Deltopyxis triangulispora gen. et sp. nov., a polysporous *Tromeropsis*-like discomycete of unclear relationship

HANS-OTTO BARAL & GUY MARSON

Abstract

The new genus and species *Deltopyxis triangulispora* is described. It is so far known from 14 sites in the south of Luxembourg and one in the neighbouring region of France. The discomycete forms very small, blackish-brown apothecia on bark, more rarely on wood, but particularly on more or less strongly senescent hymenia of *Vuilleminia* spp. The apothecia occur on dead, corticated, internally very slightly to rather strongly white-rotten, attached or broken, periodically dry branches at a height of about 1–3 m above ground. In most of the collections *Vuilleminia* was present and covered the bast on one side of the branch, while the periderm still covered the remaining areas. *D. triangulispora* is so far recorded on angiosperm shrubs of the genera *Corylus*, *Crataegus*, *Ilex*, *Prunus*, and *Salix*, which had an advanced age or were already dead. The species prefers undisturbed, usually thermophilous hedges or open woodlands, especially close to their edges, but sometimes occurs also in dense, more air-humid woods. The fungus is characterized by 64-spored, elongate saccate, short-stalked, inamyloid, rather thin-walled asci which arise from croziers and open at the apex by a broad slit-like pore. The hyaline ascospores have a distinctly triangular shape when seen in profile view, but look slightly flattened, (ellipsoid-)deltoid in front view. In the living ascus they are arranged in a dense elongate cluster, which is forcibly discharged as one entity. The position of *Deltopyxis* within the Ascomycota is unknown.

Kurzfassung

***Deltopyxis triangulispora* gen. et sp. nov., ein vielsporiger, *Tromeropsis*-ähnlicher Discomyzet von unklarer systematischer Stellung**

Die neue Gattung und Art *Deltopyxis triangulispora* wird beschrieben. Bislang sind 14 Standorte im Süden Luxemburgs und eine in der angrenzenden Region Frankreichs bekannt. Der Discomyzet bildet sehr kleine, schwarzbraune Apothezien auf Rinde, seltener Holz, und besonders auf mehr oder weniger stark gealterten Hymenien von *Vuilleminia*-Arten. Apothezien traten ausschließlich auf toten, berindeten, intern leicht bis ziemlich stark weißfaulen, ansitzenden oder gebrochenen, wiederholt trockenfallenden Ästen in einer Höhe von ungefähr 1-3 m über dem Boden auf. In den meisten Kollektionen war *Vuilleminia* auf einer Seite des Asts vorhanden, während das Periderm den Bast auf den verbleibenden Astflächen noch bedeckte. *D. triangulispora* konnte bislang auf Angiospermen-Sträuchern der Gattungen *Corylus*, *Crataegus*, *Ilex*, *Prunus* und *Salix* nachgewiesen werden, welche ein fortgeschrittenes Alter zeigten oder bereits abgestorben waren. Die Art bevorzugt ungestörte, normalerweise wärmeliebende Hecken oder offene Wälder, vorzugsweise in Waldrandnähe, kommt aber manchmal aber auch in dichten, luftfeuchteren Wäldern vor. Der Pilz ist gekennzeichnet durch 64-sporige, länglich-sackförmige, kurz gestielte, inamyloide, ziemlich dünnwandige Asci, die aus Haken entstehen und sich apikal mittels eines schlitzförmigen Porus öffnen. Die hyalinen Ascosporen haben in Profilansicht eine deutlich dreieckige Form, während sie in Rückenansicht leicht abgeflacht, (ellipsoid-)rauten- bis drachenförmig aussehen. Im lebenden Ascus sind sie in einer dichten, länglichen Traube angeordnet, welche als eine Einheit aktiv abgeschossen wird. Die Position von *Deltopyxis* innerhalb der Schlauchpilze ist unbekannt.

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1 Introduction

During various searching trips for desiccation-tolerant ascomycetes on xeric (= exposed, periodically dry), dead branches, the second author discovered in 1991 a very small blackish-brown discomycete with polysporous asci and triangular spores, which could not be identified with the consulted literature. It somewhat resembled the unispecific genus *Tromeropsis* SHERWOOD. However, a number of characteristics deviate from *Tromeropsis*, therefore, we place this apparently undescribed species in a new genus.

In most of the collections the species was found fruiting on the hymenia of slightly to very strongly decayed *Vuilleminia*. This saprobiontic corticioid

genus of basidiomycetes decorticates dead attached branches by rupturing the periderm. However, on some of the recorded hosts of *D. triangulispora* (*Salix*, *Ilex*) the presence of *Vuilleminia* could not be demonstrated. The fungus appears to have been overlooked because of its minute apothecia and its exclusive occurrence on xeric branches, a habitat which is currently neglected by collectors. Our collecting activities showed that such branches carry a vast diversity of little known ascomycetes.

2 Materials and Methods

2.1 Microscopy

Observations were made with a Zeiss Standard 14 microscope with 100× oil immersion phase contrast achromat objective, and a modified Olympus CH–2 microscope with a Zeiss 100/1.25 oil immersion planachromat objective and Zeiss Kpl W 16 ×/16 wide field oculars. All collections were examined in tap water, mostly in the living state (see Baral 1992), after rehydrating the branches which were dry when collected. IKI was added either directly to a water mount, or after treatment with KOH. The presence of gel was tested using CRB added to a water mount. CR_{SDS} was applied to a mount in KOH. Photographic images (macro- and microphotos) were obtained using a Nikon Coolpix E4500 and a Canon S70 by using different macrolenses, and all drawings were done free-hand.

2.2 Cultures and media

Pure cultures were obtained from ascospores shot on Corn Meal Agar (Sigma-Aldrich, Fluka analytical #42347). 4g CMA and 12 g Agar-Agar (Merck #1615) were dissolved in 1l water, which corresponds to a dilution of 1:4 of the recommended concentration. The agar plates where incubated at room temperature. Illumination with UV-A (365 nm) for some hours was partly applied.

2.3 Deposition of cultures and dried specimens

Type material is deposited in the public herbaria at Botanische Staatssammlung München (M) and Staatliches Museum für Naturkunde Karlsruhe (KR), further specimens in the private herbaria of the authors (H.B., G.M.). The pure culture is deposited at Centraalbureau voor Schimmelcultures (CBS). A sequence is deposited in GenBank (JQ688406).

3 Results

Abbreviations: * = living state, † = dead state. Relative lipid content: 0 = without lipid bodies (= LBs), 5 = maximum possible lipid content relative to ascospore volume. Values in { } indicate the number of collections that were examined. IKI = Lugol's solution, ~1% I_2, 2% KI, in H_2O; KOH = potassium hydroxide, 5–10%; CR_{SDS} = Congo Red with SDS (sodium dodecyl sulfate); CRB = Brilliant Cresyl Blue, ~1% in H_2O; KClO = potassium hypochlorite.

***Deltopyxis triangulispora* Baral & G. Marson gen. et sp. nov.**

Mycobank: MB 564441, 564489

Diagnosis generico-specifica: Apothecia solitaria vel gregaria, 0.07-0.35 mm diam, 60-160 µm alta, sessilia, brunneoatra, orbicularia, superficialia, margine distincta, leniter crenulata. Asci in statu vivo 30-50 × 10-13 µm, clavato-fusoidei, apice late rotundati, tenuitunicati, inamyloidei (IKI), 64-spori, saepe non stipitati, e uncis nati. Ascosporae in statu vivo 2.5-4 × 2-3 µm, triangulares, guttulis oleagineis 1-4 praeditae. Paraphyses rectae, non ramosae, dense septatae, in statu vivo 1.5-2.8 µm latae, apice clavatae vel capitatae, 2-3.3 µm latae, cellulae terminales 2.3-7 µm longae, exsudato granulato, intense flavo-brunneo tectae. Excipulum ectale e textura prismatica-angulare, 10-95 µm altum, cellulae 5-17 × 3-9 µm, angulo arduo orientatae, multi- ad eguttulatae, gelatina intercellularis pallide ochracea, extus exsudato crasso, obscure rubrobrunneo vel (olivaceo-) brunneo tectum, in solutione KOH non dissoluto, excipulum marginem versus 15-30 µm crassum, textura prismatica-globulosa. Habitat ad corticem vel lignum ramulorum durorum siccorum Coryli, Crataegi, Ilicis, Pruni vel Salicis, in societate vel supra basidiomata Vuilleminiae et algas.

Holotypus: Luxembourg, Dudelange, Därebësch, 24.XII.1991, in *Vuilleminia* senescente ad corticem ramuli sicci Crataegi, Guy Marson, holotypus in M-0190818 (ex H.B. 4576a) isotypus G.M. 4668.

Etymology: named after the triangular, in dorsal view deltoid ascospores and the resemblance with the genus *Phaeopyxis*.

Apothecia rehydrated (0.07-)0.1-0.28(-0.35) mm diam {8}, 60-160 µm thick {7}, scattered to gregarious, singly or crowded in small groups, sessile or

with an indistinct stipe-like base, superficial, round, hymenium flat, light cream to dark brownish-grey or blackish, margin dark brown to black, always indistinctly to distinctly crenulate-pustulate, hairless, protruding (0-)5-20 µm beyond hymenium {4}, exterior on flanks ± glabrous. **Asci** *(30-)32-40(-50) × (8-)9.5-12(-13) µm {6}, †(23-)35-40(-45) × (7-)8-10 µm (spores alive) {5}, †24-35(-45) × (6.5-)7-10(-11) µm {7} (spores dead), ellipsoid-clavate-fusoid, 64-spored (50-64 spores counted) {6}, spores in living mature asci forming a central column *23-33 × 6.5-9 µm, in dead asci filling the whole ascus lumen; **apex** of mature asci broadly hemisphaerical, rather thin-walled (*0.3-0.4 µm, †H_2O 0.5-0.6 µm), in KOH subapical and upper lateral wall dictinctly swollen (especially in immature asci), 0.5-1.4 µm thick {4}, with a ± distinct apical chamber (here wall 0.3-0.7 µm thick), opening by a large slit-like pore, entirely IKI- {4} (with or without pretreatment with 5 % KOH), wall surface in CRB faintly to deeply lilac {2}, ectotunica of apex in KOH+CR_{SDS} partly distinctly reddish; **base** ± unstalked, rarely stalked, arising from croziers without perforation {6}; immature living asci with fusion nucleus 5 µm diam {2}, nucleolus 2.5 µm diam, asci multinucleate prior to spore delimitation, ascoplasma staining red-brown in IKI only at the base of some submature asci. **Ascospores** slightly to strongly triangular in profile view, medium flattened and ± deltoid to ovoid in dorsal view, *(2.5-)2.8-3.5(-4) × (2-)2.2-2.8(-3) µm {7}, †2.3-3 × 1.8-2.3 µm {1}, *1.8-2.3 µm wide in dorsal view (†1.7-1.9 µm); with 1-4 LBs 0.5-1.2 µm diam {4} (often only near one end, relative lipid content 2-3), KOH-inert, CRB-; wall surface CRB-, CR-; germinating ascospores rarely seen, budding to form cylindric(-ellipsoid) **phialoconidia** *2-3 × 1-1.2 µm {1} with 1-2 small LBs. **Paraphyses** straight, consistently unbranched along their entire length, without anastomoses, laterally emerging from excipular cells at the junction of hymenium and ectal excipulum, terminal cells *2.7-6 × (2-)2.5-3(-3.3) µm {4}, †2.3l-7 {2} × (1.3-)1.8-2.7(-3) µm {4} (3-4 µm wide including gel sheath), slightly to distinctly clavate-capitate, lower cells *(4-)5-9(-10) × (1.5-)1.7-2.3(-2.7) µm {5}, †(4-)5-6.5(-7.5) × (1-)1.3-1.8(-2) µm {2}, near base *4-7× 2.2-3.5 µm (†1.6-2.3 µm wide); 2-4 µm longer than living asci, 4-9 µm longer than dead asci; living cells constricted at septa, containing a few small LBs, some terminal or lower cells with a very indistinct, transient, globose body 1-1.2 µm diam; middle part agglutinated with asci by a gel (CRB bright lilac); **exudate** directly attached or over a 0.5-1.5 µm thick gel, cloddy to granular, pale to deep yellowish- to ochraceous- or olivaceous-brown, 0.2-0.6 up to 1-2 µm thick, unchanged in KOH. **Medullary excipulum** 5-30 µm thick, of hyaline, dense, slightly gelatinized, partly horizontally oriented textura globulosa-angularis-prismatica, cells *2-5 µm wide, multiguttulate to eguttulate, indistinctly delimited from ectal excipulum. **Ectal excipulum** at base and lower flanks of textura (prismatica-) globulosa-angularis, (10-)30-95 µm thick {4}, light brown (subhyaline near base), orientation irregular or at a 30-90° angle towards surface, individual cells *(5-)6-12(-17) × (3-)4-7(-9) µm {5}, containing a few or many larger and smaller hyaline LBs (0.2-)1-2(-3) µm diam, also ± eguttulate depending on the population, at mid flanks and margin 15-30 µm thick, of textura prismatica-globulosa ± irregularly oriented at 10-90°, cells *(2.5-)3-5(-6) x (2-)2.5-3.5(-4) µm {2}; cells (†) thin-walled but agglutinated by a medium refractive intercellular gel */†1-2(-3) µm thick, lower flanks medium gelatinized, at mid flanks strongly so, gel in CRB deep lilac; inner cells at margin forming periphyses-like outgrowths; intercellular pigment at lower flanks scattered, light reddish ochre-brown, towards margin abundant, bright (olivaceous-)yellowish-ochraceous to red-brown; all parts of ascocarp inamyloid (IKI); **exudate** on excipular surface forming large, 1-2 µm thick, deep red-brown to olivaceous-brown clods, scattered on flanks, dense at margin; pigment unchanged in KOH though sometimes darker, not dissolved (even when heated), stained blue in CRB, entirely discoloured in KClO. **Anchoring hyphae** very sparse, hyaline, smooth, *1.5-2 µm wide, wall 0.2 µm thick {2}. — **Anamorph**: **Conidiomata** 0.12-0.25(-3.5) mm diam, round, densely gregarious, partly confluent and then reaching 0.8 mm diam, sessile, with a bright ochre- to red-brown peridium composed of globose, light brown cells; at first globose, black, apically closed, opening by a transversal slit, margin indistinctly crenulate, producing a whitish slimy conidial mass. **Conidiophores** subglobose to obpyriform, with a short to long neck, *4.5-8 × (2.5-)3-3.3 µm, conidiogenesis phialidic with minute collarette. **Phialoconidia** *(2.7-)3.2-4.5 × (1-)1.1-1.4(-1.5) µm, straight to slightly curved, eguttulate or with a single minute LB.

Ecology: collected in ca. 1-3 m above ground, on (9-)12-25(-34) mm thick, corticated or partly decorticated, dead, internally little to rather strongly white-rotten, attached or sometimes broken branches of *Corylus avellana* {1}, *Crataegus* sp.

{6}, *C. laevigata* {1}, *C. monogyna* {4}, *Ilex aquifolium* {1}, *Prunus spinosa* {7}, *Salix caprea* {1}, *Salix ×capreola* {1}, *Salix* (?)*cinerea* {1}, on little to mostly medium (sometimes strongly) rotten bark {3} and wood {4}, often on slightly to very strongly decayed *Vuilleminia* spp. {16}, *V. cystidiata* {3}, periderm usually present only on one side of the branch, ruptured and rolled aside by *Vuilleminia* which is either still perceptible or has disappeared, in the latter case apothecia on exposed bast, sometimes over narrow cracks of periderm, or on periderm around spines (*Prunus*), with abundant green aerophytic algae around and below apothecia, but often also without. **Assoc.**: *Capronia* aff. *chlorospora* {4}, *Catillaria nigroclavata* {1}, *Chaetosphaeria myriocarpa* {1}, *Claussenomyces* sp. (on *Vuilleminia*) {2}, *C. atrovirens* (conidia ellipsoid) {1}, *C.* aff. *atrovirens* (conidia allantoid) {1}, indet. *Corticiaceae* {2}, *Cryptocoryneum condensatum* {1}, *Dacrymyces* sp. {1}, *Dactylospora* spp. {13}, *Eutypella prunastri* {1}, *Frullania dilatata* {2}, *Gloniopsis smilacis* {1}, *Hyphodiscus* aff. *hymeniophilus* {3}, *Hyphodontia sambuci* {1}, *Hypnum cupressiforme* {3}, *Lepraria* sp. {4}, *Melanelia exasperatula* {2}, *Thyridaria* sp. {2}, *Metzgeria* sp. {1}, ?*Nitschkia* sp. {1}, *Orbilia eucalypti* {1}, *O. vinosa* {1}, *Parmelia sulcata* {2}, *Patellariopsis atrovinosa* {1}, *Peniophora* sp. {1}, *Physcia* sp. {2}, *Platismatia glauca* {1}, *Polydesmia pruinosa* {1}, *Porina aenea* {2}, *Rhizodiscina lignyota* {2}, *Ulota crispa* {1}. **Altitude**: 220–395 m. **Geology**: mostly ± calcareous: Lower Keuper (Bunte Mergel), Lower Liassic (Grès de Luxembourg, Marnes et Calcaires de Strassen), Upper Liassic (minette, Toarcien; Bettembourg bituminous shale), Lower Dogger (coral limestone). **Phenology**: throughout the year. **Desiccation tolerance**: After 7–10 weeks alive in all parts; after 17 months still many spores and excipular cells, and some paraphysis cells viable.

Specimens examined (all collected by G. Marson, ø = no specimen preserved):
France, Lorraine, Moselle: 2.8 km SE of Dudelange, 1.5 km WNW of Zoufftgen, Nachtweide, 255 m, *Crataegus monogyna*, on very old *Vuilleminia*, 4.XI.2011 (G.M. 2011.11.04. #01).
Luxembourg: Mersch, 4 km S of Larochette, 2 km E of Fischbach, E of Folkend, Wald, 350 m, *Ilex aquifolium*, on wood, 25.IV.1994 (ø); – ibid., *Ilex aquifolium*, on wood and bark, 6.XII.2011 (G.M. 2011.12.06. #01). – **Luxembourg**, 5.5 km NNW of Luxembourg, 1.5 km E of Bridel, Plakigebierg, 280 m, *Prunus spinosa*, on *Vuilleminia*, 7.III.2003 (H.B. 7316). – 6 km S of Luxembourg, 0.8 km SE of Fentange, Wënkel, 265 m, *Salix caprea*, on ?bark, 7.III.1993 (ø). – **Capellen**, 5 km ENE of Pétange, 1 km E of Hautcharage, Reischlaedchen, 328 m, *Prunus spinosa*, on *Vuilleminia cystidiata*, 27.III.1999 (ø?). – Grevenmacher, 4 km NNE of Grevenmacher, 2 km NW of Mertert, Schlaufiels, N of Schlammbaach, 220 m, *Corylus avellana*, on old *Vuilleminia*, 19.III.1995 (H.B. 5281b). – **Esch-sur-Alzette**, 2.3 km SE of Dudelange, Därebësch, 272 m, *Prunus spinosa*, on bark, 12.XII.1991 (H.B. 4571a, G.M. 4643); – ibid., *Crataegus* sp., on *Vuilleminia*, 24.XII.1991 (M [ex H.B. 4576a], **holotype**; G.M. 4668, isotype); – ibid., *Prunus spinosa*, on bark, 23.II.1992 (G.M. 4786); – ibid., ?*Crataegus*, on old *Vuilleminia*, 17.IV.1996 (ø?); – ibid., *Prunus spinosa*, on *Vuilleminia cystidiata*, 20.V.2001 (ø); – ibid., *Crataegus laevigata*, on old ?*Vuilleminia*, 28.II.1998 (H.B. 6071a); – ibid., *Crataegus* sp., on *Vuilleminia*, 2.XI.2000 (H.B. 6820a); – ibid., *Prunus spinosa* and *Crataegus* sp., on *Vuilleminia cystidiata*, also on bark, 30.X.2011 (KR 0029475, ex H.B. 9634a); – ibid., *Crataegus*, on old *Vuilleminia*, 14.I.2012 (ø). – 2.5 km NNE of Dudelange, 1.2 km S of Bettembourg, near railway area, 280 m, *Salix* (?)*cinerea*, on wood, 16.X.2000 (H.B. 6808a. – 1.5 km W of Dudelange, Haard, 345 m, *Crataegus monogyna*, on *Vuilleminia*, 30.V.2010 (G.M. 2010.05.30. #01). – 2 km SE of Dudelange, Bloklapp, 288 m, *Prunus spinosa*, on old *Vuilleminia*,, 19.I.2007 (ø?). – 1.5 km S of Differdange-Obercorn, Kiemreech, 395 m, *Crataegus monogyna*, on old *Vuilleminia*, 6.VIII.2008 (ø). – 1 km S of Differdange-Obercorn, Kallek, 380 m, *Crataegus monogyna*, on old *Vuilleminia*, 16.VI.2010 (H.B. 9626a). – 5 km SSW of Luxembourg, 0.7 km N of Kockelscheuer, 305 m, *Salix ×capreola*, on wood, 10.XI.2000 (H.B. 6836b). – 1.5 km SW of Kayl, Léiffrächen, 388 m, *Crataegus* sp., on *Vuilleminia*, 7.X.2011 (H.B. 9625b). – 1 km NW of Rumelange, Holleschbierg, 380 m, *Crataegus*, on old *Vuilleminia*, partly on apothecia of *Dactylospora* sp., 6.IV.2010 (ø).

4 Discussion

Based on its peculiar features of triangular spores and multispored asci, *Deltopyxis triangulispora* is a well-characterized fungus. In several of its characteristics, it showed some variation among the studied populations. However, this variation was never so strong that we excluded any of our collections from the description.

Apothecia. The small ascomata of *Deltopyxis triangulispora* are clearly recognizable only in the hydrated state. Their size is usually 0.1-0.2 mm but varies between 0.07-0.15 and 0.2-0.28 mm, rarely 0.35 mm, sometimes within a collection. According to the apothecial diameter, the height of the apothecia varies considerably. The hymenium is usually greyish-cream but especially in larger or older apothecia it may get dark blackish-brown due to more abundant exudate over the paraphyses.

Young (immature) apothecia of *D. triangulispora* are blackish and almost globose when rehydrated, with a diameter of 70-80 µm and a height of 60-70 µm. At their apex they possess a 15-20 µm wide pore. At this development stage no or only a few very immature asci are present. When the first asci attain maturity, the hymenium is distinctly exposed and bordered by the protruding dark margin.

Hamathecium ontogeny. In young apothecia the paraphyses grow upwards from the bottom of the young hymenium, but also from the sides of the hymenial cavity. Those that arise from the bottom form their short, capitate terminal cell only when they reach their final length.

During apothecial growth, new paraphyses were seen to be formed exclusively at the margin. At the junction of hymenium and ectal excipulum, new paraphyses emerge laterally from inner cells of the ectal excipulum (Fig. 1.3). When starting as lateral outgrowths, they resemble periphyses, but they soon bend upwards and elongate until they reach the hymenial surface. At this development stage they are still much shorter than the adult paraphyses, but they will elongate when the lateral excipulum becomes the bottom of the expanding hymenium.

Young paraphyses in *Deltopyxis* were never observed to develop between the asci, neither emerging from the subhymenium, nor by branching of adult paraphyses. This peculiar feature of marginal development of new paraphyses might support the isolated position of *Deltopyxis* in comparison to members of the *Helotiales* and *Orbiliomycetes*, in which young paraphyses generally develop also between the asci during apothecial growth.

Lipid bodies in excipular cells. In the holotype and some other collections, the cells of the ectal excipulum contained a more or less high amount of larger and smaller lipid bodies (LBs, Figs 1.1d, 1.2b, 5c). In some other collections the cells were often devoid of these droplets or contained them in lower abundance. We conclude that the lipid content in the excipular cells is subjected to external circumstances rather than having a genetical origin.

Asci. Based on our observation of living asci at different stages of development, the ascospores of *Deltopyxis triangulispora* are formed by multiple mitoses after meiosis of the fusion nucleus, i.e., the asci are “truly polysporous” in the terminology of Martens (1937). True polyspory is also observed in the genera *Sarea* Fr. and *Tromeropsis* with similar elongate-saccate, multispored asci and small ascospores (Hawksworth & Sherwood 1981).

The asci are unitunicate and open by a large apical pore which is distinctly slit-like (Fig. 5g images on the upper right). An apical thickening is only slightly developed. In very young asci the entire ascus is thick-walled in the dead state (e.g., when mounted in KOH, Fig. 5g, images on the left), except for a small region at the very apex. Later the thickened part of the wall is restricted to the apical region (Fig. 1.1i), though this wall thickening is often rather inconspicuous, and immature asci may also be uniformly thin-walled (Fig. 1.1g).

The asci are inamyloid in IKI, with or without KOH pretreatment. In CR_{SDS} the ectotunica is distinctly stained, but only in the apical region (Fig. 5g, in another collection the wall did not stain). This congophily of the apical ectotunica could not be observed in any of those taxa of *Helotiales* and *Orbiliomycetes* that we have tested so far.

The spores are arranged in a dense elongate cluster in the living ascus (Figs 1e, 5f, 6c), which is actively discharged. When rehydrated and placed in a Petri dish, apothecia started to eject spore clusters after ca. 5 min. When shot on agar, the spores form dense, single-layered heaps which allow quite exact counting of the spores (Fig. 5h-i). These heaps suggest that the spores are ejected as one entity, similar as in the *Orbiliomycetes*.

Ascus length was in some specimens consistently around 25-30 µm but in others always around 30-40 µm. Likewise, the width of the asci varied between 7-9 and 10-12 µm. These differences are only to a certain degree due to real variation, but mainly depend on the living versus dead state. However, contrary to many other ascomycetes, the asci of *Deltopyxis* shrink only insignificantly when they loose turgor, as long as the spores remain alive. In fact, adding KOH to dead asci that contain living spores provokes an unexpected ascus shrinkage for 10-17 % in length and 5-20 %

in width due to shrinkage of the included spores (a similar effect is induced by shortly heating the slide). Living asci shrink to a similar rate (10-15 % in length and 15-20 % in width) when killed by KOH or heat.

The effect is provoked by an increase in water content and volume of the living spores as soon as the ascus turgor is released. In the spore cluster the spores are rather strongly dehydrated. When the asci loose turgor, the spores fill the complete ascus by keeping the elastic ascus wall in a state of tension (Fig. 6d-e). As a consequence, such dead asci are scarcely smaller in size than living asci (Figs 5f, 6c). This peculiarity of *D. triangulispora* might be due to the saccate shape of the asci which are not much longer than the pars sporifera.

Despite the observed real variation in ascus size, the asci appear to be always 64-spored, with rare exceptions of single asci with perhaps only 32 spores or intermediate spore numbers. The variation in length is partly explained by the occasional presence of a more or less pronounced stalk.

The first collection from Folkend near Fischbach on *Ilex* consisted of only two apothecia. This specimen differed in the asci which were noted much shorter (*21 × 9 µm, with a pars sporifera of only 20 × 6 µm). Ascospore size was not evaluated; also spore number was not noted but might well have been only 32. This site was revisited in 2011, but again only a few apothecia could be detected. Here the asci were distinctly longer (†23-27 × 7.5-10 µm, containing living spores). They were clearly more than 32-spored and matched the specimens on the other hosts in every respect.

Ascospores. The living ascospores are quite consistent in size when measured outside the asci. Their shape is somewhat variable, but strongly depends on the direction of view: the characteristic triangular shape is only seen in profile view, whereas in dorsal view they look ± deltoid and especially in oblique view more ellipsoid (Figs 1.1l, 1.2d upper right). Shrinkage of the spores when killed by KOH lies in the range of 5-20 % in both length and width.

In turgescent asci the angular spore shape permits a very dense packing within the spore cluster. This is best seen in asci that lost turgor prior to spore release: the living spores completely fill the ascus and fit together like a honeycomb.

Anamorph. In one collection of *D. triangulispora* a few ascospores were seen to show yeast-like germination, i.e. to bud off conidia at one end, apparently by phialidic conidiogenesis (Fig. 1.4). This phenomenon seems to be rare because it was not observed in any of the other collections studied.

When the ascospores were shot on agar, they formed dense heaps. Germination did either not occur at all, or spores germinated only 7 days after shooting, especially when the heaps were separated with a glass rod. Often, germinated spores very soon stopped growing, but in one culture (on CMA1:4) they formed a very slow-growing mycelium, with up to 110 µm long hyphae within ca. 25 days after germination, i.e., ca. 30 µm per week. When transferred one week later to another plate, the hyaline mycelium formed a dense mat that hardly increased in diameter within the next month. Simultaneously, several dozens of ochre- to red-brown conidiomata developed (Fig. 6f-g). These somewhat resemble the ascomata in both size and shape, but open by a transversal slit. The abundantly produced phialoconidia resemble those formed on the ascospores but are longer and partly also wider.

The conidia of *Deltopyxis* resemble those obtained by Weber (2002) in pure culture of *Tromeropsis microtheca*, the ascospores of which produced a likewise slowly growing mycelium on agar (MEA). However, the small, ± cylindrical, straight to medium curved conidia of *T. microtheca* emerged from little pegs on integrated conidiogenous cells (conidiogenesis holoblastic). Also larger conidia were observed which germinated yeast-like to form small conidia.

Ecology. The sites where *Deltopyxis triangulispora* was collected are open woodlands or hedges, particularly at ± S-exposed slopes but also in areas with indistinct inclination. The geology was generally more or less calcareous or basic, and the vegetation usually thermophilous. The inhabited substrates are dead branches with a usually initial to optimal, rarely final stage of wood decay, attached to living or dead shrubs or small trees one or a few meters above ground, sometimes also broken but hanging on other branches. Quite an undisturbed vegetation over many years is a prerequisite for the detection of many of these desiccation-tolerant ascomycetes that are confined to xeric branches. Most of the branches on which *D. triangulispora* occured were previously infected by *Vuilleminia* which was, however, no more viable when this and other discomycetes appeared on their basidiomata or on the bark around. Such branches are usually entirely corticated, but with the periderm replaced by the *Vuilleminia* on one side (Figs. 2c, 3a). The branches

were usually broken only terminally, but are quite easy to break near the trunk due to an often advanced wood decay (white rot) caused by *Vuilleminia*. Sometimes they were already broken towards the trunk and hang on lower branches.

The apothecia of *Deltopyxis triangulispora* are preferably found on the very thin layer of the fruitbodies of *Vuilleminia*, either when these were still whitish to skin-coloured, or on the darker, more grey-brown to olivaceous marginal regions (Figs. 3a,e, 4b-c). *D. triangulispora* may also sparsely occur on bark remote from *Vuilleminia*, and here usually over small holes or clefts of the periderm (Figs. 3b, g, 4d).

In the collections on *Salix* and *Ilex* no *Vuilleminia* could be noted at all, however. Here the branches were partly or entirely decorticated, and the apothecia grew mainly on wood, though often close to other corticioid basidiomycetes (*Peniophora*, *Hyphodontia*). Also in a collection on *Prunus spinosa* from Därebësch (H.B. 4571a) no *Vuilleminia* could be observed, instead, the branch was entirely corticated.

Frequently, *Deltopyxis triangulispora* occurs in close association with species of *Dactylospora* and *Capronia*, which likewise preferably grow on senescent *Vuilleminia*. In a single collection, *D. triangulispora* even grew partly on the apothecia of *Dactylospora* (Fig. 4a). This raises the question whether *D. triangulispora* shows some fungicolous connection to *Dactylospora* rather than *Vuilleminia*.

Aerophytic algae and various lichens and mosses typically occur on the inhabited branches, but are not always present. Sometimes they even cover the apothecia and need to be removed by a jet of water in order to detect the apothecia.

Relationship. *D. triangulispora* was first considered by us to belong in the genus *Tromeropsis* (*Ascomycota* incertae sedis). We studied *T. microtheca* (P. KARST.) SHERWOOD from several collections and found that it differs in many points, which appears to justify separation at the generic level (see also the redescription by HAWKSWORTH & SHERWOOD 1981). *T. microtheca* resembles *D. triangulispora* in the dark apothecia and multispored, inamyloid asci that arise from croziers and open by a large apical slit. *T. microtheca* differs in (1) apothecia with an even margin, (2) an ectal excipulum of elongate, vertically oriented cortical cells, (3) paraphyses with uninflated terminal cells which are much longer than wide, young paraphyses at the margin not formed as in *Deltopyxis*, (4) strongly refractive, hyaline, KOH-soluble extracellular drops between the paraphyses in the middle and lower part of the hymenium, (5) an also laterally thickened ascus wall in the apical half of the mature ascus (dead state), (6) a negative stain of the entire ascus wall in CR_{SDS}, (7) an often long, flexuous ascus stalk, (8) 128-spored asci, (9) cylindric-ellipsoid ascospores, and (10) occurrence on coniferous substrate. Particularly the criteria 1-6 are considered diagnostic at the generic level.

The brief and rather inaccurate description of the genus *Microspora* VELEN. [non *Microspora* THURET 1850, algae] with the single species *M. dura* VELEN., reported from coniferous wood by VELENOVSKÝ (1934), resembles *Deltopyxis* in some respects. However, it was found to be a synonym of *Tromeropsis microtheca*, based on a study of syntype collections preserved at PRM (BARAL ined.).

Several members of *Lecanoromycetes* show a certain similarity with *Deltopyxis*. The genus *Dactylospora* KOERB. resembles not only macroscopically, but also in the construction of the ectal excipulum. It differs in mostly 8-spored asci with a strong hemiamyloid iodine reaction of the thin lateral wall and a thick, strongly euamyloid apical cap, also in brown, septate, elongate ascospores. The genus *Steinia* KÖRB. resembles *Deltopyxis* in its elongate-saccate, 16-spored asci and subglobose ascospores, but the hymenium is hemiamyloid, also the asci possess an euamyloid tholus, the paraphyses are much narrower and somewhat curved, and the apothecia have a convex immarginate disc (see KANTVILAS & MCCARTHY 1999). The lichenized or lichenicolous genus *Polysporina* VĚZDA resembles *Deltopyxis* in its multispored asci, but deviates in richly branched and anastomosing paraphyses with uninflated apices, and in amyloid asci (see KANTVILAS 1998). Macroscopically, *Catillaria nigroclavata* (NYL.) SCHULER can easily be confused with *Deltopyxis*, when the latter forms larger apothecia.

The resinicolous genus *Sarea* FR. closely resembles *Deltopyxis* in the saccate multispored asci and the paraphyses tipped by pigmented exudate (see also HAWKSWORTH & SHERWOOD 1981). It differs in asci with a strongly hemiamyloid thick external gel and an inamyloid tholus with a rostrate opening mechanism. The ascus wall layers are unstained in CR_{SDS}, and the paraphyses are sometimes branched above and below and are not formed in the manner as described for *Deltopyxis*. The ectal excipulum is internally hyaline, sharply delimited, heavily gelatinized, of vertically oriented elongate cells. The pycnidial anamorph of *Sarea* is quite similar to that of *Deltopyxis*.

The genus *Rhizodiscina* Hafellner (*Patellariales*, *Dothideomycetes*) is superficially rather similar to *Dactylospora* and *Deltopyxis*. It is characterized by faintly euamyloid or sometimes inamyloid, saccate, 8-spored asci with a thick inamyloid tholus, brown, 1-septate ascospores, and projecting, brown, 3-4.5 µm wide anchoring hyphae.

Quite a lot of genera of *Helotiales* (*Leotiomycetes*) with small, sessile, blackish apothecia bear some similarity with *Deltopyxis*. However, the rather saccate asci in *Deltopyxis* seem to indicate that this genus does not belong to the *Helotiales*. Five mainly lichenicolous genera with black apothecia are in the following compared with *Deltopyxis*: *Geltingia* Alstrup & D. Hawksw., *Llimoniella* Hafellner & Nav.-Ros., *Phaeopyxis* Rambold & Triebel, *Rhymbocarpus* Zopf, and *Skyttea* Sherwood, D. Hawksw. & Coppins. *Skyttea* differs in asci with strongly thickened apical and thin lateral wall, also in elongate, hyaline to brown, hair-like cells at the protruding margin (Sherwood, Hawksworth & Coppins 1981). Hair-like structures are partly also typical of *Rhymbocarpus*, in which the asci are not or only slightly thick-walled at the apex. The genus *Llimoniella* resembles *Deltopyxis* in the structure of the ectal excipulum and the rather thin-walled asci. It differs in a purplish pigment that turns violaceous in KOH, and in addition an olivaceous pigment that turns bright green herein (Diederich & Etayo 2000). The type species of *Geltingia*, *G. associata* Alstrup & D. Hawksw., strongly resembles *Deltopyxis* in median section and in the slightly thickened apical ascus wall with a slight apical chamber, according to the redescription by Diederich et al. (2010 Figs 2E, 5). Also *Rhymbocarpus aggregatus* Etayo & Diederich (2011, Fig. 1C-E) concurs in median section with *Deltopyxis*. The asci in *Phaeopyxis* have an immature overall thickened wall (laterally †1-1.5 µm thick, apically †1.5-3.5 µm) and partly show a faintly amyloid iodine reaction; the brown excipular and hymenial pigment often stains ± violet-brown in KOH (Rambold & Triebel 1990).

A specimen of *Phaeopyxis punctum* (Massal.) Rambold et al., identified by R. Santesson and G. Rambold (H.B. 4341), was examined by us: the (bluish) black-brown exudate did not change its colour in KOH. In contrast to *Deltopyxis*, the apical ascus wall did not stain in CR_{SDS}.

All five genera differ from *Deltopyxis* in their 8-spored, rather narrowly cylindrical asci, in apically not or only slightly inflated paraphyses in which the terminal cells are generally about 5-10× longer than wide, in a tendency of the paraphyses to being branched at the uppermost septum or at least towards the base, in ellipsoid-ovoid or cylindric-oblong to fusiform ascospores with a usually rather high lipid content, and in a mostly lichenicolous habitat, the apothecia being often deeply immersed or erumpent (except for *Llimoniella*).

A certain similarity is seen between *Deltopyxis* and the type species of *Patinella* Sacc., *P. hyalophaea* Sacc., which was placed with hesitation by Nannfeldt (1932) in the *Orbiliaceae* because of capitate paraphyses and angular excipular cells, and later in the *Dermateaceae* by Spooner (1987). Reexamination of the type material (Baral ined.) confirmed placement in the *Helotiales*, but its relationship to a family is quite difficult to assess. The apices of the narrow, 8-spored asci have a pronounced inamyloid apical thickening, and the strongly capitate, dark brown apices of the long terminal cells of paraphyses appear as being thick-walled. The spores have a similar size and lipid content as in *Deltopyxis* but are ellipsoid.

The genera *Claussenomyces* Kirschst. (in the current circumscription) and *Tympanis* Tode frequently possess polysporous asci, but this feature is due to budding of 8 ascospores within the premature ascus, very different from *Deltopyxis*. Also here the asci open by a large apical slit, but the ascus wall is congophilous laterally rather than apically [observed in *C. kirschsteinianus* (Kirschst.) G. Marson & Baral and *C. atrovirens* (Pers.) Korf & Abawi agg.]. Several further genera with black apothecia exist in the *Helotiales*, but none of them appears to be related to *Deltopyxis*.

There exist also some undescribed species of *Orbiliomycetes* with black-olivaceous apothecia and 8-16-spored asci (Baral et al. in prep.). Their ascospores contain spore bodies that disappear when KOH is added, whereas the drops in the spores of *Deltopyxis* resist herein and are, therefore, classified as lipid bodies. Moreover, the asci have a furcate base without croziers, unlike *Deltopyxis*. In its extraordinary spore shape, *D. triangulispora* reminds of some undescribed species of *Orbilia* Fr. in which triangular spores actually occur, but particularly the absence of a spore body and the fact that small conidia bud from the ascospores make such relationship highly improbable. Like *Deltopyxis*, the asci of *Orbilia* open by an apical slit; yet, it was never the ectotunica that stained in CR_{SDS}, but instead the endotunica showed a congophilous reaction in species with a thick-walled ascus apex.

Phylogenetic placement: A sequence of the ITS1-5.8S-ITS2 rDNA gained from pure culture (H.B.

Table 1. Alignment of *Orbiliales*-specific signature (Orb5.8s1F) in the 5.8S rDNA with the deviating signature of a few basal *Orbiliomycetes* and the signature of *Deltopyxis* and the remaining *Ascomycota*. Different bases in bold type.

Group	5.8S rDNA																
Orbiliomycetes (Orb5.8s1F)	G	C	A	G	C	G	A	A	A	**C**	G	C	G	A	T	A	**G**	**T**
Orbiliomycetes (basal)	G	C	A	G	C	G	A	A	A	**C**	G	C	G	A	T	A	**G**	**G**
Deltopyxis and other *Ascomycota*	G	C	A	G	C	G	A	A	A	**T**	G	C	G	A	T	A	**A**	**G**

9625b) was provided by S. Hermant. Two clarify the phylogenetic placement of *Deltopyxis* within the *Ascomycota* proved impossible with this gene region, but it allows at least to exclude a closer relationship with the *Orbiliomycetes*. This class is characterized by a signature in the 5.8S rDNA which was used by Smith & Jaffee (2009) as one of three *Orbiliales*-specific primers (Orb5.8s1F, see Tab. 1). *Deltopyxis* differs from this signature in three positions, while it concurs in this signature with many if not all of the remaining *Ascomycota* from which a sequence is available in GenBank. However, it must be mentioned that a few basal members of *Orbiliomycetes*, from which we got sequences, differ from this primer signature at the last position by a base that corresponds to the remaining *Ascomycota*.

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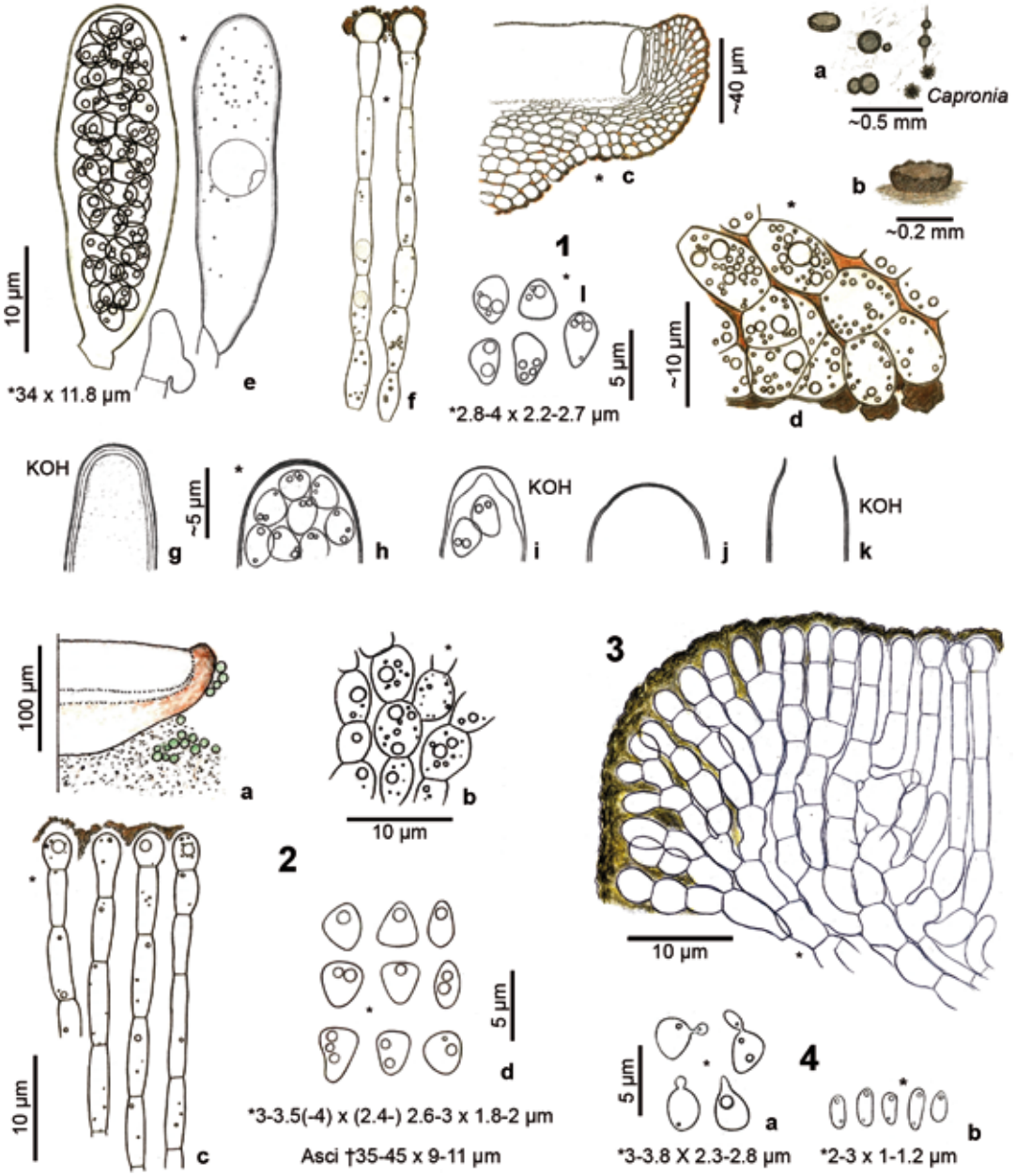

Fig. 1. *Deltopyxis triangulispora*. 1a)-b) apothecia (rehydrated, a with *Capronia* aff. *chlorospora*); 1c), 2a) median section of apothecium; 1d) median section of ectal excipulum at apothecial base (cortex); 2b) dto., inner part; 1e) mature and young ascus (with fusion nucleus), very young ascus in process of crozier formation; 1f), 2c) paraphyses; 1g)-k) ascus apices (1g) immature, 1h)-j) mature, 1k) emptied); 1l), 2d) ascospores (right spore in 1l) and two upper right spores in 2d) in dorsal view); 3. median section of margin (excipulum, marginal hymenium); 4a). yeast-like budding of ascospores; 4b) conidia formed on ascospores. – 1. from Därebësch (Dudelange), on *Crataegus*, H.B. 4576a (holotype), 2. ibid., H.B. 6071a (topotype), 3. ibid., on *Prunus spinosa*, H.B. 9634a, 4. from Léiffrächen (Kayl), on *Crataegus*, H.B. 9625b.

Fig. 2. Collection sites of *Deltopyxis triangulispora*. a) Därebësch (Dudelange, type locality), open *Crataegus* and *Prunus spinosa* bushes at the border of a pasture; b) Léiffrächen (Kayl), *Crataegus* bushes in a more dense and air-humid woodland; c) Därebësch, *Crataegus* branch in situ, with *Vuilleminia* (14.I.2012).

Fig. 3. Branches of *Crataegus* with *Deltopyxis triangulispora* (rehydrated, white arrows). a)-c), g) from Léiffrächen (Kayl, H.B. 9625b); d) from Därebësch (Dudelange, H.B. 9634a); e)-f) from Kallek (Obercorn). – a), c) on periderm over small holes (a) with *Vuilleminia* below, c) detail of a); d), g) on border of senescent *Vuilleminia*; e), f) on very old ?*Vuilleminia*. – a)-c) with *Melanelia exasperatula*; g) with *Lepraria* sp.; b), g) with *Dactylospora* sp.; f) with *Chaetosphaeria myriocarpa*.

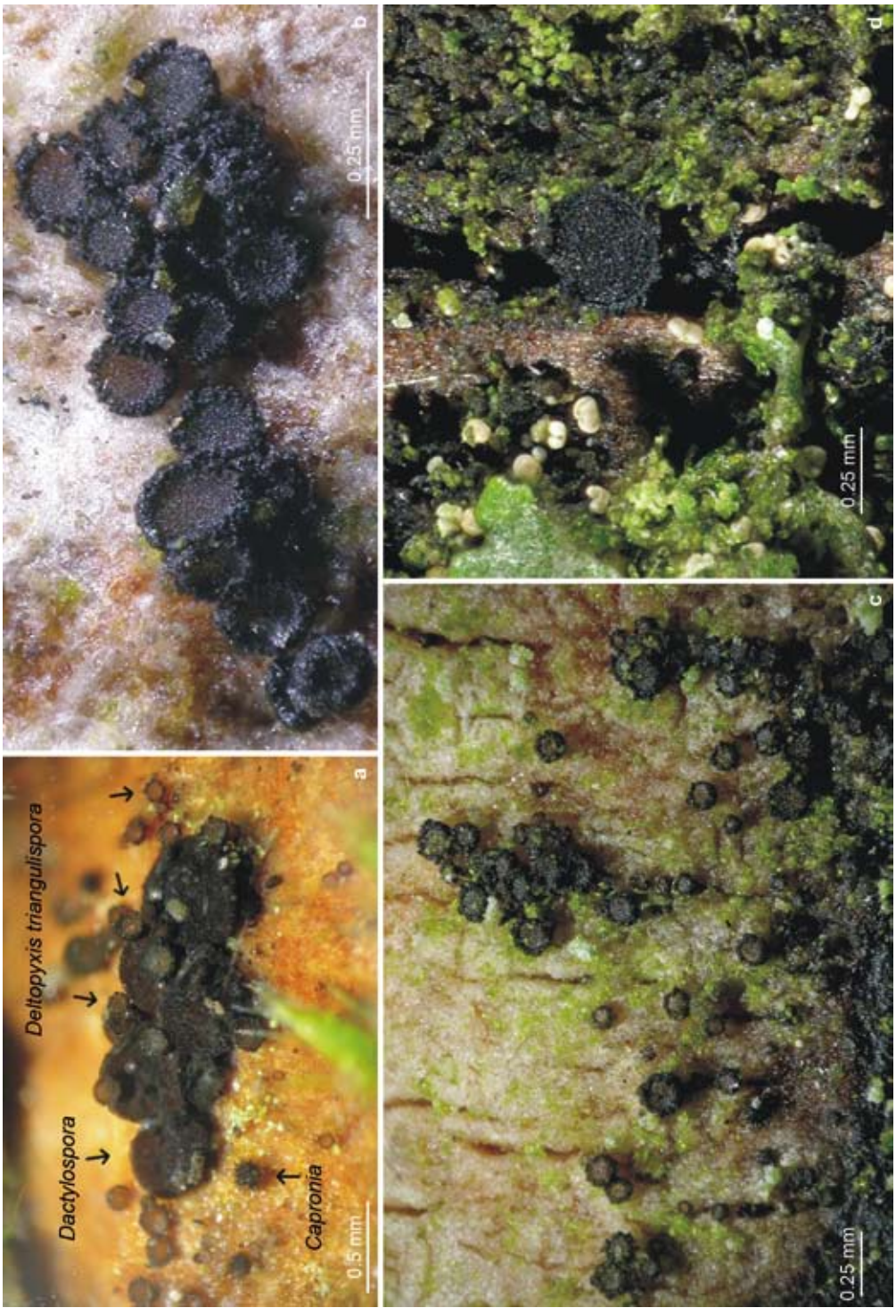

Fig. 4. Apothecia of *Deltopyxis triangulispora* on the natural substrate (rehydrated). a) from Holleschbierg (Rumelange); b) from Kiemreech (Obercorn); c) from Haard (Dudelange); d) from Därebësch (Dudelange, H.B. 9634a); – a)-c) over senescent *Vuilleminia* (a) on old apothecia of *Dactylospora* sp.); d) on bark (single oversized apothecium over split through periderm). – a)-c) *Crataegus* sp., d) *Prunus spinosa*

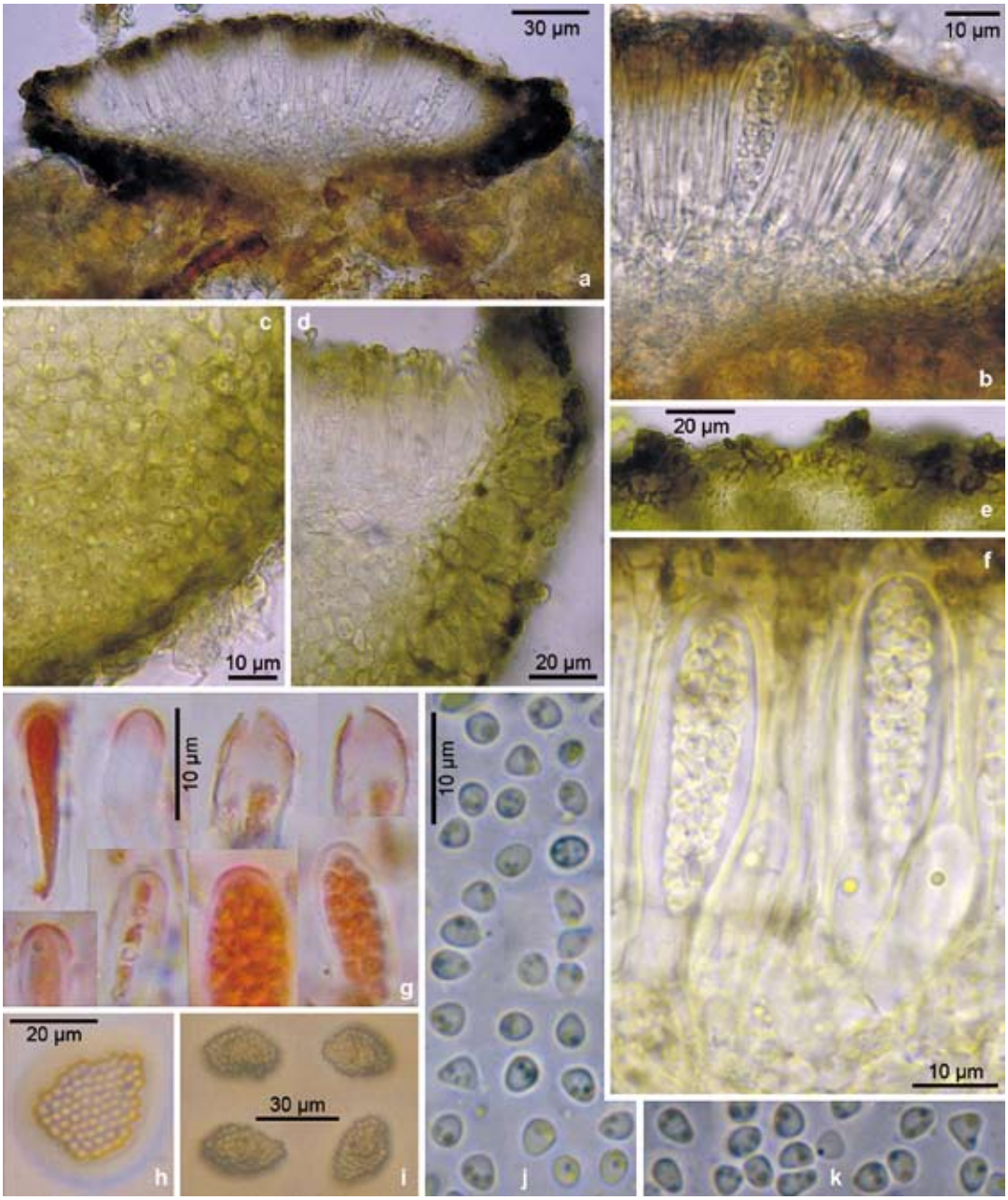

Fig. 5. Microscopic teleomorph features of *Deltopyxis triangulispora* (living state in tap water, except for g) in KOH+CR_{SDS}). a)-b) median section of apothecium on *Vuilleminia*; c)-d) dto., cells of ectal excipulum containing LBs; e. top view on crenulate, dark olivaceous-brown margin; f) turgescent mature asci in context of hymenium; g) ascus apices at different stages of development, showing thickened wall in young asci (left), slit-like broad pore after ejection (upper right), and congophilous ectotunica at apex; h)-i) spore heaps on agar; j)-k) ascospores. – a)-b), i) Léiffrächen (Kayl, on *Crataegus*, H.B. 9625b); f) from Kiemreech (Obercorn, on *Crataegus monogyna*, 6.VIII.2008); h), k). Därebësch (Dudelange, H.B. 9634a, *Prunus spinosa*); j) from Holleschbierg (Rumelange).

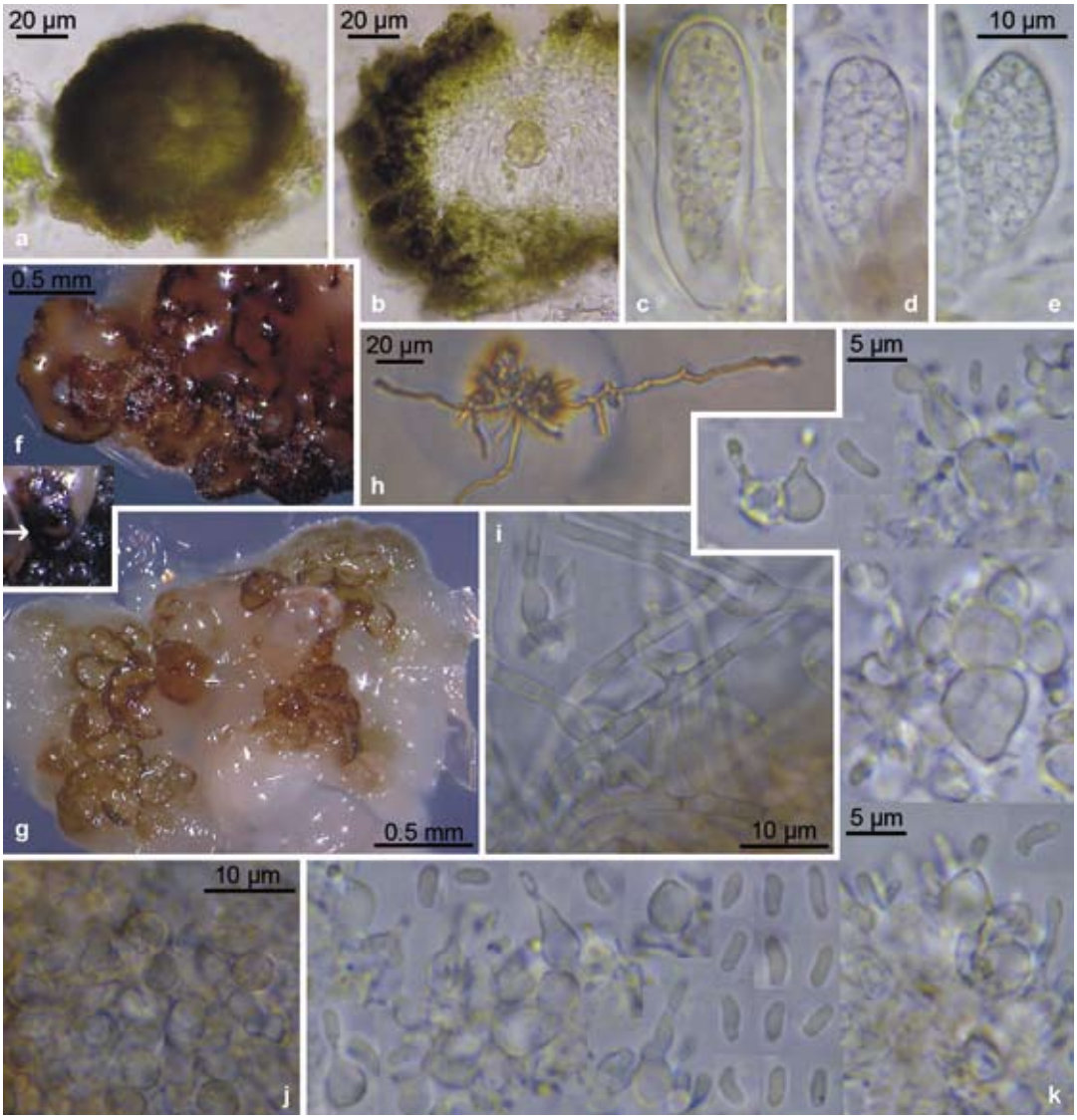

Fig. 6. Microscopic teleo- (a-e) and anamorph (f-k) features of *Deltopyxis triangulispora* (living state in tap water). a)-b) median section of young apothecia; c-e. mature asci containing living spores (c) ascus turgescent, d)-e) asci dead); f)-g) anamorph produced in pure culture, conidiomata rupturing by a slit; h) young mycelium from germinated ascospores; i) mycelium around conidiomata; j) globose cells of peridium; k) phialides and phialoconidia. – a)-c) Därebësch (Dudelange, H.B. 9634a, *Prunus spinosa*); d)-e) Wald (Koedange, on *Ilex*, 25.XII.2011); f)-k). Léiffrächen (Kayl, on *Crataegus*, H.B. 9625b).