### Byssosphaeria and Herpotrichia from Taiwan, with notes on the taxonomic relationship between these two genera

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Four species of *Byssosphaeria* and two species of *Herpotrichia*, including two new species, *B. erumpens* sp. nov. and *H. fusispora* sp. nov., are described and illustrated from Taiwan. As the separation of *Byssosphaeria* from *Herpotrichia* has been historically controversial, their relationship is discussed. Conclusively they are considered to be closely related but separate genera, and their distinctions can be morphologically recognized.

Keywords: Byssosphaeria, Herpotrichia, Taiwan, taxonomy.

Bose (1961) and Sivanesan (1972) conceived a wide concept of Herpotrichia Fuckel to involve eight synonymous genera. Barr (1984) re-established four genera from Herpotrichia, i.e. Byssosphaeria Cooke, Lojkania Rehm, Neopeckia Sacc. and Pseudotrichia Kirschst., but this segregation was not accepted by von Arx & Müller (1984), who argued that these four genera are morphologically similar, and possess a common Pyrenochaeta anamorph. Nonetheless, Barr's disposition has since been widely adopted. Among these separated genera, Lojkania, Neopeckia and Pseudotrichia are undoubtedly good genera as they can be readily distinguished from *Herpotrichia* on morphology. *Lojkania* is characterized by ascospores with germ slits (Yuan & Barr, 1994), Neopeckia by the cylindrical asci filling completely the locule (Barr, 1984), Pseudotrichia by the protruding or slit-like apex of ascomata (Barr, 1990; Huhndorf, 1994). However, the separation of Byssosphaeria and Herpotrichia remains equivocal.

Sivanesan (pers. comm.) preferred to consider *Byssosphaeria* as a synonym of *Herpotrichia*. This classification was followed by Hsieh & al. (2000), who reported three species of *Herpotrichia* from Taiwan, namely *H. rhodosticta* Berk. & Broome, *H. schiedermayeriana* (Fuckel) Barr, and *H. macrotricha* (Berk. & Broome) Sacc. However, if we follow Barr (1984), who considers the two genera to be separate, the former two species are accommodated in *Bysso-sphaeria*. Furthermore, during a continuous survey of Taiwan asco-mycetes, additional *Byssosphaeria-Herpotrichia* species were recognized, including one new record and two new species. The controversy of placing species to either genus highlights the need to clarify the concept of these two genera so as to determine to which genus the Taiwan species can be assigned.

Barr (1984) did not draw a clear delimitation between Byssosphaeria and Herpotrichia, but instead she assigned them to Melanommatales and Pleosporales respectively. Melanommatales is characterized by the trabeculate pseudoparaphyses and Pleosporales is characterized by the cellular pseudoparaphyses (Barr, 1983, 1987). This suggests that the main difference considered by Barr (1984) between Byssosphaeria and Herpotrichia concerns these two pseudoparaphysis types. However, as being proved by the sequence analyses by Winka (2000) and Liew & al. (2000), Melanommatales is a synonym of Pleosporales, as is accepted in the latest edition of 'Dictionary of the Fungi' (Kirk & al., 2001), and pseudoparaphyses are inadequate for application in taxonomy. It means the criterion used to distinguish Byssosphaeria from Herpotrichia should no longer be applied. On the other hand, in 18S rDNA sequence-based trees constructed by Berbee (1996) and Silva-Hanlin & Hanlin (1999), Herpotrichia diffusa (Schwein.) Ellis & Everh. clusters with Herpotrichia juniperi (Duby) Petrak, and the bootstrap support for the connection is strong, being 99% and 100% respectively. Herpotrichia diffusa, in fact, was treated as Byssosphaeria diffusa (Schwein.) Cooke by Barr (1984, 1990). Consequently it can be inferred that, if Byssosphaeria and Herpotrichia are separate genera, they must be closely related. It should be noted that although B. diffusa and H. juniperi are not the type species of respective genera, they do bear strong resemblances to them and can be representative of each genus. After examining materials referred by Sivanesan (1972) and deposited at IMI, we came to realize that Byssosphaeria, typified by B. schiedermayeriana (Fuckel) Barr, and *Herpotrichia*, typified bv H. rhenana Fuckel then H. herpotrichoides (Fuckel) Cannon, (Cannon, 1982), can be separated on the morphological basis, although they are similar on many aspects. Both genera share many characteristics in common. Ascomata are superficial, non-papillate, covered with hyphal appendages and seated on a subiculum. The apex of the ascomata is rounded or flattened, sometimes pallid or colored around the ostiolum. Ascospores are usually surrounded by a mucilaginous sheath which may draw out at ends to appear as appendages. Anamorph states of both genera, where known, are *Pyrenochaeta*-like coelomycetes, and are reported for eight species (Sivanesan, 1984), in which B. diffusa, B. rhodomphala (Berk.) Sacc., and B. schiedermayeriana (Fuckel) Barr are present in Taiwan. However, asci in Byssosphaeria are short to long-stalked, while in Herpotrichia asci are sessile to short-stalked. Ascospores of Byssosphaeria are pale to dark brown, while those of Herpotrichia are hyaline and seldom become brown with age. Both genera have subglobose ascomata, but turbinate ascomata occur in many species of Byssosphaeria.

In conclusion, we accept *Byssosphaeria* and *Herpotrichia* to be separate genera; however their segregation is not in the sense of Barr (1984), who attributed these two genera to two different orders on the basis of pseudoparaphysis types, but rather in our own view of recognizing their differences in asci and ascospores. We also consider they are closely related genera and should be accommodated in the same family. In the 'Dictionary of the Fungi' (Kirk & al., 2001), Byssosphaeria is placed in the Melanommataceae, and Herpotrichia in the Lophiostomataceae (syn, Massarinaceae). It is believed that their placement into different families needs to be revised by taking molecular data into account. As the generic distinction between Byssosphaeria and Herpotrichia is clarified, two new species, B. erumpens sp. nov. and H. fusispora sp. nov., are accordingly proposed from Taiwan. In addition to these two new species, one new record, B. diffusa, and three previously reported species (Hsieh & al., 2000), treated accordingly as B. rhodomphala, B. schiedermayeriana, and *H. macrotricha*, are described and explicitly illustrated so as to provide a clear comparison between Byssosphaeria and Herpotrichia.

### **Material and methods**

Specimens were collected in Taiwan. To study the generic circumscription of *Herpotrichia* and *Byssosphaeria*, specimens cited in Sivanesan's monograph (1972) in the herbarium of IMI were examined, but they will not be listed under the heading of 'specimen examined' in this paper. Measurements, microtome sections and photographs were made from Taiwan materials. Measurements of sectioned ascomata, asci and ascospores were made in water by ranges of maximal and minimal dimensions. Single spore isolations were made in an attempt to obtain their asexual states, but without success. The official acronym TNM represents the herbarium of National Museum of Natural Science, and NCHUPP represents the herbarium of the National Chung Hsing University.

### Taxonomy

Key to species of *Byssosphaeria* and *Herpotrichia* from Taiwan

1.	Asci nearly sessile, ascospores hyaline	2
1.*	Asci short to long-stalked, ascospores brown	3

2.	Ascomata less than 500 $\mu m$ wide, with a conical ostiolar cavity
2.*	Ascomata more than 500 μm wide, ostiolar cavity not as above 
3. 3.*	As cospores more than 25 $\mu m$ long $\ldots \ldots B.$ schiedermayeriana As cospores less than 25 $\mu m$ long $\ldots \ldots \ldots 4$
4.	Apex of ascomata crowned with pulverulent, reddish mass of cells
4.*	Apex of ascomata pallid or whitish around ostiolum 5
5.	Ascomata superficial, ascospores pale brown, with darker ends
5 *	Ascomata erumpent, ascospores evenly dark brown <i>B</i> erumpens

**Byssosphaeria diffusa** (Schwein.) Cooke, Grevillea 15: 81. 1887. – Fig. 1.

Syn.: *Herpotrichia diffusa* (Schwein.) Ell. & Everh., North Amer. Pyrenomyc. p. 158. 1892.

For other synonyms and descriptions see Barr (1984, 1990), Bose (1961) and Sivanesan (1972).

As comata densely gregarious, superficial on subiculum, subglobose to turbinate, 340–480  $\mu$ m wide, 300–450  $\mu$ m high, apex flattened, non-papillate, disc-like, whitish, becoming slightly depressed around the ostiolar center, periphysate, abundant hyphal appendages radiating from sides below the apical region. Peridium 35–50  $\mu$ m thick, thickened and 50–60  $\mu$ m thick at the apex. – Asci clavate, 80–110×9–12  $\mu$ m, 8-spored, long-stalked, stalk up to 40  $\mu$ m long. – Pseudoparaphyses 1.5–2.5  $\mu$ m wide. – Ascospores oblong to fusiform, 16–20×4–6  $\mu$ m, smooth, pale brown, darkened at each end, with up to 4 guttules, 1-septate, strongly constricted at the septum, surrounded by a mucilaginous sheath which protrudes as appendages at the ends of the ascospores.

Specimen examined. – TAIWAN: Chiai Hsien, Alishan, 2 Mar 2002, on dead stem of *Cyclobalanopsis* sp., C. Y. Chen (NCHUPP c0721).

DNA sequences deposited in the database of Genbank are filed under the name of *Herpotrichia diffusa* rather than *Byssosphaeria diffusa*. Caution should be taken in carrying out phylogenetic analyses with these sequences, because *Byssosphaeria* belongs in the Melanommataceae, while *Herpotrichia* belongs in Lophiostomataceae.



Fig. 1. Byssosphaeria diffusa (from NCHUPP c0721). – a. Ascomata on host surface. – b. Section of ascoma. – c–e. Asci. – f–j. Ascospores. – Bars: a = 1 mm; b = 100  $\mu$ m; c–j = 10  $\mu$ m.

### Byssosphaeria erumpens C. Y. Chen & W. H. Hsieh sp. nov. - Fig. 2.

Ascomata solitaria vel saepius 2–6 conferta, regulatim dispersa, erumpentia, basibus cortice hospitis cinctis, subglobosa, 420–520 µm lata, 450–550 µm alta, glabra, apice leniter compresso, non papillato, circum ostiolum pallido. Paries ascomatis 20–30 µm crassus, 4–6 stratis cellularum compositus, interne cellulis pallide brunneis, extreme cellulis valde pigmentiferis. Asci clavati,  $105–130 \times 12-15$  µm, 8-spori, longe pedicellati, pedicelli usque ad 45 µm longi. Ascosporae ellipsoideae vel fusiformes, 20– $25 \times 5-6$  µm, uniseptatae, atrobrunneae, laeves, imbricate biseriatae vel supra biseriatae et infra uniseriatae in asco, vaginis mucilaginosis extremis protractis obtectae.



Fig. 2. Byssosphaeria erumpens (from holotype). – a. Ascomata on host surface. Note the erumpent ascomata. – b. Section of ascoma. – c–e. Asci. – f–j. Ascospores. – Bars: a = 500 μm; b = 50 μm; c = 20 μm; d–j = 10 μm.

Holotypus: Taiwan, Nantou Hsien, Menfeng, in caulibus emortuis *Litseae* sp., 14 Oct 2002, C. Y. Chen, TNM(F) 15217; isotypus NCHUPP c0804.

Ascomata single or 2–6 in small clusters, evenly scattered, erumpent, with the ruptured host tissue around the base, subglobose,  $420-520 \mu m$  wide,  $450-550 \mu m$  high, glabrous, apex slightly com-

pressed, non-papillate, occasionally depressed, pallid around the ostiole, periphysate, usually with a cushion of thin stroma beneath individual ascomata. Peridium 20–30  $\mu$ m thick, composed of 4–6 layers cells, inner cell layers pale brown, the outermost cell layer deeply pigmented, giving a crust-like appearance in vertical section, cells elongated at sides and more or less angular at base, peridium around ostiolar region becoming thicker and hyaline, up to 70  $\mu$ m thick, composed of angular, conspicuously scleroplectenchymatous cells. – Asci clavate, 105–130×12–15  $\mu$ m, 8-spored, long-stalked, stalk up to 45  $\mu$ m long. – Pseudoparaphyses 1.5–2.5  $\mu$ m wide. – Ascospores ellipsoid to fusiform, 20–25×5–6  $\mu$ m, smooth, dark brown, overlapping, biseriate in the upper part of ascus and uniseriate below, 1-septate, strongly constricted at the septum, surrounded by a mucilaginous sheath which projects out to appear as appendages at the ends of ascospores.

Other specimens examined. – TAIWAN: Hualien Hsien, Tienshieng, on dead stem, 29 Dec 2001, C. Y. Chen (NCHUPP c0431); Nantou Hsien, Wushe, on dead stem of *Litsea* sp., 16 Feb 2002, C. Y. Chen (NCHUPP c0603).

The erumpent glabrous ascomata without underlying subiculum seem not typical of *Byssosphaeria*. However, there is a thin stromatic mat beneath the ascomata. It is thought to be the remnant of the subiculum. Moreover, the ascomata with a pallid and compressed apex and the dark brown ascospores with a surrounding mucilaginous sheath protruding at two ends, strongly suggest the disposition of this species in *Byssosphaeria*. The ascospores in this species resemble those of *B. diffusa*, but can be distinguished by being evenly dark brown and larger in size. The position (erumpent) and arrangement (evenly scattered) of ascomata on substrate are unique in this genus.

## Byssosphaeria rhodomphala (Berk.) Sacc., Syll. Fung. 2: 212. 1883. – Fig. 3.

Syn.: Herpotrichia rhodosticta (Berk. & Broome) Sacc., Syll. Fung. 2: 213. 1883.

For other synonyms and descriptions, see Barr (1984, 1990), Bose (1961), Hsieh & al. (2000), Réblová (1997), Samuels & Müller (1978) and Sivanesan (1972).

A scomata scattered or loosely gregarious, superficial on subiculum, turbinate, 300–460  $\mu$ m wide, 320–440  $\mu$ m high, apex rounded, non-papillate, usually depressed, with reddish pulverulent, projected mass of cells around the ostiole, periphysate, hyphal appendages sparsely arising from the lower sides of ascomata. Peridium laterally 20–30  $\mu$ m thick, at apex externally crowned with loosely aggregated,



Fig. 3. Byssosphaeria rhodomphala (from NCHUPP 2555). – a. Ascomata on host surface. – b. Section of ascoma. – c–e. Asci. – f–j. Ascospores. – Bars: 1 = 500  $\mu$ m; 2 = 50  $\mu$ m; 3–5 = 10  $\mu$ m; 6–10 = 5  $\mu$ m.

reddish, globose cells. – Asci clavate,  $120-150 \times 12-15 \mu m$ , 8-spored, occasionally 4–7-spored, long-stalked, stalk up to 40  $\mu m$  long. – Pseudoparaphyses 1.5–2.5  $\mu m$  wide. – Ascospores ellipsoid,  $18-23 \times 5-7 \mu m$ , smooth, pale brown, 1-septate, slightly constricted at the septum, without a mucilaginous sheath.

Specimen examined. - TAIWAN: Nantou Hsien, Kuantaochi, on decaying wood, May 1999, C. L. Wang (NCHUPP 2555). The hyphal appendages on ascomata in this species are reported to be abundant, while they are relatively sparse for this Taiwan material. However, as in the case of B. schiedermayeriana, the amount of the hyphal appendages is usually variable. It is expected to find ascomata with abundant hyphal appendages when further collections are made. This species was reported as H. rhodosticta from Taiwan (Hsieh & al., 2000), while is treated herein in Byssosphaeria according to its turbinate ascomata, long-stalked asci and brown ascospores.

**Byssosphaeria schiedermayeriana** (Fuckel) Barr, Mycotaxon 20: 34. 1984. – Fig. 4.

Bas.: *Herpotrichia schiedermayeriana* Fuckel, Jahrb. Nassauischen Vereins Naturk. 27–28: 27, 1873.

For other synonyms and descriptions, see Barr (1984, 1990), Bose (1961), Hsieh & al. (2000), Hyde & al. (1999), Samuels & Müller (1978) and Sivanesan (1972).

Ascomata densely gregarious, superficial on subiculum, turbinate or compressed globose, 720–900 um wide, 700–950 um high, apex flattened, non-papillate, disc-like, reddish, yellowish, or whitish, periphysate, covered with hyphal appendages except the apical region, occasionally appearing glabrous by sloughing off hyphal appendages with age. Peridium 60–90 µm thick, composed of angular to strongly compressed, hyaline to dark brown cells, sometimes a cluster of angular cells becoming melanized, visible as an areolate patch (Fig. 4c), with parallel, laminated cells connecting adjacent areolate patches. – Asci narrowly clavate,  $150-180 \times 12-16$  µm, 8-spored, occasionally 4-7-spored, long-stalked, stalk up to 60 µm long. – Pseudoparaphyses 1–2 µm wide. – Ascospores ellipsoid to fusiform,  $30-42 \times 5-8 \mu m$ , smooth, pale brown, 1-septate, septum slightly constricted, sometimes becoming 3-septate and dark brown, mucilaginous sheath surrounding ascospores and protruding at ends as appendages.

Specimens examined. – TAIWAN: Kaoshiung Hsien, Mienung, on dead stem of bamboo, 2 Jan 1988, A. Sivanesan (as *B. jamaicana*, IMI 322683; NCHUPP AS349); Nantou Hsien, Tsuifeng, on decaying wood, 26 Feb 1992, W. H. Hsieh (NCHUPP 2272); Hualien Hsien, Tienshieng, on decaying wood, 6 Jul 2000, C. Y. Chen (NCHUPP c0011); Taichung Hsien, Paileng, on dead stem, 20 Apr 2001, C. Y. Chen (NCHUPP c0210); Nantou Hsien, Lushan, on decaying wood, 28 Jan 2002, C. Y. Chen (NCHUPP c0509); Chiai Hsien, Alishan, on dead stem of *Deutzia* sp., 3 Mar 2002, C. Y. Chen (NCHUPP c0733).

The appearance of ascomata is variable from different collections. The surface is glabrous to hairy, and the apex is reddish, yellowish or whitish around the ostiolum. The appendages of



Fig. 4. Byssosphaeria schiedermayeriana (from NCHUPP c0210) – a. Ascomata on host surface. – b. Section of ascoma. – c. Peridium. Note the areolate patches. – d–f. Asci. – g–k. Ascospores. – Bars: a = 1 mm; b = 100  $\mu$ m; c = 10  $\mu$ m; d = 20  $\mu$ m; e–f = 10  $\mu$ m; g–k = 5  $\mu$ m.

ascospores described in Bose (1961) and Sivanesan (1972) should be actually the extension of mucilaginous sheath which is usually closely applied to the ascospores and liable to be overlooked. Neither mucous sheath nor appendages were described by Barr (1984, 1990) and Samuels & Müller (1978). Although not reported by previous researchers, the areolate patches and laminated cells in the peridium, absent in other Byssosphaeria species, are consistently present in all the material examined of this species. The specimen, IMI322683, reported as *B. jamaicana* (Sivanesan & Hsieh, 1989) from Taiwan, is actually an immature stage of *B. schiedermayeriana*. Although the ascospores are immature, the characteristic peridium with areolate patches and laminated cells is indicative of this species. This species was reported as *H. schiedermayeriana* from Taiwan (Hsieh & al., 2000), while it is treated herein in *Byssosphaeria* according to its turbinate ascomata, long-stalked asci and brown ascospores.

### Herpotrichia fusispora C. Y. Chen & W. H. Hsieh sp. nov. – Fig. 5.

Ascomata solitaria vel laxe aggregata, compresse globosa, 650–850 µm lata, 500–700 µm alta, superficialia supra subiculum, hyphis brevibus, crassitunicatis, brunneis sparsim obtecta, apice rotundo, non papillato. Paries ascomatis 50–90 µm crassus, e cellulis 6–12 stratis cellularum, inconspicue scleroplectenchymaticarum, angulatarum vel compressarum compositus, interne cellulis pallide brunneis vel flavido-brunneis, extreme cellulis atrobrunneis. Asci ellipsoidei,  $120-160 \times 13-15$  µm, 8-spori, sessiles vel breviter pedicellati. Ascosporae anguste fusiformes,  $36-48 \times 5-8$  µm, plerumque 4–8-guttulatae, uniseptatae, imbricate biseriatae in asco, typice in muco extremis protractis inclusae.

Holotypus: Taiwan, Taichung Hsien, Wuling, in ligno, 16 Oct 2002, C. Y. Chen, TNM(F) 15218.

As com at a scattered or loosely gregarious, superficial on subiculum, compressed globose, 650–850  $\mu$ m wide, 500–700  $\mu$ m high, sparsely clothed with short, brown, thick-walled, unbranched hyphae, apex rounded, non-papillate, periphyses not seen. Peridium 50–90  $\mu$ m thick, composed of 6–12 layers of inconspicuously scleroplectenchymatous, angular to strongly elongated cells, outer cell layers dark brown, becoming pale to yellowish brown towards inner layers, dark brown cell layers increasing and pale to yellowish brown cell layers decreasing towards the ostiolar region where merely dark brown cells are present. – Asciellipsoid, 120–160×13– 15  $\mu$ m, 8-spored, sessile or short-stalked. – Pseudoparaphyses 1.5–2.5  $\mu$ m wide. – Ascospores narrowly fusiform, 36–48×5–8  $\mu$ m, smooth, hyaline, usually 4–8-guttulate, overlapping, biseriate, with a median, constricted septum, mucilaginous sheath, if present, projecting at ends as appendages.

The asci and ascospores in H. fusispora are strongly similar to those of H. macrotricha. However this species is distinct in having significantly larger ascomata with a rounded and non-discolored apex. The ostiolar cavity in this species is common canal-like (Fig. 5b), instead of conical (Fig. 6b-c) in H. macrotricha.



Fig. 5. Herpotrichia fusispora (from holotype) – a. Ascomata on host surface. – b. Section of ascoma. Note the short ostiolar cavity without periphyses. – c–e. Asci. – f–j. Ascospores. – Bars: a = 1 mm; b = 100  $\mu$ m; c = 20  $\mu$ m; d–e = 10  $\mu$ m; f–j = 10  $\mu$ m.

Herpotrichia macrotricha (Berk. & Broome) Sacc., Syll. Fung. 2: 213. 1883. – Fig. 6.

For synonyms and other descriptions, see Barr (1984), Bose (1961), Hsieh & al. (2000) and Sivanesan (1972).



Fig. 6. Herpotrichia macrotricha (from NCHUPP 2364). – a. Ascomata on host surface. – b. Section of ascoma. Note the conical ostiolar cavity. – c. Hypha-like periphyses in ostiolar cavity. – d–f. Asci. – g–k. Ascospores. – Bars: a = 500  $\mu$ m; b = 50  $\mu$ m; c–d = 20  $\mu$ m; e–f = 10  $\mu$ m; g–k = 5  $\mu$ m.

Ascomata loosely gregarious, superficial on subiculum, subglobose, 320–400  $\mu m$  wide, 360–420  $\mu m$  high, hyphal appendages arising from lower sides, apex compressed, non-papillate, occasionally pallid around the ostiole, with a characteristic conical ostiolar cavity (Fig. 6b) filled with modified, hypha-like periphyses (Fig. 6c). Peridium laterally 25–35  $\mu m$  thick. – Asci ellipsoid to clavate, 110– 140  $\times$  14–16  $\mu m,$  8-spored, sessile or short-stalked. – Pseudoparaphyses 1.5–2.5  $\mu m$  wide. – Ascospores fusiform, 42–48  $\times$  4–6  $\mu m,$  smooth, hyaline, sometimes guttulate, with up to 7 guttules, 1-septate, slightly constricted at the septum, rarely becoming 3–5-septate, surrounded by a mucilaginous sheath which is drawn out to form a short appendage at each end of ascospores.

Specimen examined. - TAIWAN: Ilan Hsien, Tuling, on dead stem, 29 Apr 1994, C. Y. Chen (NCHUPP 2364); Ilan Hsien, on dead herbaceous stem of *Eupatorium formosanum* Hayata, 21 Apr 2003, C. Y. Chen (NCHUPP c1017).

The ascospores in *H. macrotricha* are reported to be hyaline and then brown and up to 5-septate with age. By examining the material in IMI, the brown or 5-septate ascospores only occur in overmature ascomata and can hardly been seen. The Taiwan materials have the persistently hyaline ascospores. This species is unique in having conical ostiolar cavity.

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