

# Hirticlavula elegans, a new clavarioid fungus from Scandinavia

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Based on material from Denmark and Norway the new clavarioid genus *Hirticlavula* with one species, *H. elegans*, is described. It produces tiny, hyaline white basidiomata with upward pointing blunt ended hairs. Phylogenetic analysis of the LSU region of rDNA strongly supports the inclusion of this fungus in the Clavariaceae sensu stricto where it occupies a position sister to all members of *Clavaria*, *Camarophyllopsis*, and *Hodophilus*. It has been found from May to October fruiting on wet, rotten hardwood bark on the ground, and its lignicolous, saprophytic nutritional mode further distinguishes it from other members of the Clavariaceae.

Key words: Clavariaceae, taxonomy, phylogeny, *Hirticlavula*

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## Introduction

One may think that the fungi of the well researched Northern Europe can no longer offer significant surprises. But this is not so: mycologists and forayers continue to discover remarkable new genera and species from this area. Here, we describe a new clavarioid taxon characterised by minute, somewhat typhuloid, hairy fruit bodies on rotten bark. It is currently known from several collections from Denmark and Norway with the first collection dating back to 1995. It has been reported provisionally by Jordal & Læssøe (2009), and has also featured

in various versions of MycoKey (Læssøe & Petersen 2003). Initial attempts at sequencing material for phylogenetic reconstruction in 2005 were unsuccessful. Following successful sequencing based on Norwegian material from 2011, a formal description can now be proposed.

Typhuloid fungi with or without sclerotia have been monographed by Corner (1950, 1970) and by Berthier (1976). In these works no fungi with characters approaching those of the fungus described below can be found. In addition, the fungus was not covered in a comprehensive

treatment of mainly Iberian cantharelloid and clavarioid fungi (Olariaga 2009) nor was anything similar treated by Birkebak et al. (2013).

## Material and methods

### *DNA sequencing and phylogenetic analysis*

Three entire basidiomata were crushed in a single Eppendorf tube using a Retsch ball mill (Retsch, Dusseldorf, Germany) and their genomic DNA was extracted using a modified CTAB-based extraction protocol in accordance to Murray & Thompson (1980) and Gardes & Bruns (1993). The large subunit (LSU) region of ribosomal DNA was amplified with the forward and reverse primer set LROR and LR7 (Vilgalys & Hester 1990) using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions. Amplicons were sequenced with an ABI 3100 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA) and the primers LROR, LR3R, LR5 and LR7 (Vilgalys & Hester 1990). A data matrix was assembled from LSU sequences of *Hirticlavula elegans* and members of the Clavariaceae sensu Dentinger & McLaughlin (2006), as well as clavarioid fungi from other groups (i.e. *Mucronella*, *Macrotiophula*, *Typhula*). The matrix was aligned using MAFFT version 6.717 (Kato & Toh 2008), the resulting alignment was manually verified, and then Gblocks v0.91b (Castresana 2000) was used to remove ambiguously aligned bases. The Bayesian information criterion in jModelTest v0.1.1 (Guindon & Gascuel 2003, Posada 2008) was used to determine the best-fit model of evolution, which was then implemented in both maximum likelihood and Bayesian analyses. GARLI v 1.0 (Zwickl 2006) was used to determine the most likely tree and maximum likelihood bootstrap support for the dataset. Bayesian analyses were conducted using Mr.Bayes version 3.2 (Ronquist & Huelsenbeck 2003) with two independent runs of four Markov Chain Monte Carlo chains with 1.0 x 10<sup>7</sup> generations each, sampling trees every 1000th generation. A final standard deviation of <0.01 for the split frequency was interpreted to reflect convergence. The first 25% of sampled trees were discarded as burn-in and posterior probabilities for each node of the 50% majority rule consensus tree were recorded.

### *Morphology*

The macroscopic images were taken with a Leica M 420 microscope equipped with various analogue and digital cameras. Pictures are stacked using Zerine Stacker and post processed with Adobe Photoshop. Observations of microscopic characters and micrographs were made with Leica Orthoplan and Olympus BX50 microscopes. Measurements were made on dead material in ca 5% ammoniacal Congo red.

## Results

The aligned matrix of LSU sequences included 94 taxa and 1288 characters, and the GTR+I+G model was selected by jModelTest as the best-fit model of evolution for the data. Results of the maximum likelihood bootstrap analyses and the Bayesian inference are shown on the maximum likelihood tree (-lnL -11336.51) (Fig. 1). *Hirticlavula elegans* is included within the well supported Clavariaceae sensu stricto (see Dentinger & McLaughlin 2006). It is supported as sister to a clade comprised of the genera *Clavaria*, *Camarophyllopsis*, and *Hodophilus* (100% Bayesian Posterior Probability, BPP and 78% Maximum Likelihood Bootstrap Proportion, BP) that is equivalent to Clade 4 as described in Birkebak et al. (2013). *Hyphodontiella* is weakly supported as sister to the *Hirticlavula*-Clavariaceae clade (59 BPP%, 53% BP). In all analyses, *Hirticlavula* is remote from all typhuloid taxa.

### ***Hirticlavula* J.H. Petersen & Læssøe, gen. nov.**

Mycobank no.: MB 808059

Basidiomata club shaped, small, stipe hirsute, without sclerotium. Hyphal system monomitic, hyphae non-inflated. Hairs unbranched, septate, cyanophilous, up to 250 µm long, widening towards the obtuse apex. Basidia holobasidia without clamps. Spores hyaline, more or less cyanophilous, smooth.

*Type: Hirticlavula elegans* J.H. Petersen & Læssøe

### ***Hirticlavula elegans* J.H. Petersen & Læssøe, sp. nova** – Figs. 2–5

Mycobank no.: MB 808060

*Holotype: Norway. Vestfold.* Sem, Gullkroneenes Naturreservat, Lille Gullkrone, 59.2854179 / 10.3826165, on *Quercus* bark on the ground in mature oak woodland, 25.X.2013 *Læssøe, JHP-13.364* (O, isotype in TUR).

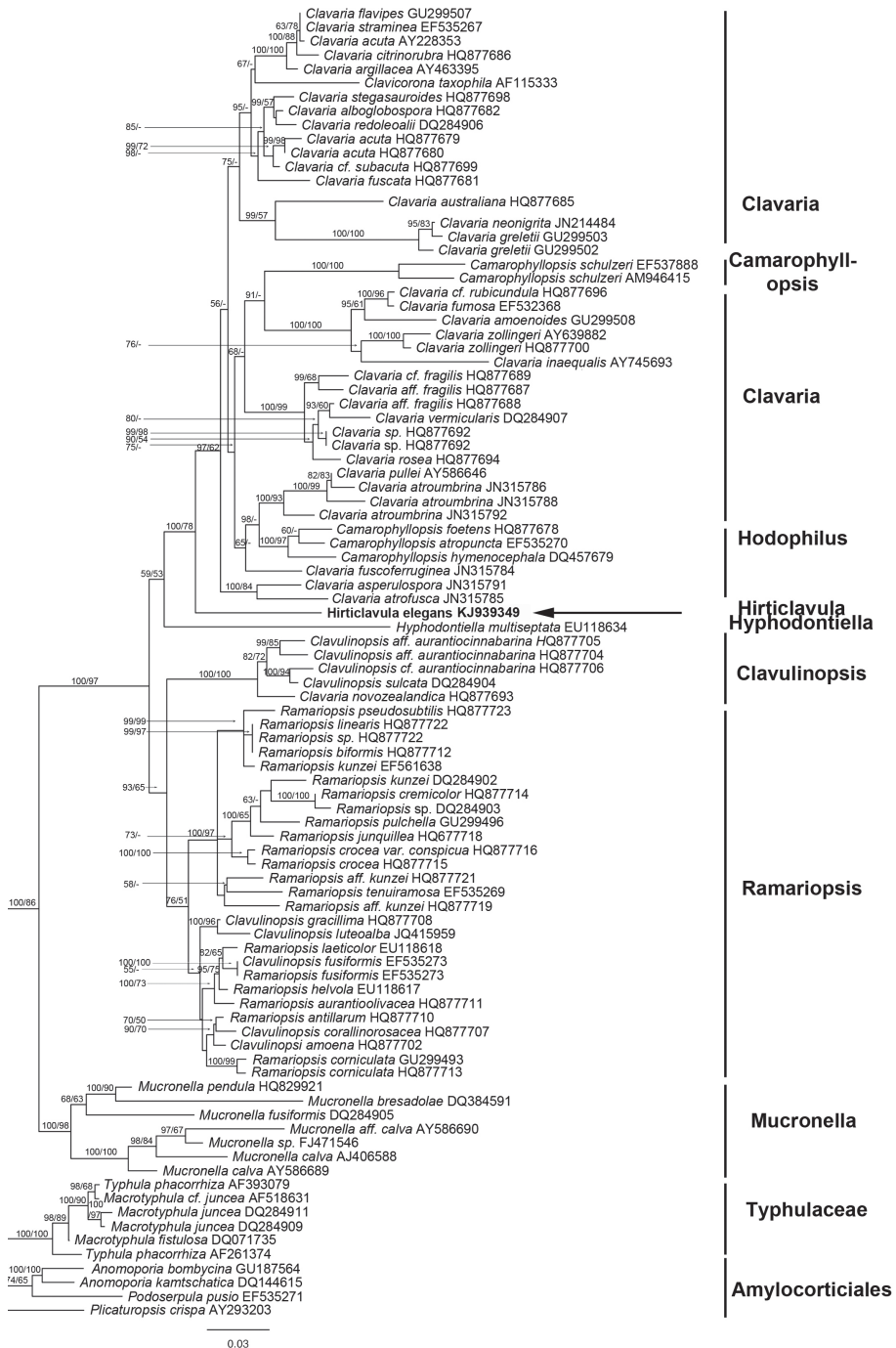


Fig 1. Maximum likelihood tree inferred from a dataset of large subunit rDNA sequences showing the placement of *Hirticlavula elegans* among other clavarioid taxa. Support values are given above the branches as Bayesian posterior probability/bootstrapped proportion. Accession numbers for sequences retrieved from GenBank are given following the sequence name.

*Basidiomata* 800–1100  $\mu\text{m}$  high, translucent white, with a 600–800  $\mu\text{m}$  long and 40–60  $\mu\text{m}$  thick, hairy stem abruptly widening into a 230–260  $\times$  90–200  $\mu\text{m}$  large, solid, fertile head. Dried material with a cream tinge and hardly collapsed. *Hyphal structure* monomitic, without clamps; hyphae in stem parallel, non-inflated, 1–2  $\mu\text{m}$  wide, more intertwined and gnarled at insertion point. *Hairs* straight, pointing upwards at an oblique angle, unbranched, slightly thick-walled, remotely septate, 150–250  $\times$  1.5–2.5  $\mu\text{m}$  (base) and 3–4.5  $\mu\text{m}$  thick at the rounded  $\pm$  clavate apex; cells 10–30  $\mu\text{m}$  long. *Basidia* without clamps, cyanophilous, 14–22  $\times$  3–5.5  $\mu\text{m}$ , with four, curved and remarkably thin, 4–5  $\mu\text{m}$  long sterigmata. *Spores* hyaline, cyanophilous or with a few cyanophilous particles inside, smooth, broadly ellipsoid, 4.5–6.5  $\times$  3–4.2  $\mu\text{m}$ ,  $Q=1.2$ –1.5, mean  $Q=1.36$  ( $n=15$ ), apiculus up to 0.8  $\mu\text{m}$  long. Crystals not prominent.

The basidiomata do not emerge from a sclerotium, but arise directly on bark or rarely wood from hard wood trees, two collec-

tions on *Quercus*, another on *Salix*, one on *Corylus* and one on undetermined hardwood.

**Additional specimens studied: DENMARK.**

**Jutland.** Skindbjerglund, south of Aalborg, 56.88889 / 9.923832, on bark on *Quercus* trunk on the ground, 30.IX.1995 *Vesterholt*, JHP-95.177 (C); Store Vildmose, Sandelsbjerg, 57.220723 / 9.759979, on rotten *Salix* bark on the ground in moist *Salix* carr, 16.V.2005 *Huhtinen* JHP-05.067/JHP-05.068 (C, TUR). **NORWAY. Akershus.** Frogn, Håøya, 59.104221 / 10.212019, on rotten hard wood bark (*Betula?*), 2.IX.2011 *Læssøe. Møre & Romsdal.* Nesset, under Rangåfjellet, on decayed *Corylus* wood, 62.461407 / 8.221528; MQ 59499 26079, 26.IX.2008 *Læssøe & Nielsen* TL-13513 (C).

**Discussion**

The distinguishing characters of this species are its very small basidiomata and peculiar hairs. The up to 1.1 mm high basidiomata are well outside



Fig. 2. *Hirticlavula elegans* (holotype). – Photo: J.H. Petersen.



Fig. 3. Basidiomata of *Hirticlavula elegans*. a = JHP-95.117, b = JHP-05.068, c-d = JHP-13.364 (holotype). – Photos: J.H. Petersen

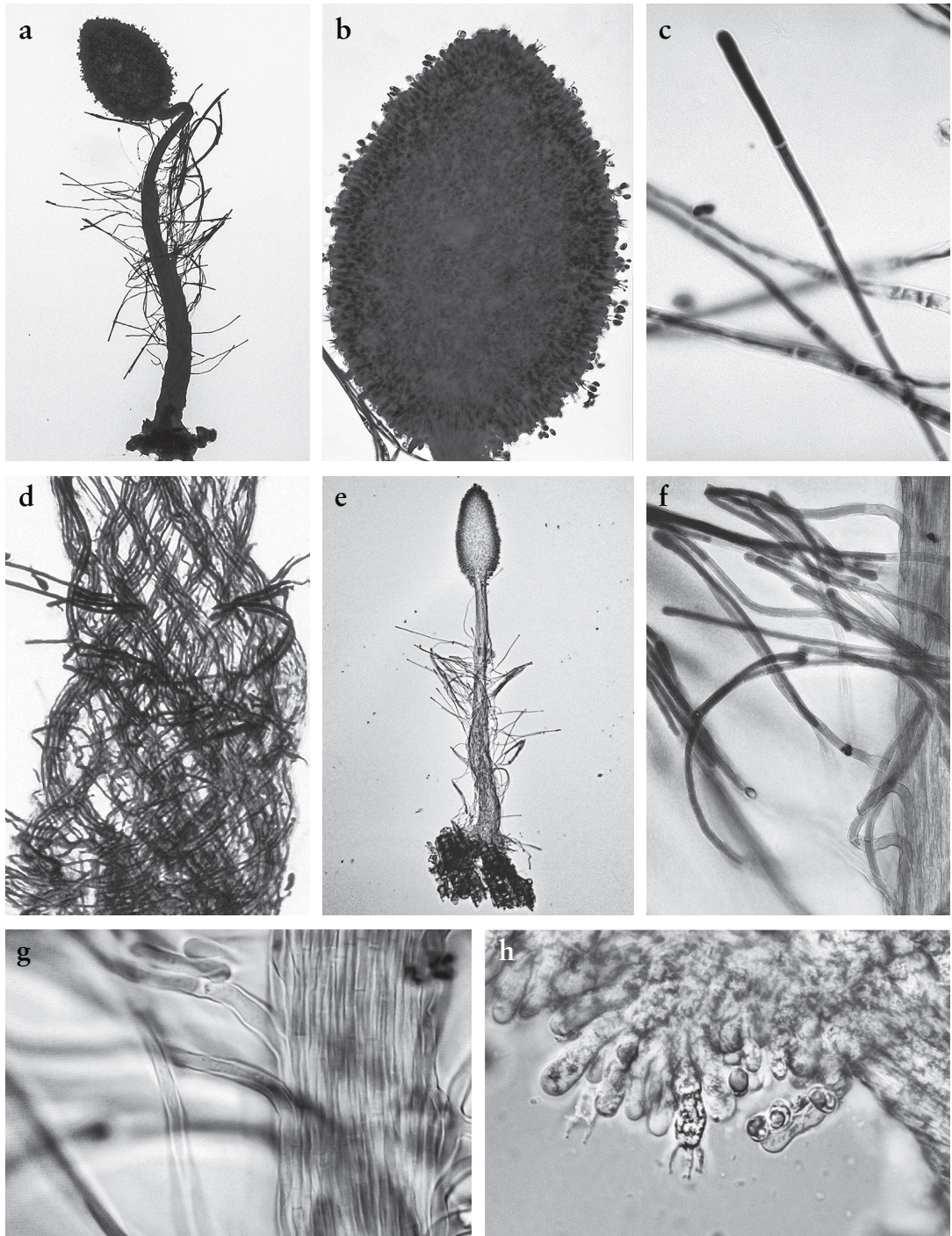


Fig. 4. Light microscope photographs of *Hirticlavula elegans*. a-c = JHP-95.117, d-h = JHP-13.364. a = whole basidiome, b = head with basidia and spores, c = rounded apices of hairs, d = spiralling hyphae of stem base, e = whole basidiome, f = hairs, g = hairs raising from stipe tissue, h = basidia and spores; a-g in cotton blue, h in water. – Photos: J.H. Petersen & T. Læssøe.

the range of all other known members of the Clavariaceae sensu stricto but are found elsewhere among more distantly related clavarioid fungi, e.g. the genera *Typhula*, *Pistillina* and *Pterula*. The hairs of *Hirticlavula elegans* originate from the outer hyphae of the stipe and point upwards, as if aiming to surround the fertile head. They are not merely branched hyphae, as the sparsely septate, rather irregular hairs often seen on the stipes of species of *Typhula*, *Pistillina* and *Pterula*, but are highly specialised structures with numerous septa and ending in a characteristic, rounded apex. While the hairs of all other known clavarioid fungi narrow towards the apex, the hairs of this species widen. *Hirticlavula elegans* is strongly supported as sister to all other members of *Clavaria*, *Camarophyllopsis*, and *Hodophilus* in phylogenetic analyses of the LSU region, and can be distinguished from these genera not only based on its minute size and diagnostic hairs, but also based on its ecology. While members of the Clavariaceae sensu stricto can be classified as biotrophic based on stable isotope analysis (Birkebak et al. 2013), *H. elegans* is lignicolous and saprotrophic. Given that *Hyphodontiella*, another wood-inhabiting genus within the clavarioid clade, is identified as sister to the *Hirticlavula*-Clavariaceae clade, further multigene analysis of the systematic positions within this group could provide valuable insight into the evolution of nutritional mode transitions in the clavarioid fungi.

It is rather surprising that *Hirticlavula* can produce basidiomata from May through to October. We do not know other tiny clavarioid fungi with such a long fruiting period. The unique morphology, ecology, and phylogenetic position of this fungus all suggest that it represents an undescribed genus closely related to the *Clavaria/Camarophyllopsis*-clade within the Clavariaceae sensu stricto.

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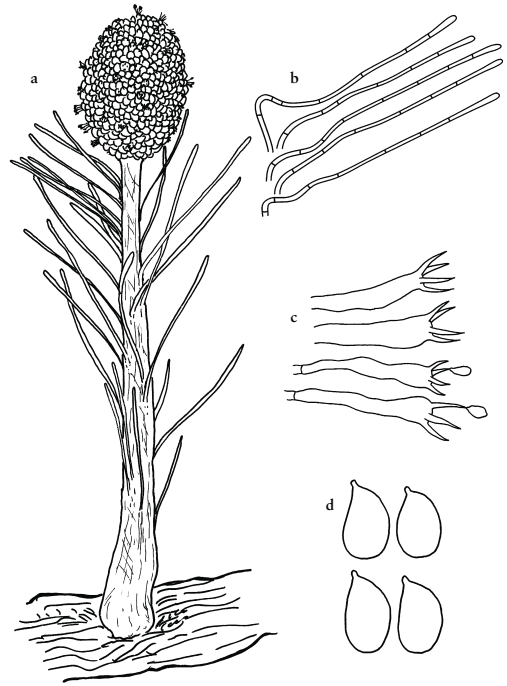


Fig. 5. *Hirticlavula elegans* (JHP-95.117). a = whole basidiome, b = hairs, c = basidia, d = spores. – Drawings: J.H. Petersen.

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