# Hyponectria buxi with notes on the Hyponectriaceae

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Hyponectria buxi, the type species of Hyponectria, and the type genus of the family Hyponectriaceae is described and illustrated in this paper. The main characteristics of the family are discussed and notes are provided on other genera included in this family.

Key words: Amphisphaeriaceae, Clypeosphaeriaceae, Hyponectriaceae, Xylariales

#### Introduction

Hyponectria buxi (DC. ex Desm.) Sacc., was originally described by Saccardo (1878, 1883). Petrak (1923) recognized that this species should be separated from the Hypocreaceae and introduced the Hyponectriaceae to accommodate both Hyponectria and Anisostomula. The Physosporelleen (Höhnel, 1919) is the earlier, but invalid name for the family. Arx and Müller (1954) included both Hyponectria and Anisostomula in the Polystigmataceae, but later Muller and Arx (1962) arranged them in the Amphisphaeriaceae, as did Dennis (1968) and Müller and Arx (1973). Barr (1976) recognized two families, the Physosporellaceae (an invalid name) and the Melogrammataceae, and united Anisostomula and Hyponectria.

Barr (1990) provided a description of the family, while the Dictionary of the Fungi (Hawksworth *et al.*, 1995) refer 17 genera to the *Hyponectriaceae*. Many genera have recently been disposed of in the family, but as Hyde *et al.* (1998a) state, until a good description of the type species is provided, the characters that make up the family are confusing. This paper therefore examines authentic material of *Hyponectria buxi* and provides a detailed description of the species. With the understanding of *Hyponectria buxi*, the family characters are outlined and genera included in the family are discussed.

#### Materials and methods

Dried material of Hyponectria buxi was borrowed from IMI, CBS and G.

All descriptions and measurements were carried on an Olympus BX50 microscope. The iodine reaction was tested with Melzer's reagent.

#### **Taxonomy**

Hyponectria buxi (Alb. and Schwein.: Fr.) Sacc., Michelia 1: 250 (1878).

(Figs. 1-13)

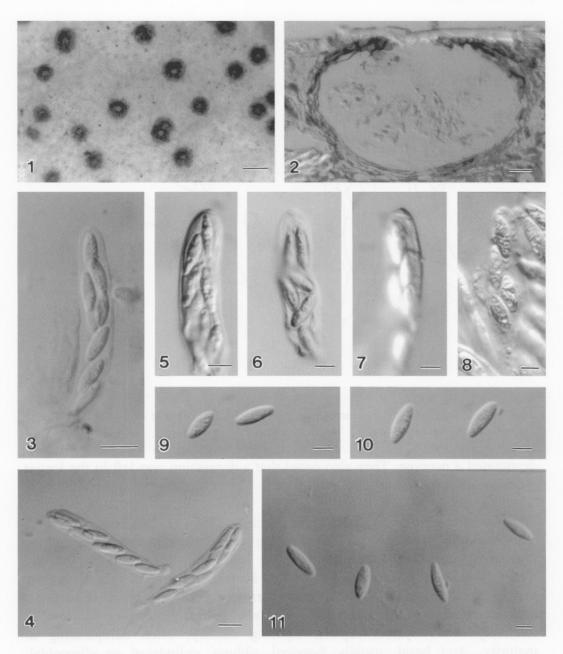
- ≡ Sphaeria buxi (Alb. and Schwein.) DC., Flore de France 6: 146 (1815).
- ≡ Sphaerella buxi (Alb. and Schwein.) Fuckel, Symbolae Mycologicae: 100 (1870).
- ≡ Guignardia buxi (Alb. and Schwein.) Lindau, Hifsbuch 2: 21 (1903).
- ≡ Sphaeria atrovirens Alb. and Schwein. var. (β) buxi Alb. and Schwein.: Fr., Albertini and Schweinitz, Conspectus Fungorum in Lusatiae: 48 (1805): Fries, Systema Mycologicum 2: 501 (1823).
- = Sphaeria buxifolia Cooke, Journal of Botany 21: 69 (1883).
- = Laestadia buxifolia (Cooke) Sacc., Sylloge Fungorum 2: XXXI (1883).

Ascomata 140-214 μm diam., 90-156 μm high, immersed, depressed globose, visible as orange to brown dots on the host surface, coriaceous, ostiolate, solitary or mostly gregarious. Ostiole aperiphysate. Peridium 10-12 μm thick, comprising 3-4 layers of flattened thin-walled cells, clypeal tissues lacking. Paraphyses up to 6 μm wide at the base, sparse, hypha-like, septate, tapering to 1.5 μm at the apex, not embedded in a gelatinous matrix. Asci 48-70 × 7-12 μm, 8-spored, cylindric-clavate to clavate, short pedicellate, unitunicate, with an indistinct J- apical thickening, 4-6 μm diam., 1-1.5 μm high, and invaginations into the plasmalemma, giving a ring-like appearance. Ascospores 10-16 × 3-5 μm, overlapping biseriate or obliquely uniseriate in the ascus, hyaline, ellipsoidal or oblong, straight or inequilateral, unicellular, contents minutely guttulate, lacking a sheath.

*Neotype*: FRANCE, Caen, "ad flo. *Buxi*, legit. Roberge, dedit Curtis", Desmazières, Pl. Crypt. Fr. no 1280. 1843 (PH, neotype designated by Rossman *et al.*, 1999).

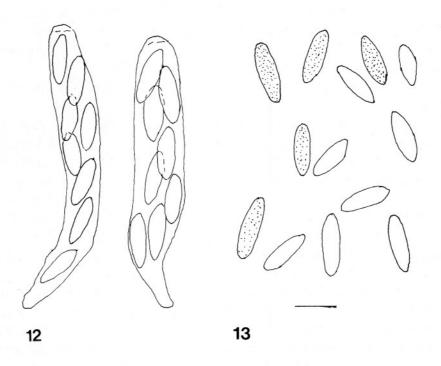
Material examined: FRANCE, Genève, on leaves of Buxus sp., 1921, De Candolle et ses enfants, Herbier de Candolle no 245 (G, labelled as Sphaeria buxi Desm. and Sphaeria (subtecta) buxi Desm.; the material is in poor condition); nr Prost, 1819, Mendel, (G); The Netherlands: Frankrijk, Mt Ventoux, Massif des Cedres, in leaves of Buxus sempervirens, 21 Oct. 1974, H.A. van der Aa (CBS 4433); Suisse, Egerkingen, Jakobsleiter, in leaves of Buxus sempervirens, 25 June 1974 (CBS 000016); UK: Surrey, Mickleham, in leaves of Buxus sp., 1927, E.W. Mason no. 365 (IMI 16895, this material is in good condition); Surrey, Box Hill, in leaves of Buxus sempervirens, 4 May 1952, B.A. Stone (IMI 49466, this material is plentiful and in good condition); Northampton, Pitsford, in leaves of Buxus sempervirens, 30 June 1963, K.A. Pirozynski (IMI 101345a; mostly immature); Yorkshire, Clapham, in leaves of Buxus sp., 3 May 1963, C. Booth (IMI 102003); Derbyshire, near Dovedale, Ilam Park, in leaves of Buxus sempervirens, 11 June 1966, G. Morgan Jones (IMI 119802).

Hyponectria buxi has often mistakenly been attributed to "DC. Ex Desmazières" but as discussed by Rossman et al., (1999), the taxon was



Figs. 1-11. Light micrographs of *Hyponectria buxi* (from IMI 16895). 1. Appearance of ascomata on host surface. 2. Section of ascoma. 3-8. Asci. 9-11. Ascospores. Bars: 1-2=40  $\mu m$ ; 3-11=6  $\mu m$ .

originally published by Albertini and Schweinitz (1805) and sanctioned by Fries at an infraspecific rank. Rossman et al. (1999) illustrated the species and



Figs. 12, 13. Diagrammatic representation of *Hyponectria buxi* (from IMI 16895). 12. Asci. 13. Ascospores. Bars =  $10 \mu m$ .

designated a neotype. They also noted that a depression was left in the leaves when the ascomata dry.

## Hyponectriaceae Petr., Annales Mycologici 21: 305 (1923), emend.

Stromatic tissues if present, forming clypei over immersed ascomata. Ascomata immersed, erumpent or nearly superficial, sphaeroid, globose or ovoid, small or medium, upright or horizontal. Papilla short, ostiole periphysate or aperiphysate. Peridium narrow, of compressed rows of cells, dark externally, pallid internally or entirely pallid. Paraphyses narrow or relatively wide, sparse, septate, tapering distally, often deliquescing at maturity. Asci basal, usually 8-spored, oblong, cylindrical or ellipsoidal, pedicellate, with an apical thickening and invaginations into the plasmalemma, giving a ring-like appearance. Ascospores overlapping biseriate, or in fascicle in the ascus, hyaline, yellow to pinkish or light brown, obovoid, oblong, fusoid, isthmoid or elongate filiform, asymmetric or symmetric, one celled or one to

several septate, wall thin or thickened and firm, smooth or verruculose, surrounded by mucilaginous sheath, at times guttulate (Barr, 1990).

Anamorphs: unknown (Beltraniella reported in Pseudomassaria carolinensis M. Barr and Hodges: Hodges and Barr, 1971).

*Habitat*: Hemibiotrophic in leaves, stems, culms or saprobic in herbaceous substrates.

The major differences between the *Amphisphaeriaceae* and *Hyponectriaceae* are ascus shape and position in the centrum, and probably anamorphs. Barr (1976, 1983) also suggested that the family could be arranged with the *Phyllachoraceae* in the order Phyllachorales. The *Hyponectriaceae* appears to be most closely allied to the *Phyllachoraceae*, although both families were assigned to the Xylariales (Barr, 1990). Then again, Barr (1994) restricted the Xylariales to exclude among others, the *Hyponectriaceae*. Barr (pers. comm.) now believes that the *Hyponectriaceae* are more related to the *Phyllachoraceae* and would accept a separate Order, the *Phyllachorales* again.

Hawksworth *et al.* (1995) included 17 genera and 19 synonyms in the *Hyponectriaceae*, however, many of the genera have been disposed of elsewhere in subsequent publications e.g. (Hyde *et al.*, 1998). With the detailed descriptions of *Hyponectria*, it is now possible to review the genera included in the family. *Palmomyces* and *Charonectria* have recently been added to the family (Hyde *et al.*, 1998a; Rossman *et al.*, 1999).

## Key to genera in the Hyponectriaceae

1. Ascospores with more than one cell......5 Ascomata clypeate, asci broadly cylindrical, with a distinct discoid apical ring, ascospores ellipsoidal and usually surrounded by a mucilaginous sheath, on petioles of Ascomata aclypeate, asci clavate to ellipsoidal, without an apical ring or with an 2. indistinct ring, ascospores ellipsoidal, lunate or bacilliform, usually lacking a sheath, Ascospores ellipsoidal or oblong, asci with an indistinct apical ring, appearing as Ascomata immersed, small to medium sized (up to 400 µm diam), asci J+ (or J-), ascospores small to large, thick walled, with the wall at times ornamented ... Physalospora Ascomata immersed, small, rarely more than 200 µm diam., asci with a J- apical thickening and invaginations into the plasmalemma, giving a ring-like appearance, 

5.	Ascospores apiosporous6
5.	Ascospores with a central septum
6.	Ascomata lie horizontally between the cuticle and epidermis of the host, with a laterally placed ostiole, lacking a J+ ascal ring
6.	Ascomata perpendicular to the host surface with a central ostiole, asci with or without a J+ ring
7.	Ascospores with spine-like appendages at each end
7.	Ascospores oblong to ellipsoidal, lacking spine-like extensions9
8.	Asci with an unusually large J+ apical ring, ascomata forming under individual cyplei  Ceriospora
8.	Asci lacking a J+ apical ring, ring indistinct, ascomata forming under a stroma
9. 9.	Ascospores 1-septate, oblong or ellipsoidal, ascomata orange to yellow <i>Charonectria</i> Ascospores 1-5-septate, cylindrical or fusiform, ascomata darker
10. 10.	Ascospores 2-septate, ascomata forming under a clypeus

#### Notes on genera included in the Hyponectriaceae

### 1. Apiothyrium Petr., Sydowia 1: 1 (1947).

This is a poorly known monotypic genus represented by *A. arcticum* Petr. Ascomata lie horizontally between the cuticle and epidermis of the host and has a laterally placed ostiole. The asci lack a J+ apical ring and the ascospores are hyaline, two-celled and apiosporous (Wehmeyer, 1975). Further studies on this species are required in order to ascertain its taxonomic affinities, although we tentatively include it in the *Hyponectriaceae*.

## 2. Arecomyces K.D. Hyde, Sydowia 48: 227 (1996).

Several species were described in this genus by Hyde (1996a), which was referred to the *Hyponectriaceae*. Ascomata are immersed under a clypeus or pseudostroma, asci are broadly cylindrical, with a J- discoid apical ring and ascospores are unicellular, hyaline and mostly with a sheath. In all cases the hosts were palms. Because of the thin-walled peridium of the immersed ascomata, the form of the ascal ring, and unicellular hyaline ascospores we agree with Hyde (1996a), that *Arecomyces* should be accommodated in the *Hyponectriaceae*.

#### 3. Arwidssonia B. Eriksson, Svensk Botanisk Tidskrift 68: 199 (1974).

Arwidssonia was introduced to accommodate Heterosphaeria empetri Rehm, which could not be accommodated in any existing genus (Eriksson, 1974). The genus contains two species with immersed ascomata having thin walls and opening by 3-5 lobes. Asci are cylindrical to slightly saccate, and are J+ only after treatment with Minks' method (see Eriksson, 1974). Ascospores are (1-)3(-5)-septate and hyaline. This appears to be appropriately accommodated in Hyponectriaceae (Barr, 1990).

# 4. *Ceriospora* Niessl, Verhandlungen des Naturforschenden Vereines in Brünn 14: 169 (1876).

In *Ceriospora*, asci are cylindric-clavate with an unusually large J+ apical (as opposed to subapical) ring. Ascomata are immersed in host tissue with a blackened papilla and ascospores are 2-celled with spine-like appendages (Hyde, 1993a). The inclusion of *Ceriospora* in the *Hyponectriaceae* appears to be a logical step. It can no longer to be included in the *Amphisphaeriaceae* (Kang *et al.*, 1999b).

### 5. Chamaeascus L. Holm, K. Holm and M. Barr, Blyttia 3-4: 121 (1993).

Chamaeascus is a monotype genus represented by C. arcticus L. Holm, K. Holm and M. Barr (Holm and Holm, 1993). Ascomata are immersed and non-papillate, but darker around the pore. Asci are ellipsoidal and lack an apical ring, while ascospores are bacilliform to lunate, 1-celled, with obtuse or slightly pointed ends (Holm and Holm, 1993). Although no reasons were given for the inclusion of the species in the *Hyponectriaceae*, its inclusion seems possible.

## 6. Charonectria Sacc., Michelia 2: 72 (1880).

Charonectria was introduced for Hyponectria-like species with immersed solitary ascomata and 1-septate ascospores. This was considered to be a unique genus of the Hyponectriaceae (Rossman et al., 1999). Based on the thin walled membraneous ascomata immersed in leaves, pallid clypeus, and large 2-celled ascospores the inclusion seems correct. A similar depression as with Hyponectria buxi, is left in the leaves when the ascomata dry.

# 7. *Frondispora* K.D. Hyde, Sydowia 45: 208 (1993).

Represented by *Frondispora bicalcarata* (Ces.) K.D. Hyde, this monotypic genus has ascomata immersed under a pseudostroma, a narrow peridium, cylindric-clavate asci with an inconspicuous apical ring and 2-celled ascospores with spine-like appendages (Hyde, 1993a). Kang *et al.* (1999b)

tentatively placed this genus in the Clypeosphaeriaceae. Many characters are in common with those in Hyponectria buxi, and the inclusion of Frondispora in the Hyponectriaceae, on reflection, appears to be logical.

### Hyponectria Sacc., Michelia 1: 250 (1878).

In species of Hyponectria, ascomata are immersed, small, rarely more than 200 µm diam., depressed globose, with a very short papillate or plane ostiole. The peridium is thin and soft and composed of two or three layers of thinwalled cells. Asci are unitunicate, clavate, oblong, or cylindric, sometimes pedicellate, with an apical thickening and invaginations into the plasmalemma, giving a ring-like appearance. Paraphyses are sparse, thin walled, broad, taper distally, and are attached at both top and base of the centrum. Ascospores are one celled, thin walled, and usually small (rarely over 20 µm long). This genus represents the type of the Hyponectriaceae, which was reviewed by Barr (1977).

9. Physalospora Niessl, Verhandlungen des Naturforschenden Vereines in Brünn 14: 170 (1876).

Barr (1976) considered Hyponectria and Physalospora to be similar and placed them both in the Physosporellaceae. The genus has not to our knowledge been studied recently although, Kohlmeyer et al. (1995) described a new species P. citogerminans Kohlm., Volkm.-Kohlm. and O. Erikss. from Juncus. This new species has deeply immersed ascomata with a peridium of brown flattened cells. Asci are cylindric-clavate with a refractive discoid apical ring and ascospores are hyaline, unicellular and surrounded by a sheath. This species differ from the type Physalospora alpestris Niessl, which has apapillate ascomata and yellowish-greenish ascospores (Kohlmeyer et al., 1995). In the type species asci are also J+ (Nograsek, 1990). We concur with the inclusion of *Physalospora* in the *Hyponectriaceae*.

Species of *Physalospora* have immersed ascomata with an apex which is barely or widely erumpent, small to medium sized (up to 400 µm diam), globose or conical, and with a well-developed papillate apex. The peridium is firm, thin or thick. A clypeus may be present, or in species with widely erumpent apices, the apex may be blackened and at times setose. Asci and paraphyses are similar to those of species of Hyponectria. The ascospores in Physalospora species are small to large, thick walled, with the wall at times ornamented. Further studies are required to establish the differences between

Hyponectria and Physalospora.

#### 10. Pseudomassaria Jacz, Bulletin dl' Herbier Boissier 2: 663 (1894).

The type species of *Pseudomassaria*, *P. chondrospora* appears to be typical of the *Hyponectriaceae*, although in some species of *Pseudomassaria*, asci have J+ rings (Hyde *et al.*, 1998a). We suspect that the genus is presently polyphyletic.

#### 11. Rachidicola K.D. Hyde and Fröhl., Sydowia 47: 217-222 (1995).

In *Rachidicola* the asci are similar to those of *Hyponectria buxi*, in having an indistinct apical ring visible due to indentation of the plasmalemma (Hyde and Fröhlich, 1995). The ascomata are relatively thin-walled and immersed under the clypeus. The ascospores are unique in being 3-celled. These characters indicate that *Rachidicola* can be accommodated in the *Hyponectriaceae*.

#### Notes on genera excluded from the Hyponectriaceae

# 1. *Apioclypea* K.D. Hyde, Botanical Journal of the Linnean Society 116: 316 (1994).

In *Apioclypea*, ascomata are immersed under a poorly developed clypeus, and asci and paraphyses are organized in a basal layer. The asci are cylindrical and have a J- discoid, or J- subapical ring. Hyde *et al.* (1998a) and Kang *et al.* (1999b) place *Apioclypea* in the *Clypeosphaeriaceae*. The information on *Hyponectria* provided here indicates that these taxa are unrelated, as the ascal apices and their staining reactions are very different.

# 2. *Exarmidium* Karst., Bidrag Kännedom Finlands Natur Folk 23: 29, 222 (1873).

Exarmidium as revised by Barr and Boise (1985), has ascomata which are immersed in clusters under a clypeus. The thin peridium is composed of compressed cells, and asci are basal, elongate to clavate, with a poorly defined apical ring, bluing in iodine in some species. Ascospores are hyaline or faintly yellowish, with 3 or more transverse septa (Barr and Boise, 1985). In the type species, E. hysteriiforme (P. Karst.) P. Karst., the hymenium also blues in iodine and this is atypical of the Hyponectriaceae.

# 3. *Lasiobertia* Sivan., Transactions of the British Mycological Society 70: 383 (1978).

Lasiobertia is closely related to Oxydothis (Hyde, 1993a) and should therefore be placed in the Clypeosphaeriaceae, until more obvious affinities can be established (Hyde et al., 1998a).

4. *Leiosphaerella* Höhn., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Mathematisch - naturwissen-schaftliche Classe Abteilung 128: 579 (1919).

Because of the similarities of this genus to *Oxydothis*, Kang *et al.* (1999b) concluded that *Leiosphaerella* should be placed in the *Clypeosphaeriaceae*, but admitted that this family was probably polyphyletic.

### 5. Linocarpon Syd and P. Syd, Annales Mycologici 15: 210 (1917).

In *Linocarpon* ascomata are immersed beneath a clypeus and asci are cylindric-clavate with a J- apical ring. The ring has been examined at the ultrastructural level and is most similar to some species in the Xylariales (Poonyth *et al.*, 1999). It is unlike the ring found in the asci of *Hyponectria buxi*, and although the organization of the centrum is basal, it is unlikely that *Linocarpon* can be included in the *Hyponectriaceae*.

6. *Mangrovispora* K.D. Hyde and Nakagiri, Systema Ascomycetum 10: 20-25 (1991).

This monotypic genus was described by Hyde and Nakagiri (1991) and is represented by *M. pemphii* K.D. Hyde and Nakagiri. Ascomata are immersed beneath a dark thin stroma with their axis horizontal to the host surface with neck at one end bending upwards to piece the surface. Asci are cylindrical with an internal ring-like structure (tube) and apical thickening. Ascospores are 4-celled with a sheath. The arrangement of the centrum and ascus structure is unlike that in *Hyponectria buxi* and therefore the inclusion of *Mangrovispora* in the *Hyponectriaceae* seems doubtful.

# 7. Monographella Petr., Annales Mycologici 22: 144 (1924).

This is a widespread genus with 4 species (Hawksworth et al., 1995). Both Monographella albescens (Thümen) Parkinson, Sivanesan and C. Booth and M. stoveri (C. Booth) Samuels and Hallett have been illustrated in detail (Parkinson et al., 1981; Samuels and Hallett, 1983) and have many similarities with species in the Amphisphaeriaceae and Clypeosphaeriaceae. The anamorph of both these species were synonymised with Microdochium (Samuels and Hallett, 1983) and in culture form conidia on salmon coloured pinnotes. The conidiogenious cells and conidia of M. oryzae (Hashioha and Yokogi) Samuels and Hallett are vaguely reminiscent of the anamorphs of some taxon in the Amphisphaeriaceae (Parkinson et al., 1981). They differ, however, as there is no conidiomata in M. oryzae, but there is a tendency to form sporodochia as colonies become older. Monographella cannot be included

in the *Hyponectriaceae* as the asci, ascospores and anamorph are atypical. Inclusion in the *Amphisphaeriaceae sensu stricto* or *Clypeosphaeriaceae* (Kang *et al.*, 1999a, b) should be considered.

8. *Neolinocarpon K.D.* Hyde, Botanical Journal of the Linnean Society 110: 104 (1992).

*Neolinocarpon* is related to *Linocarpon* (Hyde *et al.*, 1998b) and therefore its inclusion in the *Hyponectriaceae* is doubtful.

9. Oxydothis Penz. and Sacc., Malphigia 11: 505 (1898).

The asci of *Oxydothis* has recently been examined at the ultrastructural level (Wong and Hyde, 1999) and comprised a subapical electron-dense ring with a faint canal leading to the apex. The arrangement of the ascomata, structure of the asci and ascospores are unlike *Hyponectria buxi*. Kang *et al.* (1999b) placed *Oxydothis* in the *Clypeosphaeriaceae*, but regard this family polyphyletic.

10. Palmomyces K.D. Hyde, J. Fröhl. and J.E. Taylor, Sydowia 50: 59 (1998).

This monotypic genus was introduced in the *Hyponectriaceae* by Hyde *et al.* (1998a). Asci are cylindric-clavate with a J-, subapical ring and ascospores are 1-septate and apiosporous and unlike those of *Hyponectria buxi*. The inclusion in the *Hyponectriaceae* is unjustified because of the subapical tubelike ring in the ascus.

11. *Pemphidium* Mont., Annales des Science Naturelles, Botanie Serie 2, 14: 329 (1840).

Pemphidium contains four species, all with long cylingrical asci and a small refractive discoid subapical ring. Ascomata are generally immersed under a distinct shining blackened clypeus or stroma and ascospores are filiform (Hyde, 1993b, 1996b). Pemphidium species have few similarities with Hyponectria, and therefore inclusion in the Myelospermaceae may be suitable (Hyde and Wong, 1999).

12. *Physosporella* Höhn., Annales Mycologici 16: 54, *nom. nud.*; 161 (1918). This is considered as a synonym of *Physalospora* (Barr, 1976), although it was listed as an accepted genus in Hawksworth *et al.* (1995). This poorly known genus needs further study.

#### Glomerella and Colletotrichum

Glomerella Spauld. and Schrenk., U.S. Department of Agriculture Bureau Plant Industries Bulletin 44: 29 (1903).

Glomerella was studied by Arx and Müller (1954) and placed in their family, the *Polystigmataceae*. Recent treatments have considered that *Glomerella* is related to the *Phyllachoraceae* (Barr, 1990; Hawksworth *et al.*, 1995). Molecular studies suggest that the Phyllachorales is polyphyletic and that *Phyllachora* and *Coccodiella* are only the true Phyllachorales sampled (Silva-Hanlin and Hanlin, 1998).

In species of *Glomerella* ascomata are immersed and have a thin-walled peridium. Asci are cylindric-clavate to fusiform with an indistinctive apical discoid ring, and ascospores are hyaline and unicellular (Fröhlich *et al.*, 1997). This genus may be an appropriate candidate for inclusion in the *Hyponectriaceae*, however the *Colletotrichum* anamorph may be problematical.

### Sarcophoma miribelii (Fr.) Höhn.

In the process of searching for type material of *Hyponectria buxi* deposited by De Candolle, the following specimens of *Sarcophoma miribelii* were identified, and is the anamorph of *Discosphaerulina miribelii* (Van der Aa) Siyanesan.

Material examined: FRANCE, Genève, on leaves of Buxus sp., 1921, De Candolle et ses enfants, Herbier de Candolle no 245 (G, labelled as Sphaeria atrovirens and Sphaeria buxi).

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