

Lepidotia hispida: a spectacular member of the *Pezizaceae*

Nicolas VAN VOOREN
Anneke VAN DEN BERG-BLOK
Gerard VERKLEY

Ascomycete.org, 7 (6) : 303-306.
Novembre 2015
Mise en ligne le 30/11/2015



Summary: A recent collection of *Lepidotia hispida* (syn. *Peziza quelepidotia*), a very rare species, characterized in particular by stipitate ascomata, has been studied in depth. Description of macro- and micro-characters, illustrations, and comments are provided. Pure cultures have been obtained and deposited in the CBS Collection, and sequences deposited in GenBank.

Keywords: Ascomycota, *Pezizales*, peat-bog fungi, cultures, taxonomy.

Résumé : une récolte récente de *Lepidotia hispida* (syn. *Peziza quelepidotia*), une espèce très rare, caractérisée en particulier par des ascomes stipités, a été étudiée en profondeur. La description des caractères macro- et microscopiques, ainsi que des commentaires, sont fournis. Des cultures pures ont été obtenues et déposées dans la collection du CBS, et des séquences ont été déposées dans Genbank.

Mots-clés : Ascomycota, *Pezizales*, champignons des tourbières, cultures, taxinomie.

Introduction

Lepidotia hispida (Quélet) Boud., also known as *Peziza quelepidotia* Korf & O'Donnell, is a remarkable species. It has been rarely cited and illustrated since its publication by QUÉLET (*in* COOKE, 1879, fig. 402). It occurs in peat-bogs. The second author collected this species in June 2013, on a rehydrated block of potting soil, and submit pictures on the internet forum Ascofrance where it was identified. This note provides a detailed description of this collection, pure cultures isolated from it, and some additional taxonomic information for this spectacular cup-fungus.

Material and methods

The description of the apothecia is based on fresh and dried material of the same collection; the dried specimens were rehydrated in tap water over several hours. Microscopic characters were studied with an optical microscope under various magnifications. Water mounts were used for the observation of microscopic characters and measurements. Additional reactive agents were used to stain specific features: Lugol's solution (IKI) for testing the amyloid reaction of asci, and cotton blue (CB) for observation of spore ornamentation.

To obtain pure cultures, parts of fresh apothecia collected in the field were placed above 2% malt extract agar (MEA) with 30 ppm penicillin and streptomycin to shoot off their ascospores. Germinating ascospores were transferred to fresh media and incubated on the laboratory bench under diffuse daylight. Two single-ascospore isolates were deposited in the collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS 135943, 135944). For culture description, these isolates were transferred to fresh plates of malt extract agar (MEA) and oatmeal agar (OA) and incubated for 10 d in diffuse daylight. Media were prepared according to CROUS *et al.* (2009), and colony colors described according to RAYNER (1970). DNA extraction and sequencing of the internal transcribed spacer (ITS) and large subunit (LSU) of nuclear ribosomal RNA gene followed VERKLEY *et al.* (2013).

Taxonomy

Lepidotia hispida (Quélet) Boud., *Hist. class. Discom. Eur.*: 43 (1907).

Nomenclatural synonyms: *Peziza hispida* Quélet, *in* Cooke, *Mycographia*, 1 (4): 238 (1879), illeg., non *Peziza hispida* Huds. 1778; *Lachnea hispida* Quélet, *Bull. Soc. bot. Fr.*, 25: 291 (1879); *Neottiella hispida*

(Quélet) Sacc., *Syll. fung.*, 8: 192 (1889); *Peziza quelepidotia* Korf & O'Donnell, *in* Korf, *Persoonia*, 7 (2): 211 (1973).

Type specimen: *leg.* L. Quélet, Hérimoncourt (Doubs, France), ex herb. Cooke under voucher no. K(M) 199507 *sub nom.* "*Neottiella hispida*". Although this collection is not explicitly cited by COOKE (1879), the mention "Figured from specimens communicated by Dr. Quelet" referring the figure 402 leads us to think that this sample must be considered the holotype.

Apothecia at first tuba-like, more or less ventricose, with a pruinose surface, pale cream-beige, somewhat splayed at the top and more intensely colored, pinkish brown, becoming cupulate, fleshy, measuring 10–15 mm in diameter, often spread out, with a small trough in the center, hymenium dull yellowish to tan at maturity; outer surface concolorous, furfuraceous and marked by small reddish brown warts. **Stipe** 4–5 (10) × 0.3–0.8 mm, furfuraceous, at first concolorous, becoming pale reddish brown or vinaceous.

Medullary excipulum of *textura globulosa/subangularis*, with large cells, up to 70 µm in diameter. **Ectal excipulum** similar to the medullary excipulum, with cells somewhat smaller and more colored (brownish wall). **Subhymenium** thin, of *textura intricata*. **Asci** cylindrical, 190–220 × 9–11 µm, more or less truncate at the top, narrowed at the base, without croziers, 8-spored, appearing inamyloid in fresh specimens but diffusely blue (top and wall) in Lugol's solution (IKI) in rehydrated material. **Paraphyses** not enlarged at the top, × 2–3 µm, more or less curved, containing a yellowish vacuolar pigment, becoming brown with age. **Ascospores** ellipsoid-fusoid, 14–16 × 6.5–7.5 µm [$X = 14.8 \times 7.1 \mu\text{m}$ ($n=25$), $Q = 2.1$], hyaline, without oil drops but containing small guttules, quite thick-walled (~0.5 µm wide), appearing smooth in water, with low ornamentation in cotton blue, but not really verrucose as stated by KORF (1973).

Colonies on MEA reaching a diameter of 58–70 mm in 10 days, spreading with a regular margin, immersed mycelium pale luteous to luteous, aerial mycelium absent or diffuse, pure white to pale luteous; reverse concolorous. Colonies on OA 70–85 mm diam. in 10 days, similar as on MEA but without aerial mycelium. Subcultures sometimes produced masses of irregularly inflated hyphae, which on the surface produced terminal, globose to pyriform, hyaline, smooth-walled blastospores, 12–18 × 5–9 µm. No fruitbodies were formed.

Studied material: THE NETHERLANDS: numerous specimens growing on a sample of commercial Latvian potting peat, composed entirely of *Sphagnum balticum*. The fruiting occurred over several days from the beginning of June 2013, *leg.* A. van den Berg-Blok, *det.* N. Van Vooren, pers. herb. NV 2013.06.00; living cultures CBS 135943 and 135944; Sequences from CBS 135944 deposited in GENBANK (ITS



Plate 1 – *Lepidotia hispida*, different aspects. Photos: A. Van den Berg-Blok

KT869020, LSU KT869021). USA, Michigan, turfed press pot ('Tiffany pot'), leg. K. O'Donnell, 1970, living culture MUCL 18986 = CBS 136222 (? = NRRL 22205), authentic culture of *Lepidotia hispida* (sub *Peziza quelepidotia*) isolated by R.P. Korf.

Comments: The particular morphology of this fungus allows a quick determination, although some *Peziza* species can share such a habitus. This is the case for *P. varia* (Hedw. : Fr.) Alb. & Schwein. that can produce long-stipitate specimens, representing "sterigmate" forms (HÄFFNER, 1992). Such a form has been well illustrated by BOUDIER (1905-1910, pl. 266) under the name *Aleuria asterigma* Vuill. Of course, the color of apothecia in *L. hispida* is different, the ascospores are smaller and its ecology is special.

Discussion

The LSU sequence of CBS 135944 matches 99.86% with a GenBank accession AY640959 ("NRRL M80"¹, REEB *et al.*, 2004) and 98.4% with that of Genbank U42693 (NRRL 22205², O'DONNELL *et al.*, 1997; HANSEN *et al.*, 2001) both identified as *Peziza quelepidotia*, and it is furthermore 99.8% similar to AF335152 identified as *Peziza natrophila* Khan (HANSEN *et al.*, 2001). The ITS sequence of CBS 135944 does not match with any sequences in Genbank, except AF133174,

also identified as *P. quelepidotia*, a short and incomplete ITS sequence (96% homology). We have no doubt that our material corresponds to *Lepidotia hispida*.

BOUDIER (1885) proposed the new genus *Lepidotia* in the Aleuriés for species with eguttulate ascospores ("sans sporidioles") and stipitate or obconical apothecia ("sensiblement pédiculés ou obconiques"), covered by triangular scales ("à squames triangulaires"). BOUDIER (*loc. cit.*) included *Peziza hispida* Qué. and, with some doubts, *Peziza subrepanda* Cooke & W. Phillips as the two taxa belonging to this genus. The Aleuriés, in the sense of BOUDIER (1907: 43), correspond to a group of cup-fungi with amyloid asci, and thus it is considered a synonym of the family of *Pezizaceae* Dumort. ECKBLAD (1968: 171) selected *Peziza hispida* Qué. as the type-species, but he did not give a precise systematic position for the genus *Lepidotia*. KORF (1973), after the study of a modern collection he identified as *Peziza hispida*, considered *Lepidotia* not to be distinct from the genus *Peziza* Fr. and proposed a new name for the species, *Peziza quelepidotia*, due to the earlier competing name *P. hispida* Huds.

In their phylogenetic works, HANSEN *et al.* (2001) confirm the placement of *Lepidotia hispida* (as *Peziza quelepidotia*) in the *Pezizaceae*, but its position within the genus *Peziza* was unresolved. In a more recent phylogenetic study, as shown on Fig. 1 (HANSEN *et al.*, 2005), the species forms an independent clade and must be excluded from

¹ This number is not known by the curator of NRRL, Dr James Swezey (pers. comm.)

² This material comes from a culture presented in O'DONNELL & BENEKE (1973).

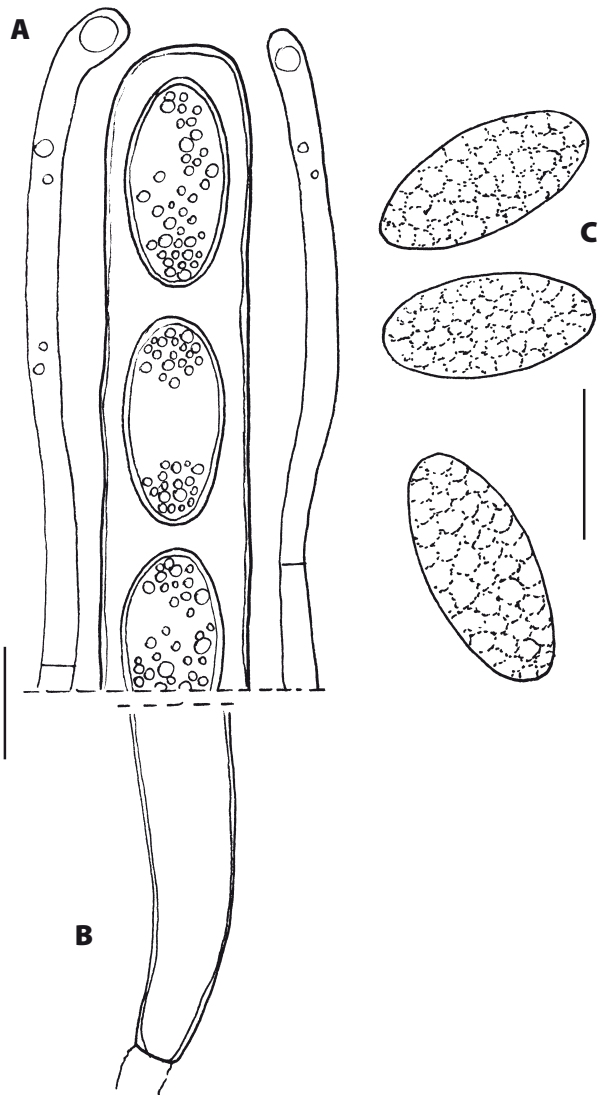


Plate 2 – *Lepidotia hispida*. Microscopic characters. Coll. NV 2013.06.00. A. Ascus top and paraphyses (in water). B. Ascus base. C. Ascospores in cotton blue. Scale bars = 10 µm. Drawing: N. Van Vooren.

the genus *Peziza*. In their analysis, it is associated with *Peziza natrophila* (NOWSHER & KHAN, 1976), another very rare taxon which shares some features with *L. hispida*, but which has strongly ornamented spores. *P. natrophila* was described from an area that was regularly treated with “alkali carbonate” (NOWSHER & KHAN, *op. cit.*). Such a place can be considered an extreme environment. The known collections of *L. hispida* suggest also that a particular environment is required for its development. We confirmed this placement by comparing Genbank sequences of LSU from the culture CBS 135944 (see above). The conspecificity of the two species, as raised by HANSEN *et al.* (2001, 2005), needs further investigations morphologically and phylogenetically.

Acknowledgements

We thank Dr Begoña Aguirre-Hudson (Royal Botanic Gardens Kew) for the information about the type-specimen of *Lachnea hispida*. We acknowledge gratefully Donald H. Pfister (Harvard University) for the pre-submission review of the manuscript.



Plate 3 – *Lepidotia hispida*. Mature ascomata. Photo: A. Van den Berg-Blok.

References

- BOUDIER E. 1885. — Nouvelle classification naturelle des discomycètes charnus connus généralement sous le nom de Pezizes. *Bulletin de la Société mycologique de France*, 1: 91-120.
- BOUDIER E. 1905-1910. — *Icones mycologicae ou Iconographie des champignons de France*. Paris, Paul Klincksieck, 4 vol.
- BOUDIER E. 1907. — *Histoire et classification des discomycètes d'Europe*. Paris, Paul Klincksieck, 222 p.
- COOKE M.C. 1879. — *Mycographia, seu icones fungorum*. Vol. I. Discomycetes. Part 6. Londres, Williams and Norgate.
- CROUS P.W., VERKLEY G.J.M., GROENEWALD J.Z. & SAMSON R.A. 2009. — *CBS Laboratory manual Series 1*. Utrecht, CBS-KNAW Fungal Biodiversity Centre.
- ECKBLAD F.-E. 1968. — The genera of the operculate Discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. *Nytt magasin for Botanikk*, 15 (1-2): 1-191.
- HÄFFNER J. 1992. — Rezente Ascomycetenfunde – XI. Sterigmate Formen in der Gattung *Peziza* (1. Teil). *Persoonia*, 14 (4): 597-602.
- HANSEN K., LÆSSØE T. & PFISTER D.H. 2001. — Phylogenetics of the Pezizaceae, with an emphasis on *Peziza*. *Mycologia*, 93 (5): 958-990.
- HANSEN K., LOBGLIO K.F. & PFISTER D.H. 2005. — Evolutionary relationships of the cup-fungus genus *Peziza* and the Pezizaceae inferred from multiple nuclear genes: RPB2, β -tubulin, and LSU rDNA. *Molecular Phylogenetics and Evolution*, 36 (1): 1-23.
- KORF R.P. 1973. — On Boudier's genus *Lepidotia* (Pezizaceae). *Persoonia*, 7 (2): 205-212.
- O'DONNELL K. & BENEKE E.S. 1973. — Apothecial formation by *Peziza quelepidotia* in pure culture. *Mycologia*, 65 (4): 913-915.
- O'DONNELL K., CIGELNIK E., WEBER N.S. & TRAPPE J.M. 1997. — Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18s and 28s ribosomal DNA sequence analysis. *Mycologia*, 89 (1): 48-65.
- QUÉLET L. 1879 [1878]. — Quelques espèces nouvelles de champignons. *Bulletin de la Société botanique de France*, 25: 287-292 + pl. III.
- NOWSHER A.Z.M. & KHAN A. 1976. — *Peziza natrophila* sp. nov. *Transactions of the British Mycological Society*, 67 (3): 540-543.
- RAYNER R.W. 1970. — *A mycological colour chart*. Kew, CMI and British Mycological Society.

REEB V., LUTZONI F. & ROUX C. 2004. — Contribution of RPB2 to multi-locus phylogenetic studies of the euascomycetes (*Pezizomycotina, Fungi*) with special emphasis on the lichen-forming *Acarosporaceae* and evolution of polyspory. *Molecular Phylogenetics and Evolution*, 32 (3): 1036-1060.

VERKLEY G.J.M., QUAEDVLIEG W., SHIN H.D. & CROUS P.W. 2013. — A new approach to species delimitation in *Septoria*. *Studies in Mycology*, 75: 213-305.



Plate 4 – Pure cultures CBS 135944: a. Colonies on MEA. b. Colonies on OA. c, d, e. Blastospores from colonies on MEA. Photos: G. Verkley.



Nicolas Van Vooren
36 rue de la Garde
69005 Lyon
France
nicolas@vanvooren.info



Anneke Van den Berg-Blok
Merellaan 192
2902 JK Capelle aan den IJssel
The Netherlands
ae.vdberg.blok@hccnet.nl



Gerard Verkley
CBS-KNAW Fungal Biodiversity Centre
Uppsalalaan 8, 3584CT Utrecht
The Netherlands
g.verkleij@cbs.knaw.nl