

# Three new species of *Hydropisphaera* (*Bionectriaceae*) from Europe and French Guiana

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**Abstract:** Three new species of *Hydropisphaera* are described and illustrated, based on material collected in Europe and French Guiana. The three species were cultured and cultures were sequenced. Based on morphological and phylogenetic comparison with the known *Hydropisphaera* species, we propose *H. cirsi*, *H. pseudoarenula* and *H. saulensis* as new species. An updated dichotomous key to the worldwide known species of *Hydropisphaera* is proposed.

**Keywords:** *Acremonium*, Ascomycota, *Hypocreales*, ribosomal DNA, taxonomy.

**Résumé :** trois nouvelles espèces d'*Hydropisphaera* sont décrites et illustrées à partir de récoltes effectuées en Belgique, en France métropolitaine et en Guyane française. Les trois espèces ont été cultivées et les cultures ont été séquencées. Sur la base d'une comparaison morphologique et phylogénétique avec les espèces d'*Hydropisphaera* connues, nous proposons *H. cirsi*, *H. pseudoarenula* and *H. saulensis* comme espèces nouvelles. Une clé dichotomique des espèces d'*Hydropisphaera* connues dans le monde est proposée.

**Mots-clés :** *Acremonium*, ADN ribosomal, Ascomycota, *Hypocreales*, taxinomie.

## Introduction

ROSSMAN *et al.* (1999) resurrected *Hydropisphaera* Dumort. as a distinct genus in the *Bionectriaceae* (*Hypocreales*) for species of nectria-like fungi that had previously been placed in the “*Nectria peziza* group” (BOOTH, 1959; SAMUELS, 1976b; ROSSMAN, 1983) and recognized eighteen species of *Hydropisphaera*. Since then, eleven additional species were introduced from both temperate and tropical areas by LECHAT & FOURNIER (2016; 2017a; 2017b), LECHAT & GARDIENNET (2009), LECHAT *et al.* (2010), LUO & ZHUANG (2010), NONG & ZHUANG (2005), ROSSMAN *et al.* (2008), TAYLOR & HYDE (2003), ZENG & ZHUANG (2016) and ZHUANG (2000). Within the *Bionectriaceae*, *Hydropisphaera* is characterized by non-stromatic, perithecial, superficial ascomata with an ascotal wall up to 110 µm thick, composed of two distinct regions with outer region composed of large, thin-walled cells and inner region of flattened hyaline cells, involving a cupulate collapse of ascomata upon drying; the asexual morph is acremonium-like or gliomastix-like (LECHAT *et al.*, 2010; ROSSMAN *et al.*, 1999).

The three new species documented herein conform well to this morphological concept and their placement in *Hydropisphaera* is supported by our phylogenetic comparison of their LSU sequences with thirteen species representing this genus (Fig. 1). We present in this paper the results of our macro- and micromorphological observations coupled with cultural characteristics and molecular data. Their affinities and differences with their closest relatives are discussed, leading to their recognition as new species.

## Materials and methods

Dry specimens were rehydrated and examined using the method described by ROSSMAN *et al.* (1999). Microscopic observations and measurements were made in water. The holotype specimen and paratypes were deposited in LIP herbarium (University of Lille) and living cultures in the CBS Collection of the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands,) or at CIRM (Centre International des Ressources Microbiennes, Marseille, France). Cultures of the living specimens were made on PDA (Potato Dextrose Agar) with 5 mg/L of streptomycin in Petri dishes 5 cm diam. incubated at 25 °C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Oviedo, Spain). Total DNA was extracted from pure cultures, blending a portion of them using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65°C. A similar volume of chloroform:isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a

new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH<sub>2</sub>O. PCR amplification was performed with the primers LR0R and LR5 (VILGALYS & HESTER, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) and a final 72°C step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected.

Analyses were performed online at [www.phylogeny.lirmm.fr](http://www.phylogeny.lirmm.fr) (DEREEPER *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (ZWICKL, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (ANISIMOVA & GASCUEL, 2006). Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

## Taxonomy

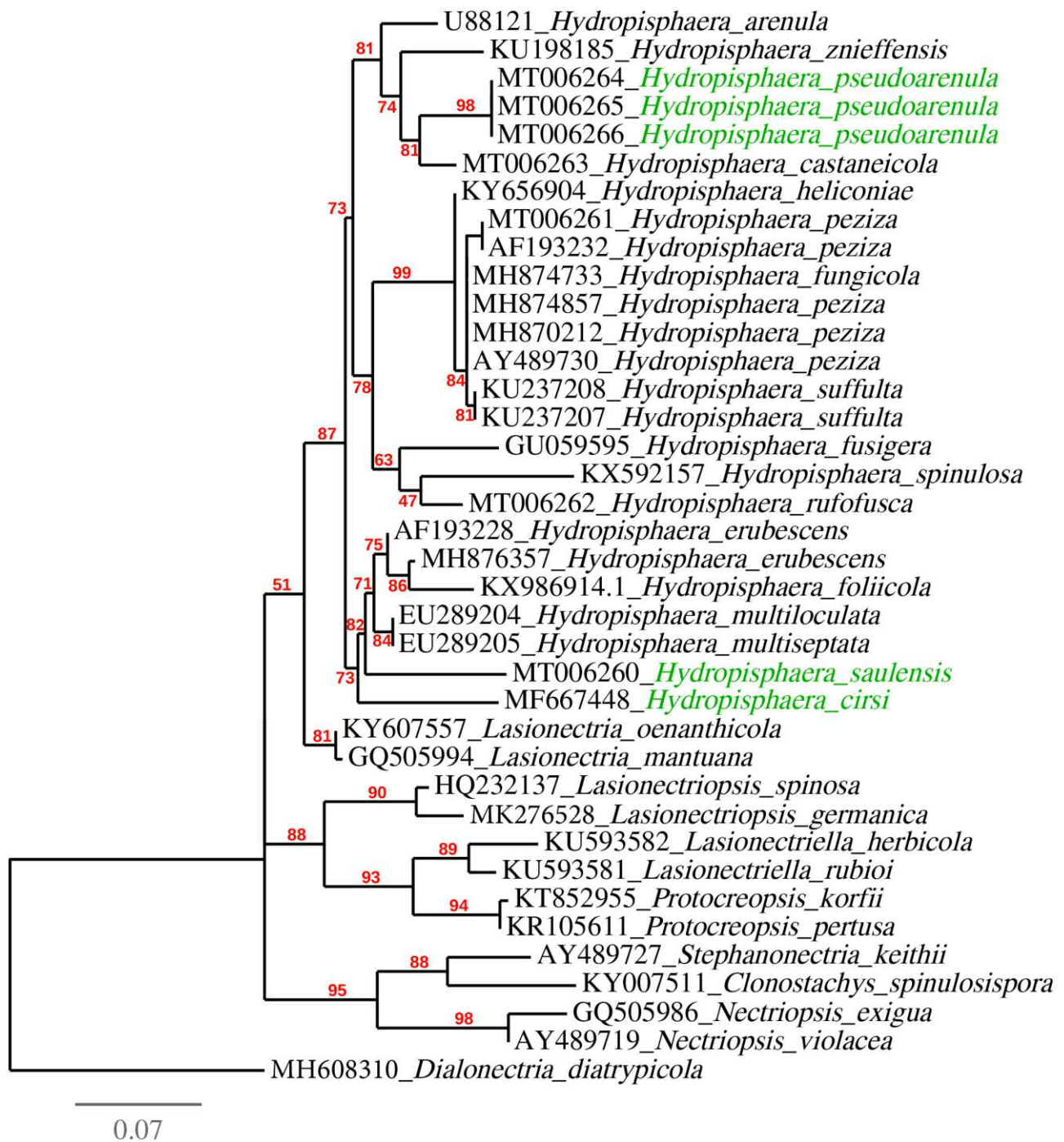
*Hydropisphaera cirsi* Lechat & J. Fourn, *sp. nov.* – MB 834181 – Fig. 2

**Diagnosis:** *Hydropisphaera cirsi* differs from known species of *Hydropisphaera* in having erect, glassy hairs scattered on lateral ascotal wall, finely spinulose ascospores and occurrence on *Cirsium arvense*.

**Holotype:** GERMANY: North Rhine-Westphalia, MTB 4506/441 Duisburg, city-forest, Uhlenhorstweg, S of cultural monument ‘Steinbruch’, 51°41’27”N, 6°80’41”E on *Cirsium arvense* (L.) Scop. (*Asteraceae*), 17 Feb. 2013, *leg.* K. Müller, communicated by K. Siepe, CLL13012 (LIP), ex-holotype culture CBS135615; GenBank LSU sequence: MF667448.

**Etymology:** The epithet “*cirsi*” refers to the host *Cirsium*.

**Ascomata** gregarious, superficial, subglobose, (160–)180–240 (–260) µm high × (150–)160–220(–240) µm diam. (Me = 210 × 195 µm, n = 20), pale yellow to pale brownish orange, cupulate when dry, not changing colour in 3% KOH or lactic acid, with erect, glassy hairs covering lateral ascotal surface. **Ascomatal apex** conical, composed of a palisade of cylindrical cells rounded at tip. Hairs 14–47 µm long, 3–3.5(–4) µm wide, hyaline, cylindrical, thick-walled, rounded at tip, septate. **Ascomatal wall** 30–45(–50) µm thick, composed of two regions: outer region 25–30 µm wide, of globose to ellipsoidal cells 4.5–10 × 3.5–7.5 µm, with pale brownish orange walls 1–1.5 µm thick; inner region 10–15 µm wide, of ellipsoidal, flattened cells 6–11 × 1.5–3 µm, with hyaline walls 0.5–



**Fig. 1** – Maximum likelihood phylogeny ( $-\ln L = 2025.63218$ ) of *Hydropisphaera* spp. inferred by PhyML 3.0, model HKY85 from a 820 bp matrix of 28S rRNA sequence, rooted with *Dialonectria diatrypicola*.

1.5  $\mu\text{m}$  thick. **Asci** (65–)70–80(–85)  $\times$  (8–)9–10(–12)  $\mu\text{m}$  (Me = 74.5  $\times$  9.5  $\mu\text{m}$ , n=30), short-stipitate, clavate, attenuated at apex with a ring-like apical thickening, containing eight irregularly biseriolate ascospores completely filling each ascus. **Ascospores** (15–)16–18(–19.5)  $\times$  4.5–5  $\mu\text{m}$  (Me = 17.4  $\times$  4.8  $\mu\text{m}$ , n=30), fusiform with rounded ends, slightly curved, 1-septate, hyaline, finely spinulose.

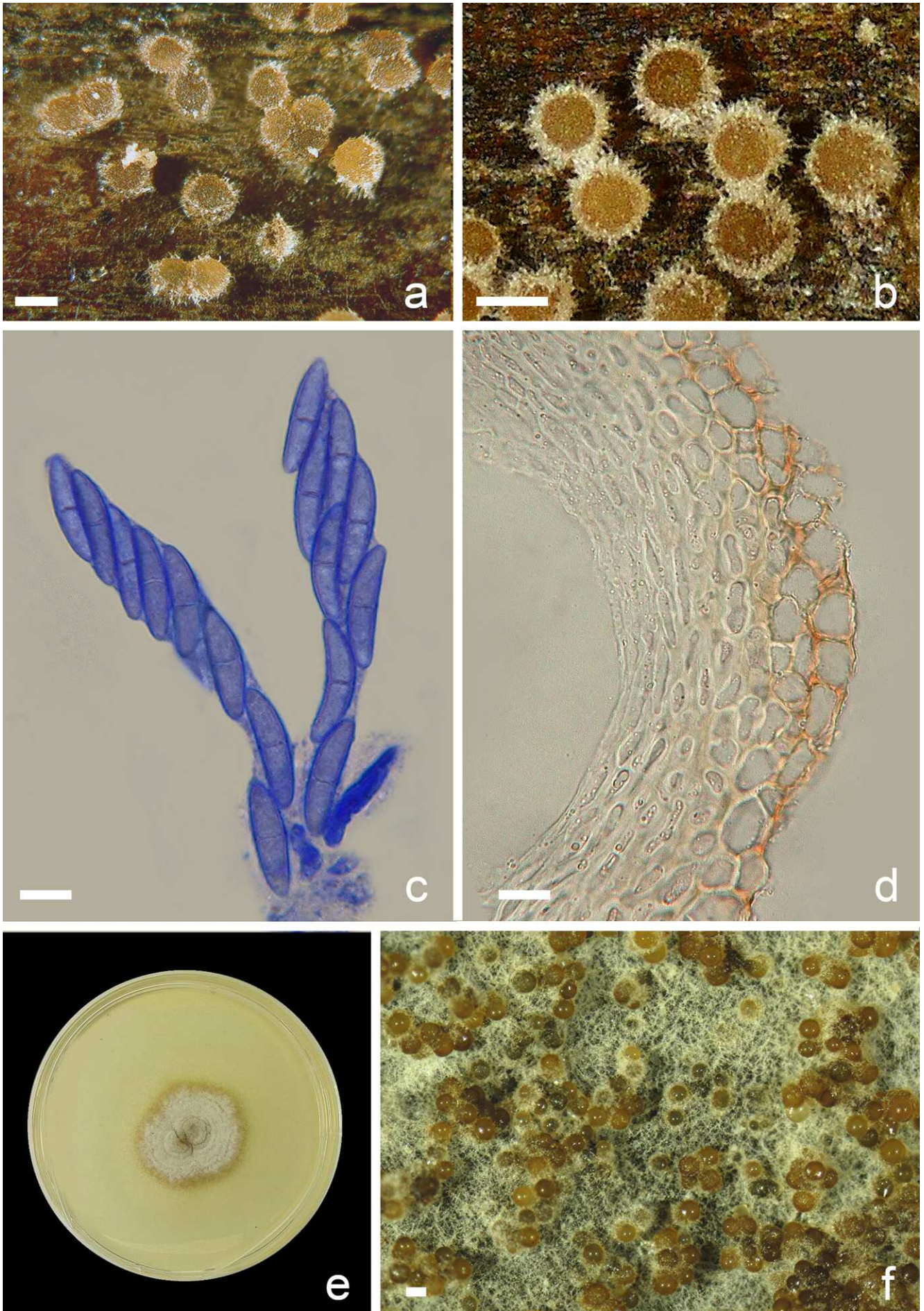
**Cultural characteristics:** After two weeks at 25°C on Difco PDA containing 5 mg/L streptomycin, colony 2.5–3.5 cm diam., not diffusing coloration in medium, mycelium white to pale greyish brown in centre, greyish brown at margin. No conidia produced in culture after three weeks, fertile ascomata appearing after five weeks.

***Hydropisphaera pseudoarenula*** Lechat & J. Fourn., sp. nov. – MB 834182 – Fig. 3

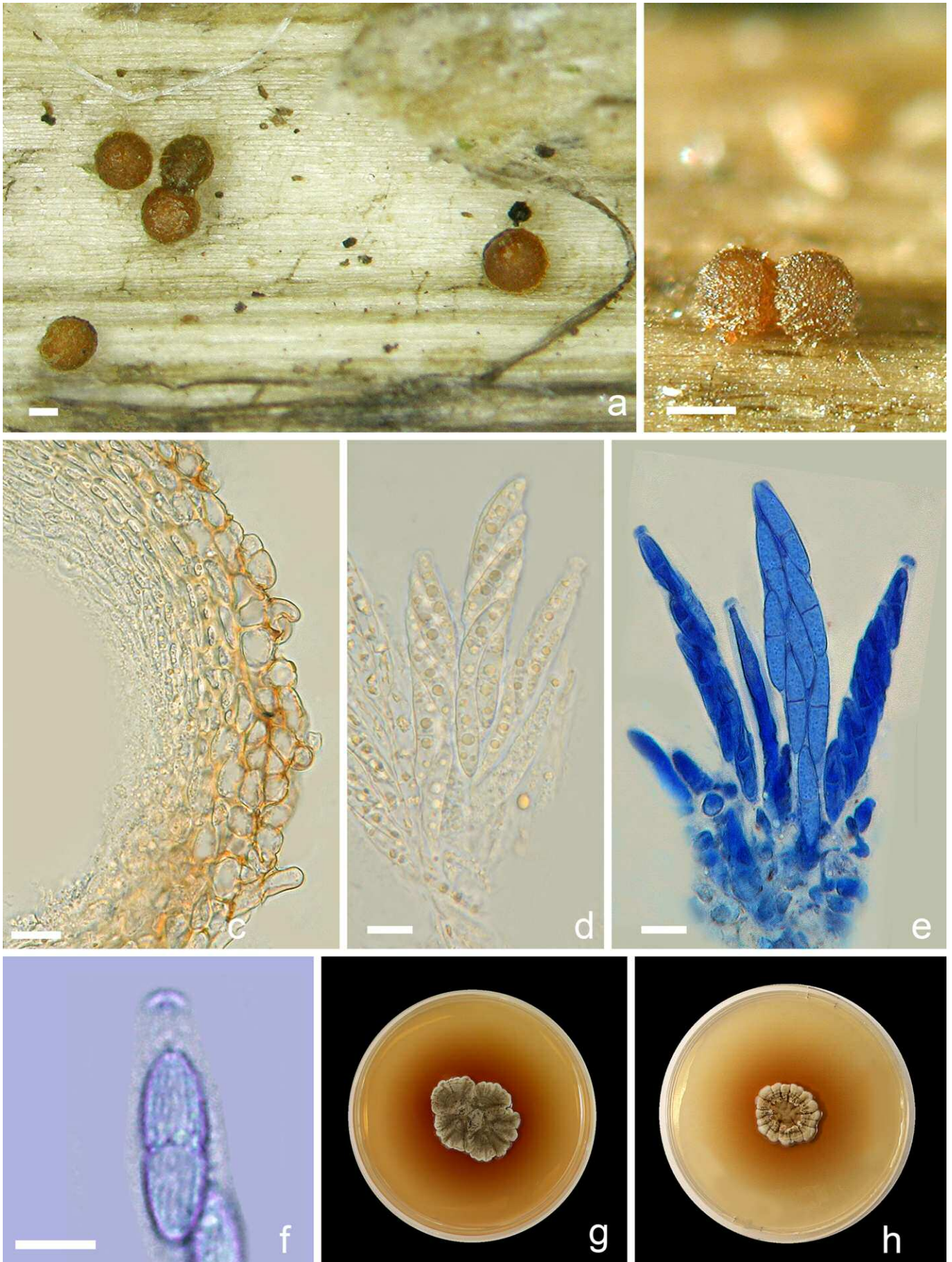
**Diagnosis:** *Hydropisphaera pseudoarenula* resembles *H. arenula*, from which it differs in having finely spinulose and larger ascospores (17–)18–20(–22)  $\times$  4.5–5  $\mu\text{m}$  vs. striate ascospores (12.5–)14–16(–21)  $\times$  3.5–4(–4.5)  $\mu\text{m}$ .

**Holotype:** BELGIUM, Stekene, on a dead stem of *Urtica dioica* L. (*Urticaceae*), 26 Sep. 2019, leg. M. Hairaud CLL19022 (LIP); GenBank LSU sequence MT006264.

**Etymology:** from Greek *pseudo-* = lying, false, in reference to the macro-morphological similarity to *H. arenula*.



**Fig. 2** – a-f: *Hydropisphaera cirsi*; a: Rehydrated ascomata on the substrate; b: Close-up of fresh ascomata in natural environment (Photo: K. Siepe); c: Asci and ascospores in lactac cotton blue; d: Vertical section through lateral ascomatal wall; e: Culture at three weeks; f: Ascomata from pure culture after five weeks. Scale bars: a-b, f = 200  $\mu$ m; c-d = 10  $\mu$ m.

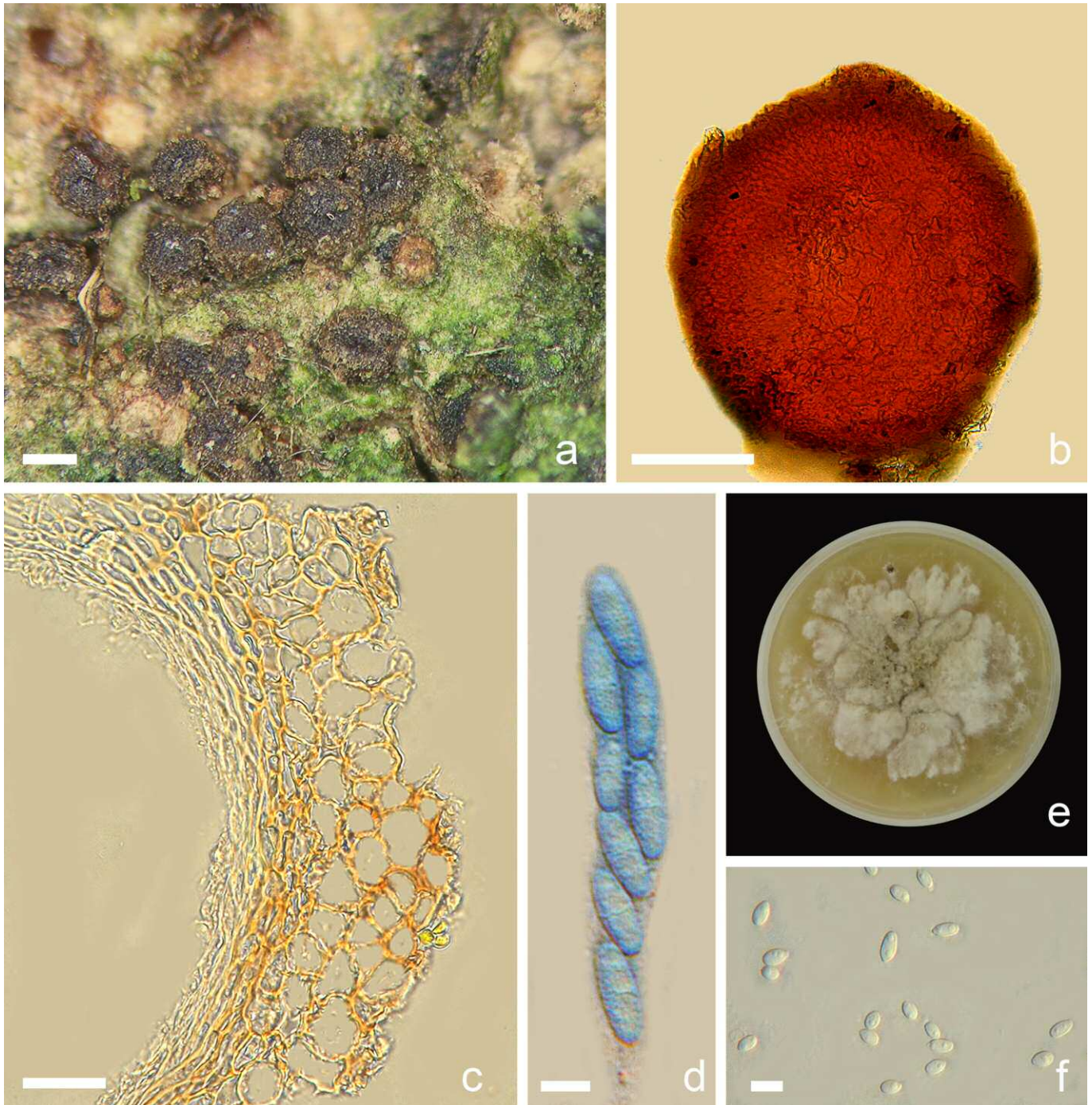


**Fig. 3** – a-e, g, h: *Hydropisphaera pseudoarenula*; a: Ascomata on the substrate; b: Close-up of ascomata in natural environment; c: Vertical section through lateral ascumatal wall; d: Asci and ascospores in water; e: Asci and ascospores in lactic cotton blue; f: *H. arenula* (JF08061), tip of ascus and striate ascospore (in dilute blue ink); g, h: Cultures at three weeks; g: CLL19020, h: CLL11052. Scale bars: a = 100  $\mu$ m; b = 200  $\mu$ m; c-e = 10  $\mu$ m; f = 5  $\mu$ m.

**Ascomata** solitary to gregarious, superficial, subglobose, (160–)180–230(–240)  $\mu\text{m}$  high  $\times$  (150–)160–200(–230)  $\mu\text{m}$  diam. (Me = 220  $\times$  190  $\mu\text{m}$ , n = 20), pale brownish orange, becoming dark brownish orange to dark brown, cupulate when dry, not changing colour in 3% KOH or lactic acid, with cylindrical, septate hairs, 10–30  $\times$  4–5  $\mu\text{m}$ , rounded at free ends, scattered on lateral wall of ascomata. **Ascomatal apex** rounded with a minute papilla, composed of a palisade of cylindrical to narrowly clavate, hyaline to pale yellow cells, rounded at tip. **Ascomatal surface** composed of globose to subglobose, angular, thin-walled cell up to 17  $\mu\text{m}$  in greatest dimension. Ascomatal wall 35–45(–50)  $\mu\text{m}$  thick, composed of two regions: outer region 25–30(–35)  $\mu\text{m}$  wide, of globose to ellipsoidal cells 5–12  $\times$  4–9  $\mu\text{m}$ , with pale brownish orange walls 1–1.5  $\mu\text{m}$  thick; inner region 10–15  $\mu\text{m}$  wide, of ellipsoidal, flattened cells 6–11  $\times$  1.5–3  $\mu\text{m}$ , with hyaline wall 0.5–1.5  $\mu\text{m}$  thick. **Asci** (50–)55–65(–70)  $\times$  8–

10(–12)  $\mu\text{m}$  (Me = 60  $\times$  9.5  $\mu\text{m}$ , n=20), clavate, slightly attenuated at apex, with an apical thickening when immature, containing eight irregularly biseriolate ascospores completely filling each ascus. **Ascospores** (16–)17–20(–22)  $\times$  4.5–5  $\mu\text{m}$  (Me = 18.5  $\times$  4.8  $\mu\text{m}$ , n=50), fusiform with rounded ends, slightly curved, 1-septate, with 2(–3) pale orange oily droplets in each cell, hyaline, appearing smooth in water but proving finely spinulose when observed in lactic cotton blue.

**Cultural characteristics:** After three weeks, colony 2.5–3.5 cm diam., diffusing a rust to reddish brown coloration in medium, mycelium grey to pale greyish brown in centre, pale brown in middle area and white at margin. No conidia produced in culture after three weeks.



**Fig. 4** – a-f: *Hydropisphaera saulensis* (CLLG19028-d, Holotype); a: Ascomata in natural environment; b: Close-up of an ascoma in side view, in water; c: Vertical section through lateral ascomatal wall; d: Ascus and ascospores in lactic cotton blue; e: Culture at three weeks; f: Conidia from culture, in lactic acid. Scale bars: a = 200  $\mu\text{m}$ ; b = 100  $\mu\text{m}$ ; c = 20  $\mu\text{m}$ ; d, f = 5  $\mu\text{m}$ .

## Key to species of *Hydropisphaera*

(The numbers in brackets after species names refer to the bibliography)

Ascomata with fasciculate or solitary hairs on the ascomatal wall; ascospores smooth, striate or spinulose.....	1
Ascomata glabrous; ascospores striate, spinulose or verrucose.....	13
1. Ascomata with solitary, erect, glassy hairs 14–47 µm long, 3–3.5(–4) µm wide; ascospores 16–18 × 4.5–5 µm, spinulose (Germany) .....	1
..... <i>H. cirsii</i> (this paper)	
1. Ascomata with fasciculate hairs .....	2
2. Ascospores more than 25 µm long on average .....	3
2. Ascospores less than 25 µm long on average .....	6
3. Ascomata dark red with red hairs; ascospores spinulose-striate (Rwanda, Congo) .....	<i>H. haematites</i> (12)
3. Ascomata pale orange, dark orange to brown, with concolorous or hyaline hairs; ascospores smooth-walled to finely striate .....	4
4. Ascomata brown with brown hairs; ascospores 25–38 × 5–7 µm (Colombia, Indonesia) .....	<i>H. dolichospora</i> (12; 14)
4. Ascomata orange to dark orange, with orange to hyaline hairs; ascospores more than 38 µm long .....	5
5. Ascomata dark orange with orange hairs; ascospores 48–55 × 6–7 µm (Argentina) .....	<i>H. gigantea</i> (12)
5. Ascomata pale orange with hyaline hairs; ascospores 38–50 × 5–6.4 µm (China) .....	<i>H. sinensis</i> (17)
6. Ascospores ≤ 17 µm long on average .....	7
6. Ascospores > 17 µm long on average .....	10
7. Ascospores spinulose, 12.5–17.5 × 3.5–4 µm; ascomata pale brownish orange .....	<i>H. rufofusca</i> (14)
7. Ascospores striate .....	8
8. Ascomata dark brownish orange; ascospores 14.5–17.8 × 4.5–5 µm (French West Indies) .....	<i>H. heliconiae</i> (5)
8. Ascomata pale orange to orange with white or orange fasciculate hairs .....	9
9. Ascospores ellipsoid, 12–17 × 4–5 µm, smooth to striate (tropical regions) .....	<i>H. suffulta</i> (11; 12)
9. Ascospores narrowly fusiform, spinulose, 15–22 × 2.4–3.6(–4) µm (China) .....	<i>H. yunnanensis</i> (6)
10. Ascospores aseptate; asexual morph gliomastix-like (French West Indies) .....	<i>H. fusigera</i> (1)
10. Ascospores one-septate .....	11
11. Ascomata orange with orange hairs; ascospores 17–23 × 5–7 µm, striate (New Zealand) .....	<i>H. cyatheae</i> (11)
11. Ascomata yellow to nearly brown with white hairs; ascospores verrucose or striate .....	12
12. Ascospores striate, (12–)16–22(–26) × 4–5(–6) µm (Indonesia) .....	<i>H. leucotricha</i> (14)
12. Ascospores verrucose, 16–18 × 7–8 µm (French West Indies) .....	<i>H. znieffensis</i> (3)
13. Ascospores spinulose to verrucose .....	14
13. Ascospores striate .....	19
14. Ascospores verrucose, 1–3-septate .....	15
14. Ascospores spinulose, 1-septate .....	16
15. Ascospores 1-septate, 10–11(–12) × 3.5–4 µm; ascomata dark brownish orange to blackish brown (French Guiana) .....	<i>H. saulensis</i> (this paper)
15. Ascospores 1–3-septate, 15–17 × 4–4.7 µm; ascomata pale yellow (French West Indies) .....	<i>H. foliicola</i> (4)
16. Ascospores 17–20 × 4.5–5 µm; ascomata pale brownish orange to dark brown (Europe) .....	<i>H. pseudoarenula</i> (this paper)
16. Ascospore less than 16 µm long .....	17
17. Ascospores less than 3.5 µm wide on average; ascomata pale dirty orange; ascospores narrowly ellipsoid-fusiform, 9.4–13.7 × 2.5–3.5 µm (China).....	<i>H. jigongshanica</i> (7)
17. Ascospores more than 4 µm wide on average .....	18
18. Ascospores (10–)12–15 × (3–)3.2–5 µm; ascomata orange yellow to reddish brown (China) .....	<i>H. spinulosa</i> (16)
18. Ascospores 8–11 × 5–6 µm; ascomata orange to reddish brown (France) .....	<i>H. castaneicola</i> (2)
19. Ascospores 1-septate .....	20
19. Ascospores 3-septate to multiseptate .....	27
20. Ascospores less than 20 µm long on average .....	21
20. Ascospores more than 20 µm long on average .....	24
21. Ascospores 8.9–10.6 × 4.3–5.9 µm; ascomata yellow to dark brown (U.S.A.) .....	<i>H. fungicola</i> (9)
21. Ascospores more than 11 µm long .....	22
22. Ascospores 14–16 × 3.5–4 µm; ascomata orange to brownish orange (cosmopolitan) .....	<i>H. arenula</i> (13)
22. Ascospores more than 4 µm wide .....	23
23. Ascospores 11–14 × 5–6 µm; ascomata orange to reddish orange, with the base immersed in the substrate (Indonesia, Mexico) .....	<i>H. hypoxantha</i> (14)
23. Ascospores 11–14 × 5–7 µm; ascomata yellow to orange, easily removed from the substrate (cosmopolitan) .....	<i>H. peziza</i> (10; 12)
24. Ascospores 19–22 × 4–4.5 µm; ascomata orange (New Zealand) .....	<i>H. arenuloides</i> (11)
24. Ascospores more than 22 µm long .....	25
25. Ascospores with lateral cilia, 23–32 × 5–7 µm; ascomata smooth to scaly (Australia) .....	<i>H. ciliata</i> (15)
25. Ascospores without cilia .....	26
26. Ascospores 23.5–30 × 5.6–7.3 µm; ascomata orange, on a minute basal stroma (New Zealand, Indonesia) .....	<i>H. macrarenula</i> (14)
26. Ascospores 23–27 × 5–6 µm; ascomata reddish orange, lacking a basal stroma (Indonesia) .....	<i>H. nymaniana</i> (12; 14)
27. Ascospores 3-septate .....	28
27. Ascospores 5–19-septate .....	29
28. Ascospores 18–26 × 4–5 µm; ascomata pale orange (temperate regions) .....	<i>H. erubescens</i> (8; 13)
28. Ascospores 65–92 × 6–8 µm; ascomata orange to brown-vinaceous (Colombia) .....	<i>H. pachyderma</i> (8)
29. Ascospores 5–7(–9)-septate, 28–38 × 4–6 µm (New Zealand) .....	<i>H. multiseptata</i> (8; 13)
29. Ascospores 11–19-septate, 50–70(–80) × 6–7(–8–11) µm (Ecuador, New Zealand, Peru) .....	<i>H. multiloculata</i> (8; 13)

**Additional specimens examined:** FRANCE, Charente Maritime, Île de Ré, on dead stem of *Smyrniolum olusatrum* L. (*Apiaceae*), 28 Apr. 2011, *leg.* M. Pennamen CLL11052 (LIP), culture CBS 130334, GenBank LSU sequence MT006265; Côte-d'Or, Véronnes, on dead stems of *Galium aparine* L. (*Rubiaceae*), 20 Jun. 2009, *leg.* A. Gardiennet AG09162 (LIP), GenBank LSU sequence MT006266.

*Hydropisphaera arenula*: FRANCE, Deux-Sèvres, L'Hermitain, la Dame de Chambrille, ruisseau de l'Hermitain, 17 Apr. 2008, on submerged wood of *Alnus glutinosa* (L.) Gaertn. (*Betulaceae*), *leg.* J. Fournier and M. Delpont, *det.* A. Rossman, JF 08061.

***Hydropisphaera saulensis*** Lechat & J. Fourn., *sp. nov.* – MB 834184 – Fig. 4

**Diagnosis:** Differs from all known species of *Hydropisphaera* in having dark brown ascomata with large, hyaline to pale yellow cells partially covering lateral wall, and verrucose ascospores.

**Holotype:** FRENCH GUIANA, Saül, Gros Arbres trail, 18 Jun. 2019, on bark of unidentified dead tree, *leg.* C. Lechat CLLG19028-d (LIP), ex-holotype culture BRFM3053, GenBank LSU sequences: MT006260.

**Etymology:** *saulensis* refers to the village of Saül (French Guiana) where this species was collected.

**Ascomata** scattered on substratum or in groups of 5–15, coalescent, non-stromatic, globose, 280–320 µm diam., dark brownish orange, becoming blackish brown collapsing cupulate when dry, not changing colour in 3% KOH or lactic acid, partially covered by a scurf of large, globose to ellipsoidal, hyaline to pale yellow cells up to 20 µm in greatest dimension. **Ascomatal apex** rounded with short, acute papilla composed of cylindrical to narrowly clavate, hyaline cells. **Ascomatal wall** 45–60 µm thick, of two regions: outer region 30–40 µm thick, composed of subglobose to globose, thin-walled cell, 8–15 × 4–10 µm, with pale orange wall 1–1.5 µm thick; inner region 10–20 µm thick, composed of ellipsoidal, elongate cells with pale orange wall, becoming hyaline towards interior. **Asci** unitunicate, clavate, short-stipitate, apex simple, 45–50 × 8–10 µm, containing 8 ascospores, biseriate above, uniseriate, overlapping below, filling each ascus. **Ascospores** (9–)10–11(–12) × 3.5–4 µm, narrowly ellipsoidal with obtusely rounded ends, 2-celled, hyaline, verrucose.

**Culture characteristics:** After three weeks on PDA at 25°C, colony 40–45 mm diam., irregularly and deeply lobate, white, pale greyish yellow at centre, not diffusing colouration in medium. Mycelium composed of septate, hyaline, smooth hyphae 2–3 µm diam. producing an abundant acromonium-like asexual morph at margin. Conidiophores simple, erect, flexuous, smooth 20–40 µm high, arising from aerial hyphae, hyaline, bearing cylindrical, subulate conidigenous cells 10–22 µm long, 2–2.5 µm diam. with a flared collarette. Conidia hyaline, aseptate, ellipsoidal with rounded apex, attenuated at base, smooth-walled, 3.5–5 × 2–2.3 µm.

## Results and discussion

The three species described above are characterized by globose non-stromatic ascomata not changing colour in 3% KOH or lactic acid, collapsing cupulate when dry, with walls 40–60 µm thick, composed of two regions with outer region composed of large, thin-walled cells that account for the cupulate collapse of ascomata upon drying, and an acromonium-like asexual morph observed in culture for one species. This combination of morphological and cultural characteristics clearly places these species in *Hydropisphaera* with the type species *H. peziza* (Tode) Dumort. as defined by ROSSMAN *et al.* (1999). This placement is confirmed by the phylogenetic analysis of their LSU sequences (Fig. 1). *Hydropisphaera cirsii* (Fig. 2) is distinct in having erect, glassy hairs covering the lateral ascomatal wall, an unusual feature in *Hydropisphaera* whose species with hairy ascomata typically have flexuous, white to orange hairs usually arranged in fascicles. Our phylogenetic analysis (Fig. 1) showed that *H. cirsii* is

nested in *Hydropisphaera* on a sister branch to *H. saulensis*, but the latter differs in having dark brown ascomata and smaller verrucose ascospores (this paper); both species differ phylogenetically by only 96.5% similarity of their LSU sequences. *Hydropisphaera pseudoarenula* (Fig. 3) is macro-morphologically similar to *H. arenula* (Berk. & Broome) Rossman & Samuels with which it could easily be confused, but is mainly distinguished by its larger, smooth to finely spinulose ascospores, instead of striate ascospores in *H. arenula* (Fig. 3). These morphological divergences are well supported by our phylogenetic analysis, placing the three studied collections of *H. pseudoarenula* on a branch distant from *H. arenula*, both species having only 97% similarity of their LSU sequences. *Hydropisphaera saulensis* (Fig. 4) is characterized by its dark brown ascomata, verrucose ascospores and occurrence in a tropical area. The most similar species *H. heliconiae* Lechat & J. Fourn., which also has dark brown ascomata and occurs in tropical area, primarily differs in having fasciculate hairs forming triangular teeth, arranged in an irregular stellate fringe around the upper margin of ascomata, and striate ascospores (LECHAT & FOURNIER, 2017b). Phylogenetic analysis indicates that these two species have only 96.3% similarity of their LSU sequences. Based on the morphological characteristics of the sexual-asexual morphs and phylogenetic analysis of their LSU sequences, *H. cirsii*, *H. pseudoarenula* and *H. saulensis* are accordingly proposed as new species, raising the number of known species of *Hydropisphaera* to thirty-two. We propose above an updated dichotomous key to species of *Hydropisphaera* based on morphology of the sexual morph, including the recently described species. Morphological characters used to distinguish these species are the ascomatal colour recorded in the fresh state, the presence of hairs and their morphology, and ascospore morphology, septation, dimensions and ornamentation observed in lactic cotton blue. For each species, the reader is referred to the original description or to an updated description in the literature cited, which often brings additional information about distinguishing characters.

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