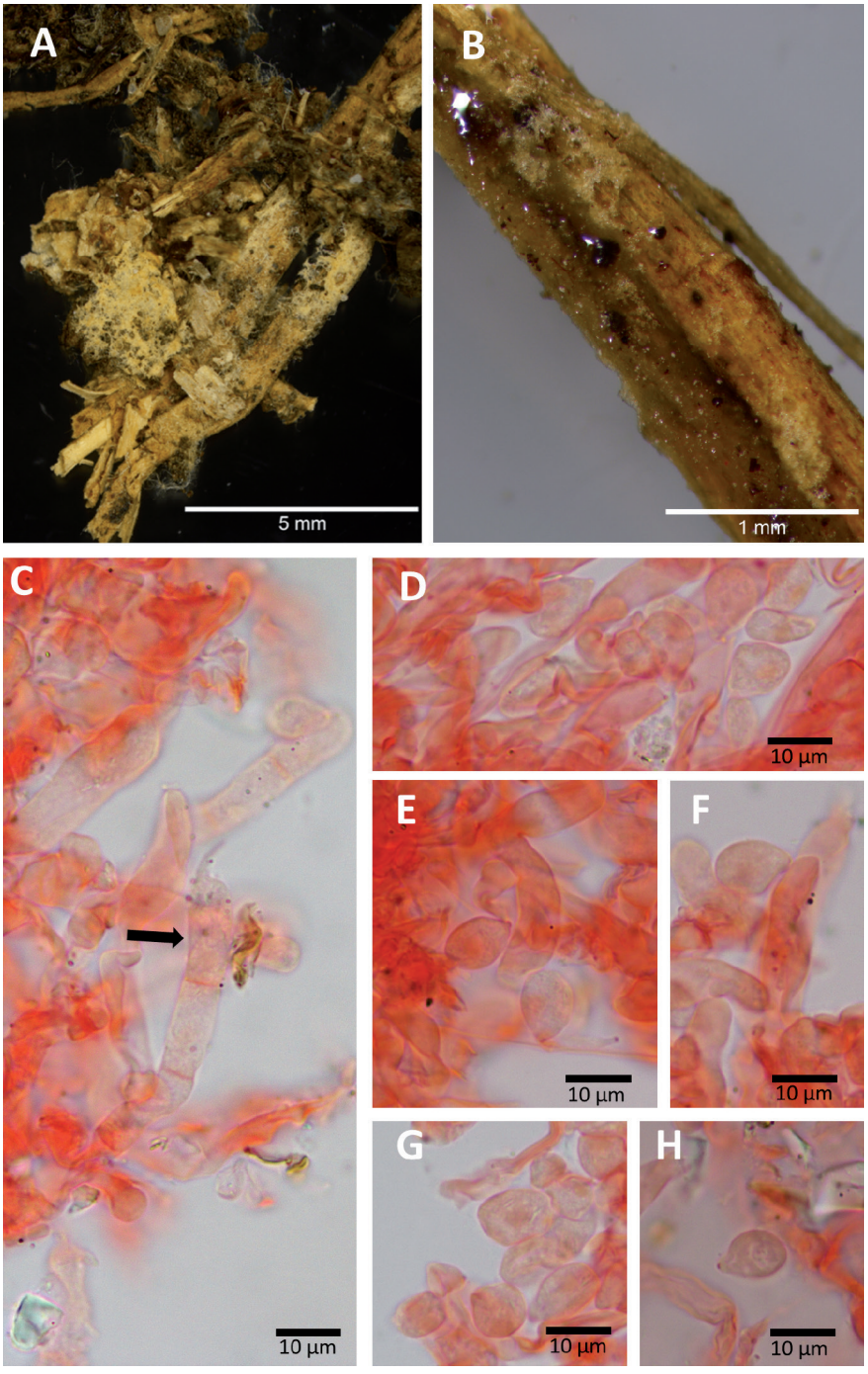

Fig. 3. *Helicogloea graminicola* (holotypus). **a.** Dried basidiocarp. **b.** Rehydrated basidiocarp. **c.** Black arrow points to basidium. **d-h.** Spores.

differences in basidiocarp texture: muco-gelatinous for *Helicogloea*, floccose-hypochnoid for *Saccoblastia* (Donk, 1966; Jülich, 1984), or they placed *Saccoblastia* in synonymy with *Helicogloea* (Baker, 1936). *Helicogloea* species are saprotrophic, decaying a wide range of substrates.To date about 25 species are known world-wide. Only six species, including *H. jozefii*, have clamp-connections which are usually abundant and distinct. The species most resembling *H. jozefii* is *H. graminicola*; the other clamped species have (sub)globose or much larger spores or cystidia. *Helicogloea graminicola* is still only known from the type locality (Poland) growing on some dry grasses and earth. The major distinguishing characters between *H. jozefii* and *H. graminicola* are the spores and the basidiocarp. The spores of *H. graminicola* have a rather ellipsoid form and are never longer than 13 µm. Those of *H. jozefii* are much more elongate, reaching 17 µm in length (some even up to 21µm) and have a more slender appearance because of the small width, resulting in a larger Q value. The basidiocarp of *H. jozefii* is much more floccose and thinner, not forming an easily detachable muco-gelatinous pellicular coating as observed in *H. graminicola*. The basidiocarp seems to be whitish in *H. jozefii* whereas it is more distinctly coloured in *H. graminicola*.

Key to the clamped species of *Helicogloea* and *Saccoblastia*.

1. Cystidia present, slightly thick-walled (type: St Helena) .....*S. media*
1. Cystidia absent 2
    2. Probasidium of variable shape and size, often constricted and forked, spores as a rule > 15 µm in length(type: Austria) .....*S. farinacea*
    2. Probasidium not forked, spores as a rule ≤ 15 µm in length 3
3. Spores (sub)globose, Q < 1.2 4
3. Spores ellipsoid to elongate-lacrymoid, Q > 1.2 5
    4. Spores 6-8 µm diam. (type: Brazil) .....*H. sphaerospora*
    4. Spores 9-12.5 x 8.5-11.5 µm (type: Taiwan).....*H. globispora*
5. Spores 8.5-13 x 5-8 µm, Q 1.22-*1.54*-1.73 (type: Poland).....*H. graminicola*
5. Spores 9.5-17 x 4.5-7.5 µm, Q 1.57-*2.08*-2.61 (type: Belgium).....*H. jozefii*

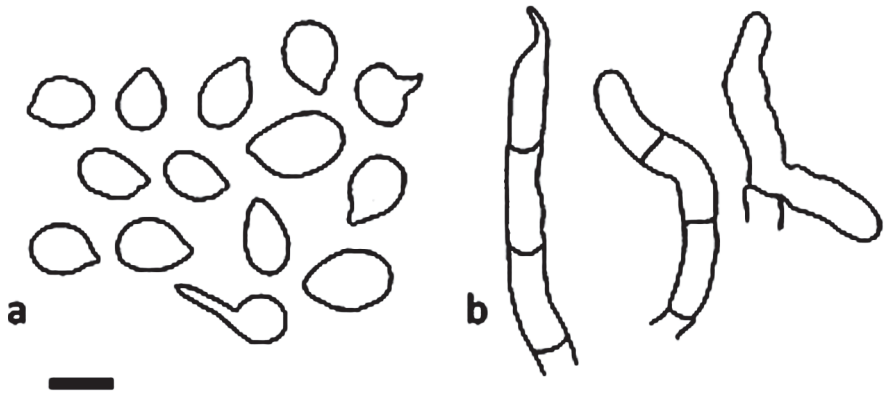

Fig. 4. *Helicogloea graminicola* (holotypus). **a.** Spores. **b.** Basidium and basidioles. Scalebar = 10 µm.

Spiculogloea inaequalis Schoutteten & Verbeken **sp. nov.** **Figs 5-6**

Mycobank: MB825551

Etymology: Named after the unequal basidial cells in the mature septate basidia.

Diagnosis: differs from all other described *Spiculogloea* species by its rather short but wide, spiculate basidia and the comparatively wide, (sub)fusoid spores.

Holotypus: Belgium. Prov. Oost-Vlaanderen, Ronse, Sint-Pietersbos, coordinates: N50.762233, E3.659919, inside hymenial tissue of *Sistotrema* sp. on a dead branch of deciduous wood, 13/03/2017, N. Schoutteten 17-132a (GENT).

Basidiocarp absent.

Hyphae thin-walled, hyaline, 1-1.5 µm diam. **Haustorial cells** growing at hyphal ends, slightly thickened at their base, 1.5-1.8 µm wide. **Basidia** stichic, transversally septate, triseptate, often with one of the cells remarkably broader and swollen, 20-26 x 6-9 µm, sometimes very shortly stalked (stalk of variable length), cyanophilous, slightly thick-walled, spiculate, cylindrical to curved, not clustered, sometimes with oil inclusions in the cells; sterigmata very rarely observed, laterally attached, slightly curved to sinuous, up to 17 µm long, up to 1.2 µm wide. **Basidiospores** scarce, smooth, thin-walled, some slightly curved, 10.0-*11.7*-13.0 x 3.5-*3.8*-4.2 µm, subcylindric to (sub)fusoid, apiculate, non-amyloid, forming secondary spores, rarely one-septate. **Cystidia** absent. **Clamps** present. Asexual stage not observed.

Additional studied material: BELGIUM. Prov. Oost-Vlaanderen, Ronse, Sint-Pietersbos, coordinates: N50.762233, E3.659919, in *Sistotrema* sp. on a dead branch of deciduous wood, 13/03/2017, NS-17-133 (GENT); Berlare, Donkmeer, coordinates: N51.027847, E3.974454, in *Sistotrema* sp. on a dead branch of deciduous wood, 06/03/2018, NS-18-040 (GENT).

Commentary: The genus *Spiculogloea* was originally described from Majorca (Roberts 1996) to accommodate the mycoparasite *S. occulta*, parasitic on *Kurtia argillacea* (Bres.) Karasiński. The genusname refers to the spiculated aspect of the basidial outer wall. The same author (Roberts 1997) described *S. minuta* from England, parasitic on *Tulasnella violea* (Quél.) Bourdot & Galzin. Hauerslev (1999) described *S. subminuta* from Denmark, parasitic on *Botryobasidium subcoronatum* (Höhn. & Litsch.) Donk. Finally, Trichies (2006) described *S. limonispora* from France, parasitic on *K. argillacea*.

Since then, multiple collections of *Spiculogloea* species were made by several authors in different European countries: Belgium (Van De Put 1998a, 2005), Germany (Langer & Oberwinkler 1998; Grosse-Brauckmann 2002; Rödel 2014), France (Trichies 1997, 2002), England (Roberts 1997, 2001), Finland (Kotiranta *et al.* 2009), Russia and Sweden (Spirin *et al.* 2016), as well as in Canada (Bandoni *et al.* 2002). As with most other intrahymenial fungi, specimens are inconspicuous and can only be found by chance as a result of microscopic examination of the host species or by sequencing. The genus is characterised by its very narrow, clamped hyphae, the (stalked) spiculate, cyanophilous basidia arising from small probasidia, and *Tremella*-like haustorial cells. These haustorial cells are attached to the hyphae of the host, as illustrated by Bauer *et al.* (2006). It should be noted that there is some variation in the degree of the spiculated aspect of the basidia in *S. subminuta*. Hauerslev (1999) indicated this in his type description (basidia "smooth or often asperulate"), though his drawings all showed spiculated basidia. Grosse-Brauckmann (2002) reported *S. subminuta* as new to Germany, but the basidia in her specimen

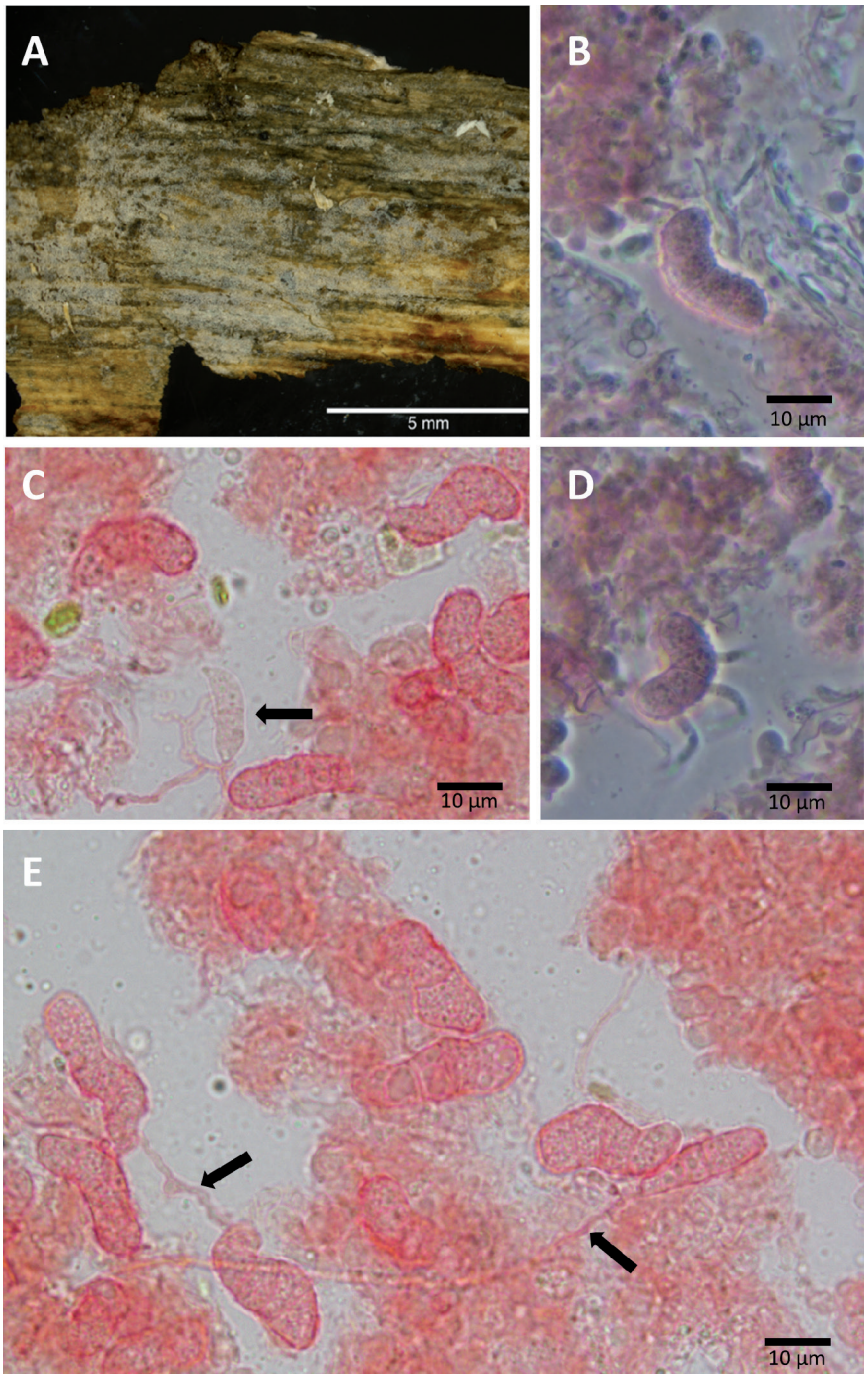

Fig. 5. *Spiculogloea inaequalis* (holotypus). **a.** Fresh basidiocarp of *Sistotrema* sp. **b.** Basidium. **c.** Basidia and septated spore (black arrow). **d.** Basidium with sterigmata. **e.** Basidia and hyphae (black arrows).

were described and illustrated as smooth. Also Van De Put (1998b, as *Occultifur* sp.) reported the same taxon but identified it incorrectly because of the smooth basidia.

A comparison of *S. inaequalis* with all previously described species is given in Table 1. Our species is characterised by its relatively short but rather wide basidia, not appearing in dense clusters. All basidia were septate but only some were producing sterigmata, as in most species. The spores are also broader than those of *S. minuta*, the species most closely resembling *S. inaequalis*.

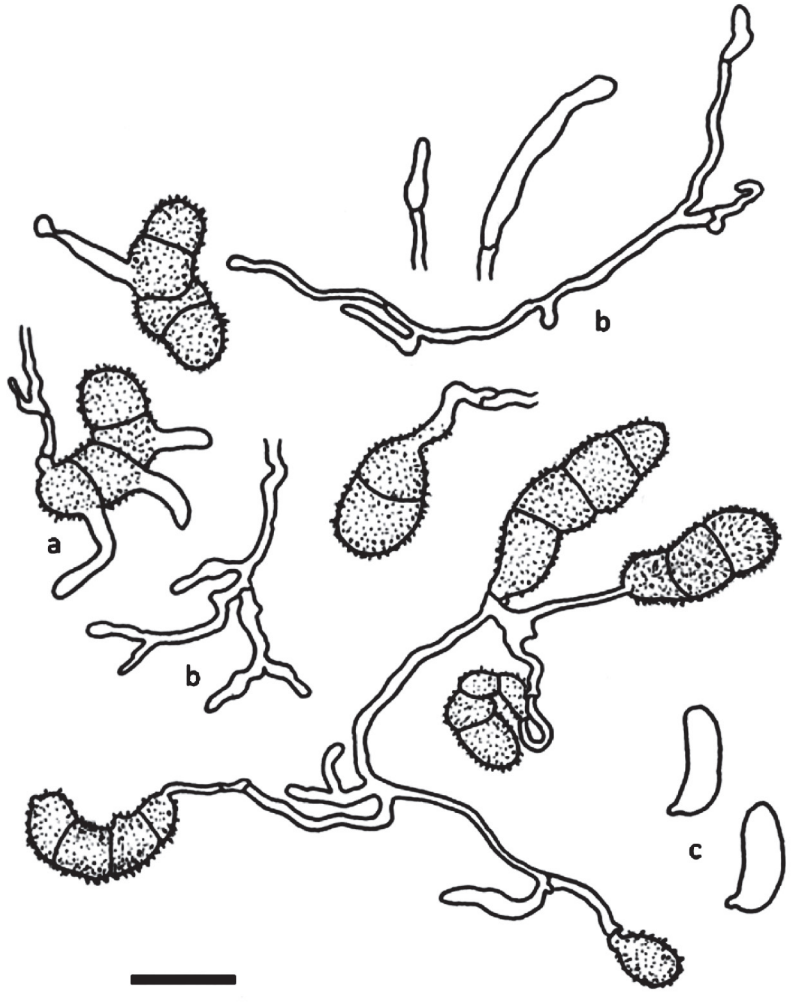

Fig. 6. *Spiculogloea inaequalis* (holotypus). **a.** Basidia. **b.** Hyphae with haustoria. **c.** Spores. Scalebar = 10 µm.

Table 1. Comparison of *Spiculogloea* species. The data included in this table are derived from the original descriptions (Roberts, 1993; Roberts, 1997; Hauerslev, 1999; Trichies, 2006)

Species	*Spores*	*Basidia*	*Conidia*
S. occulta	3.5-7.5(-8.5) ξ 2.5-4.0(-5.0) µm	< 45 µm	3.5-5.0 ξ 2.0-2.5 µm
S. minuta	(6.5-)8.5-12 ξ 2-2.5(-3) µm	12-25 ξ 3-3.5 µm	-
S. subminuta	8.5-10(-13) ξ 2-3 µm	30-65 ξ 3-4 µm	4.5-5 ξ 2.5-3 µm
S. limonispora	6-7.75-9 ξ (3.5) 4-5 (6) µm	(27)30-45 ξ 4.5-6(-7) µm	-
S. inaequalis	10-13 ξ 3.5-4 µm	20-26 ξ 6-9 µm	-

Key to the species of *Spiculogloea.*

1. Basidia as a rule > 30 µm in length.....2
1. Basidia as a rule < 30 µm in length.....4
   2. Spores widely citriform, clearly biapiculate, 6-9 x (3.5)-4-5(-6) .....*S. limonispora*
   2. Spores subfusoid to subcylindric, subglobose to ovoid, amygdaliform 3
3. Spores narrowly cylindric to (sub)fusoid, < 3 µm wide. Basidia smooth to (finely) spiculate.....*S. subminuta*
3. Spores variable in shape: subglobose, ovoid-elliptic, amygdaliform, usually > 3 µm wide. Basidia always spiculate .....*S. occulta*
   4. Basidia as a rule < 4 µm wide. Spores < 3 µm wide.....*S. minuta*
   4. Basidia ≥ 6 µm wide. Spores ≥ 3 µm wide .....*S. inaequalis*

DISCUSSION

Unfortunately we were not able to perform a molecular analysis. Our attempts to extract DNA from the *Helicogloea* specimens failed and has not been attempted for the intrahymenial *Spiculogloea*. Furthermore, publicly deposited sequences for correctly identified specimens in either genus are lacking for the moment.

Molecular studies in *Helicogloea* have only been undertaken for a few species to date. These findings indicate that the genera *Helicogloea*/*Saccoblastia* belong to the ecological and morphologically diverse class Atractiellomycetes (Pucciniomycotina), together with e.g. *Atractiella* spp. and *Phleogena faginea* (Weiss *et al.*, 2004; Bauer *et al.*, 2006; Aime *et al.*, 2006). A distinction between *Helicogloea* and *Saccoblastia* based on hymenial texture has not been confirmed, but species assigned to the two genera have been referred to two separate families (Phleogenaceae and Saccoblastiaceae) based on both molecular and ultrastructural characters (Weiss *et al.* 2004). In recent studies incorporating SSU, LSU and ITS sequences, three endopytic fungal species belonging to the Atractiellomycetes were described (Aime *et al.*, 2018; Bonito *et al.*, 2017) which provided more insights in the evolutionary relationships within the Atractiellomycetes. Aime *et al.* (2018)

described some new genera to accommodate species previously placed in *Helicogloea,* distributed over the families Helicogloeaceae and Phleogenaceae. Incorporation of a larger diversity of *Helicogloea* species in molecular studies will result in a better understanding of generic limits and relationships among species and genera.

The genus *Spiculogloea* was provisionally placed within the Platygloeales (Roberts, 1996). Molecular studies by Weiss *et al.* (2004) based on SSU and LSU sequence data indicated that an unnamed *Spiculogloea* sp. clustered together with an equally unnamed *Mycogloea* sp., forming a well-supported clade which was later referred to as the new order Spiculogloeales (Bauer *et al.* 2006). Together with Agaricostilbales*,* Spiculogloeales composed the class Agaricostilbomycetes. Aime *et al.* (2006) confirmed these relationships and added some anamorphic species of *Sporobolomyces* to the Spiculogloeales. A recent molecular study based upon seven genes (Wang *et al.* 2015a) indicated that the systematic position of the Spiculogloeales varied. However, hitherto, the single available sequence for the genus *Spiculogloea* is still the original LSU sequence from Weiss *et al.* (2004). Wang (2015a) suggested that the order might be a deep/ancient lineage, basal to the remainder of Agaricostilbomycetes and, therefore, that it represents a distinct class, formally describing the Spiculogloeomycetes (Wang *et al.* 2015b). This view adds support to an early hypothesis formulated by both Bauer (2006) and Aime (2006) that the Agaricostilbomycetes are not monophyletic. More sequencing, including of the type species, for most of these genera of heterobasidiomycetous Pucciniomycotina is needed to confirm monophyly of the various genera.

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