An approach towards a new phylogenetic classification in *Diatrypaceae*

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The ascal morphology and its taxonomic significance were explored in almost all species of octosporous *Diatrypaceae*: *Cryptosphaeria*, *Diatrype*, *Echinomyces*, *Eutypa* and *Eutypella*. The resulting character information along with other morphological characters were used to infer phylogenetic relationships among species and genera in the family. *Peroneutypa*, is resurrected to accommodate seven species and the following new combinations are proposed: *Peroneutypa alsophila*, *Peroneutypa arecae*, *Peroneutypa comosa, Peroneutypa curvispora*, *Peroneutypa gliricidiae*, *Peroneutypa kochiana*, *Peroneutypa scoparia* and *Peroneutypa obesa*. A key to species of *Peroneutypa* is provided. These new taxonomic arrangements, based on phylogenetic evidence, are proposed here for the first time.

Key words: asci, *Diatrypaceae*, *Peroneutypa*, phylogeny.

Introduction

The generic concept in *Diatrypaceae* (Tiffany and Gilman, 1965; Glawe and Rogers, 1984; Rappaz, 1987, Vasilyeva and Stephenson, 2004) is principally based on stromatic characters, such as: degree of stromatal development, configuration of perithecial necks and type of host tissue in which stromata occur. The number of ascospores per ascus is the only aspect of the ascus which has often been used as diagnostic characters. These features have been widely used although the derived taxonomy at the generic level is artificial and, in several cases, difficult to apply. Vasilyeva and Stephenson (2004, 2005, 2006) gave a clear description of the problematic situation about the limits of the genus *Cryptovalsa*, *Diatrype* and *Diatrypella*. They adopted a restricted concept for *Diatrype*, based, again, on the morphology of the stroma.

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Vasilyeva (1986) pointed out a significant parallelism within *Diatrypaceae*. This point of view had been supported by some previous studies (Wehmeyer, 1926; Croxall, 1950) suggesting the development of stromatic characters is influenced by some factors for example: host species and environmental humidity. Stromata however, vary within the same species, according to the tissue they occupy (Rappaz, 1987).

Using fluorescence microscopy, Romero and Minter (1988) observed the asci of some species of ascomycetes. They described the ascus of *Diatrype disciformis* (Hoffm.) Fr. in detail and showed the utility of this technique for interpreting problematic microscopic features.

More recently, Carmarán and Romero (1992) and Romero and Carmarán (2003) have studied the Argentinean collections of the family *Diatrypaceae* using fluorescence microscopy, and observed four types of asci. They proposed this differential morphology of the ascus may have an important role in the taxonomy of this group of fungi.

Considering the importance of microscopic characters of the asci, the ascal morphology and its taxonomic significance were explored in almost all the species of the octosporous genera of *Diatrypaceae*: i.e. *Cryptosphaeria* Ces. de Not., *Diatrype* Fr., *Echinomyces* Rappaz, *Eutypa* Tul. & C. Tul. and *Eutypella* (Nitschke) Sacc. The resulting character information was used to infer phylogenetic relationships among species and genera of the family.

Materials and methods

Morphological analysis

Sampling and collecting methodologies were explained in a previous paper by Carmarán (2002). Specimens are preserved in BAFC. Holotypes from the following herbaria, BR, CUP, DAOM, IMI, LIL, LPS, NY, PC, PH, PRE, S, W and WSP, were also studied (herbarium abbreviations follow Holmgren *et al*., 1990). Morphological characters were investigated in 118 specimens of 68 species (Table 1). Twenty-four taxa were selected to represent the diversity of the family when inferring phylogenetic relationships, among these, the morphological characters of *Eutypella arecae* (Syd. & P. Syd.) Rappaz, *Eutypa lata* (Pers.: Fr.) Tul. & C. Tul., *Eutypa leptoplaca* (Mont.) Rappaz, *Diatrype flavovirens* (Pers.: Fr.) Fr., *Eutypella aulacostroma* (Kunze: Fr.) Berl. and *Eutypella canodisca* (Ellis & Holway) Sacc. were extracted from descriptions by other authors.

Samples for conventional light microscopy were prepared from moistened specimens mounted in common mycological media: distilled water,

Table 1. Specimens examined: organized by type of asci, names of the species according to Rappaz, 1987 ([1]N. spms. = number of studied specimens; * selected species for phylogenetic analysis).

Species	[1]N. spms.	Material
SPECIMENS WITH TYPE 1 ASCI		
Cryptosphaeria eunomia (Fr.: Fr.) Fuckel	2	Switzerland. BR Rabenh.; Check Republic. BR Petrak as *Cryptosphaeria millepunctata* Grev.
**Cryptosphaeria lignyota* (Fr.: Fr.) Auersw.	4	Argentina. BAFC 50924; Germany, BR-Syd., as *C. populina*; BR-Rehm; as *C. lignyota* BR 1269 Neotype
Cryptosphaeria philippinensis Rehm	1	Philippines. Holotype S-Syd.
Cryptosphaeria pullmanensis Glawe	1	USA. Holotype, WSP 67333
**Cryptosphaeria sulcata* A.I. Romero & Carmarán	1	Argentina. Holotype BAFC 50923
**Diatrype albopruinosa* (Schwein.) Cooke	2	?, Neotype of *Sphaeria albopruinosa,* PH-Schwein; USA. LIL
Diatrype bullata (Hoff.: Fr.) Fr	2	Germany. LIL, No data LIL
**Diatrype chlorosarcha* Berk. & Broome	2	Venezuela. CUP 1060 CUP 1063
Diatrype costesi (Speg.) Petr. & Syd.	2	Chile. Holotype of *Ectosphaeria costesi* Speg LPS 425*;* Argentina. BAFC 51111.
**Diatrype disciformis* (Hoff.: Fr.) Fr.	4	England. IMI 317165 & LIL; Check Republic. LIL, Austria LIL
Diatrype doryalidis (Doidge) Rappaz	1	South Africa. PRE 31060
Diatrype enteroxantha (Berk.) Berl.	5	Venezuela. CUP 746; Argentina. BAFC 34555, BAFC 34733; South Africa. PRE 31071, 31073 as *Diatrype auristroma* Doidge
Diatrype falcata (Syd & P. Syd.) Rappaz	1	Japan. Holotype S-Syd. of *Eutypa falcata* Syd. & P. Syd.
Diatrype leonotidis Doidge	1	South Africa. Holotype PRE
Diatrype macowaniana Thum.	2	South Africa. Holotype PRE 1264; Argentina. Holotype of *Eutypa andicola* Speg LPS 2070.
Diatrype microstoma Syd. & P. Syd & Hara	2	Japan. Holotype S-Syd.; Argentina. BAFC 34734
Diatrype oregoniensis (Wehmeyer) Rappaz	1	USA. Holotype of *Eutypella oregoniensis* Whemeyer, DAOM-Wehmeyer 121086.
**Diatrype patagonica* (Speg.) Rappaz	3	Chile. Holotype LPS 2060; Argentina. Holotype of *Eutypa peraffinis* Speg. LPS 2068; BAFC 51152
Diatrype petrakii F. Rappaz	1	Spain, Holotype of *Eutypa canariensis* Petr., W 16808

Table 1 continued. Specimens examined: organized by type of asci, names of the species according to Rappaz, 1987 ([1]N. spms. = number of studied specimens; * selected species for phylogenetic analysis).

Species	[1]N. spms.	Material
SPECIMENS WITH TYPE 1 ASCI		
Diatrype praeandina (Speg.) Rappaz	1	Argentina. Holotype of *Eutypa praeandina* Speg., LPS 2061
Diatrype puiggarii Speg.	1	Brasil. Holotype LPS 2141
**Diatrype spilomea* Syd.	1	Russia. S-Syd. Isotype Exs. 424
Diatrype standleyi Fairm	1	USA. Lectotype CUP
**Diatrype stigmaoides* Kauffman	1	USA. Holotype MICH-Kauff
Diatrype subaffixa (Schwein.) Cooke	1	USA. Neotype PH-Schwein.
Diatrype tremellophora Ellis ex Ellis & Everh.	2	USA. LIL; USA Lectotype NY-Ellis as *D. disciformis var. magnoliae* Thum.
Diatrype valdiviensis Speg.	2	Chile. Holotype LPS 2074; Argentina. BAFC 51123
Diatrype virescens (Schwein.) M.A. Curtis	1	Argentina. BAFC 51124
Diatrype weinmaniae Rehm	1	Brazil. Holotype S-Rehm
Diatrype whitmanensis J.D. Rogers & A.D. Glawe	1	USA. Holotype WSP 67330
**Diatrype sp.*	1	Argentina. BAFC 34610
Eutypa consobrina (Mont.) Rappaz	1	Argentina. BAFC 34606
**Eutypa lejoplaca* (Fr.: Fr.) Fr.	2	Argentina. BAFC 34634; USA LIL as *D. stigma* (Hoff: Fr.) Fr.
Eutypa mela (Schwein.) Cooke	1	USA. Neotype of *S. mela* Schwein.
Eutypa sparsa Romell	1	USA. LIL as *Eutypa acharii* Tul.
**Eutypa* spinosa (Pers.:Fr.) Tul. & C. Tul.	2	USA. LIL, ? LIL
Eutypella acaciae Doidge	1	South Africa. Holotype PRE 30476
Eutypella andicola Speg.	2	Argentina. Holotype LPS 2124; Holotype of *Eutypella praeandina* Speg, LPS 2127
Eutypella androssowii Rehm	1	Turkestan. Holotype S-Rehm,
Eutypella anthracina Speg.	1	Argentina. Holotype LPS 2123
**Eutypella atropae* (Mont.) Sacc.	1	Algeria. Holotype PC-Mont.
**Eutypella cerviculata* (Fr.: Fr.) Sacc.	2	?, LIL; USA LIL
Eutypella chilensis Speg.	1	Chile. Holotype LPS 2125
Eutypella citricola Speg.	1	Argentina. Holotype LPS 2120
Eutypella corynostomoides (Rehm) Rappaz	1	Brazil. Holotype of *Peroneutypella corynostomoides* Rehm, S-Rehm
Eutypella doidgeae Syd.	2	South Africa. Isotype PRE 30378; PRE 33966
Eutypella erythrinicola Rappaz	1	Argentina. Holotype of *Eutypa erythrinae* Speg., LPS 2079

Table 1 continued. Specimens examined: organized by type of asci, names of the species according to Rappaz, 1987 ([1]N. spms. = number of studied specimens; * selected species for phylogenetic analysis).

Species	[1]N. spms.	Material
SPECIMENS WITH TYPE1 ASCI		
Eutypella juglandicola (Schwein.: Fr.) Ellis & Everh.	1	USA. LIL as *Eutypella fraxinicola* (Cooke & Peck) Sacc.
**Eutypella leprosa* (Pers. ex Fr.) Berl.	6	Argentina. BAFC 34626, 34627, 34628, 34630, 34631, 34633,
Eutypella leucaneae Rehm	1	?, Holotype, S-Rehm
Eutypella lophiostomoides (Speg.) Berl.	1	Brazil. Holotype of *Eutypa lophiostomoides* Speg., LPS 1937.
Eutypella ludens (Speg.) Rappaz	1	Paraguay. Holotype LPS 1935
Eutypella murrayae Syd. & P. Syd.	1	Brazil. Holotype S-Syd.
Eutypella padina (Nitschke) Nannfeldt	1	Austria. LIL
Eutypella paraphysata (Speg.) Rappaz	1	Argentina. Holotype of *Eutypa paraphysata* Speg., LPS 2062
Eutypella riograndensis (Rehm) Rappaz	1	Brasil. Holotype of *Diatrype riograndensis* Rehm, S-Rehm
Eutypella ruficarnis (Berk. & M.A. Curtis) Rappaz	1	Argentina. BAFC 34 625
Eutypella staphyleae Dearn & House	1	USA. Holotype DAOM 3965
Eutypella stenocalicys Syd. &. P. Syd.	1	Brazil. Holotype S-Syd., Baker 374
Eutypella wisteriae Syd. & P. Syd.	1	Japan. Holotype S-Syd.
TOTAL OF SPECIMENS	**91**	
SPECIMENS WITH TYPE 2 ASCI		
**Echinomyces obesa* (Syd.) Rappaz	2	Zaire. Holotype of *Peroneutypella obesa* Syd., S-Syd.; Argentina BAFC 51.139.
Eutypella alsophila (Mont.) Berl.	1	Argelia. Lectotype of *Sphaeria alsophila* PC-Mont 73
**Eutypella comosa* (Speg.) Rappaz	5	Argentina. Lectotype LPS 2080; Argentina, BAFC 34605, 34603, 34609, S/N
Eutypella curvispora (Starb.) Rappaz	1	?, Neotype. S-Starb sub. *Cryptosphaeria curvispora* "M.s. 58"
**Eutypella gliricidiae* Rehm	1	Philippines. Holotype, S-Rehm
Eutypella kochiana Rehm	1	Russia. Holotype, S-Rehm

Table 1 continued. Specimens examined: organized by type of asci, names of the species according to Rappaz, 1987 ([1]N. spms. = number of studied specimens; * selected species for phylogenetic analysis).

Species	[1]N. spms.	Material
SPECIMENS WITH TYPE 2 ASCI		
**Eutypella scoparia* (Schwein.: Fr.) Ellis & Everh.	14	Argentina. Holotype of *Eutypa tuyutensis* Speg., LPS 2063; Holotype of *Eutypa auranticola* Speg., LPS 2082; Paraguay. holotype of *Eutypella pusilla* Speg., LPS 2118; Chile. Holotype of *Peroneutypa valdiviana* Speg., LPS 2069; Argentina. BAFC 34604, 34602, 32454; 32455; 34629; 34611, 34557, 34554, 34655, 34657.
TOTAL OF SPECIMENS	**25**	
SPECIMENS WITH TYPE 3 ASCI		
**Diatrype phaselina* (Mont.) F. Rappaz	**2**	Argentina. BAFC 32456; Philippines. sub *Eutypa bambusina*, LIL

5% KOH, phloxine and Melzer 's reagent. Samples for epifluorescence light microscopy (EFM) were made in 0.05 % calcofluor (Romero and Minter, 1988). Drawings were performed using a camera lucida and photographs were taken with an Olympus c-5060 wide zoom Digital camera.

Phylogenetic analysis

Based on 24 species (Table 1, species marked with *) and 36 morphological characters (Table 2; TreeBase), a maximum parsimony analysis was performed using NONA version 2.0 (Goloboff, 1993a) with all characters equally weighted and informative characters considered unordered. The order of taxa was randomised, creating a Wagner tree and submitting it to branch-swapping by means of tree-bisection reconnection (TBR); this process was performed ten thousand times, retaining two thousand trees per replica. All retained trees were swapped until complexion using command max*.

We ran another analysis using a concavity function of the homoplasy to apply weight to all characters, a method for estimating implied weight (Goloboff, 1993b). This analysis was carried out with Pee-Wee ver. 3.0 (Goloboff, 1993b) using the same strategies as in NONA. For implied weight analyses we used concavities 1 to 6.

The consensus tree of the most parsimonious trees was calculated for each analysis. To evaluate the relative support of clades, branch support (Bremer, 1988, 1994) and bootstrap analysis were calculated. For the bootstrap

Table 2. List of morphological characters and their states and codification (* ascospores volume was calculated vol = $0.5236.d^3 + 0.7854.d^2.(D - d)$ (D = spore length; d = spore width, Corner, 1948).

Morphological characters	states	COD.
C0- Stromata effuse, blackened surface,	absent	0
interior of stromata not developed	present	1
C1- Stromata effuse, not blackened surface,	absent	0
interior of stromata not developed	present	1
C2- Stromata discrete, interior variable	absent	0
or slightly developed	present	1
C3- Stromata effuse, interior of	absent	0
stromata strongly developed	present	1
C4- Stromata discrete, interior of	absent	0
stromata strongly developed	present	1
C5- Interior of stromata white	absent	0
	present	1
C6- Interior of stromata pigmented	absent	0
	present	1
C7- Interior of stromata two colours	absent	0
	present	1
C8- Black line or dark marginal zone in the substrate	absent	0
	present	1
C9- Stromatic surface with transverse fissures	absent	0
	present	1
C10- Configuration of perithecial necks	emerging separately	0
	emerging in groups	2
C11- Length of perithecial necks	short	0
	long	1
C12- Prominence of necks	not prominent	0
	prominent	1
	very prominent	2
C13- Distance among perithecia in the stroma	scattered	0
	close, seldom in touch	1
	in touch or compressed	2
C14- Ostioles sulcate	absent	0
	present	1
C15- Asci fusiform	absent	0
	present	1

Table 2 continued. List of morphological characters and their states and codification (* ascospores volume was calculated vol = $0.5236.d^3$ + $0.7854.d^2.(D - d)$ (D = spore length; d = spore width, Corner, 1948).

Morphological characters	states	COD.
C16- Asci claviform	absent	0
	present	1
C17- Asci urniform	absent	0
	present	1
C18- Asci cylindric	absent	0
	present	1
C19- Apical citoplasmatic channel	absent	0
	present	1
C20- Apical wall of asci short	absent	0
	present	1
C21- Apex dome-like	absent	0
	present	1
C22- Apical wall of asci thickened	absent	0
	present	1
C23- Apical aparattus	slightly developed	0
	strongly developed	1
C24- Apical ring amyloid	absent	0
	present	1
C25- Asci pedicel length	absent	0
	present	1
C26- Ascospore length	0-6 µm	0
	6.1-12 µm	1
	12.1-20 µm	2
	from 20.1 µm	3
C27- Ascospore volume *	0-20.9 μm^3	0
	21 - 50.9 μm^3	1
	51 - 120 μm^3	2
	from 120 μm^3	3
C28- Ascospores colour	slightly pigmented	1
	pigmented	2
C29-Allantoid ascospores	absent	0
	present	1
C30- Fusiform ascospores	absent	0
	present	1

Table 2. List of morphological characters and their states and codification (* ascospores volume was calculated vol = $0.5236.d^3 + 0.7854.d^2.(D - d)$ (D = spore length; d = spore width, Corner, 1948).

Morphological characters	states	COD.
C31- Curvature of ascospores	cylindrical to slightly curved	0
	curved	1
	strongly curved	2
C32- Germ slit	absent	0
	present	1
C33- Substrate	bark	0
	wood	1
C34- Uniseriate ascospores	absent	0
	present	1
C35- Massive stromata	absent	0
	present	1

analysis a total of 3,000 replicates of heuristic searches were carried out using random taxon entry followed by tree bisection-reconnection (TBR). Winclada version 1.00.08 (Nixon, 2002) was used as interface to construct trees.

The proposed combinations in this paper are ascribed to Carmarán and A.I. Romero. Mycobank numbers (MB) are given in brackets after the name of new combinations.

Results

Morphological analysis

The asci of *Diatrypaceae,* generally, are described as clavate, spindle-shaped, with a truncate or blunt apex, with or without citoplasmatic strands in the apex, generally with a thicker-walled region above the ascospores. A minute ring-like structure is present and is frequently, but not always, iodine-positive using Melzer ´s reagent. Asci have typically very long stalks and ascogenous hyphae with many ascus stalks still attached are commonly observed.

Nevertheless, Carmarán and Romero (1992) and Carmarán (2002) recognized more than one morphological type of ascus. In this paper these morphologies are described:

Type 1 (Figs 1-11)

These asci are spindle-shaped with eight ascospores arranged in a cluster in the swollen upper portion. All of these asci have a thick-walled apical region, penetrated by a narrow channel with cytoplasmic strands connecting the apex with ascus cytoplasm. It is possible to see opened asci (empty asci) in several collections of different species.

Using the reflected light fluorescence attachment on calcofluor preparations, unopened asci frequently show a strong fluorescence at the thickened apical wall, a central channel can be observed in the middle of this strongly fluorescing wall. In some asci, this channel is slightly constricted at the upper third of the apical portion. The ring-like apical structure fluoresces more strongly than the surrounding wall material.

This type of ascus is the most common in the family.

Observations: Our observations are according to the descriptions by Glawe and Rogers, 1984, Rappaz, 1987 and Romero and Minter 1988. We propose to name this ascus morphology as **Type 1**.

Specimens examined: see Table 1.

Type 2 (Figs 12-18)

These asci are urn-shaped, but with truncate apex and are wider in the middle where ascospores tend to be clustered. The apical portion has a thick wall, with a very small apical ring, without any channel. This type of ascus is quite small (10-30 μm).

Using the reflected light fluorescence attachment for calcofluor preparations, the thickened apical wall fluoresced strongly. A ring-like apical structure fluoresced more strongly than the surrounding wall material.

It is restricted to a few species in the family.

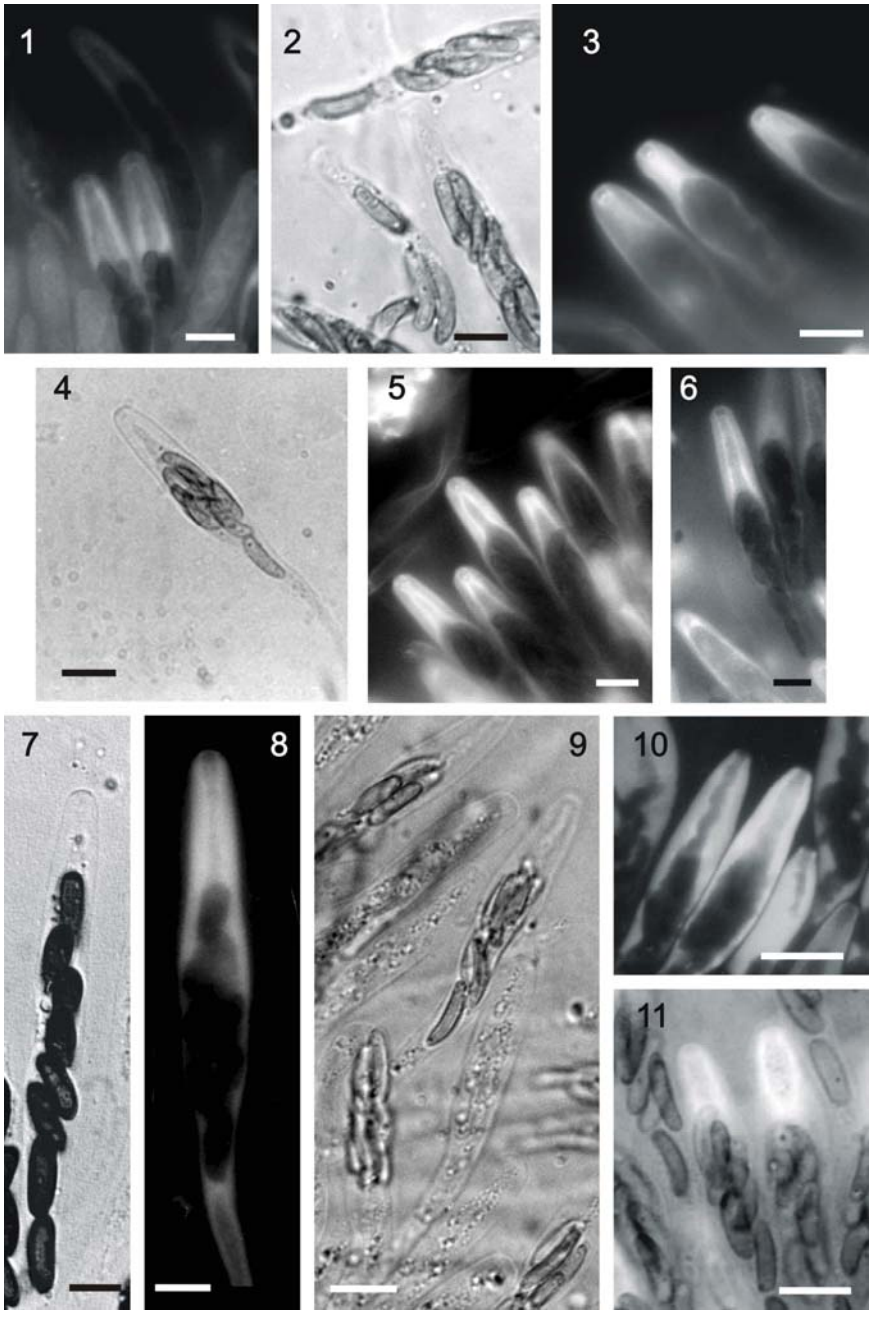
Observations: Eight species of *Diatrypaceae* have this type of ascus, *Eutypella alsophila* (Mont.) Berl., *Eutypella arecae* (Syd. & P. Syd.) Rappaz, *Eutypella comosa* (Speg.) Rappaz, *Eutypella curvispora* (Starb.) Rappaz, *Eutypella gliricidiae* Rehm, *Eutypella kochiana* Rehm, *Eutypella scoparia* (Schwein: Fr.) Ellis & Everh. and *Echinomyces obesa* (Syd.) Rappaz.

Eutypella scoparia has frequently been found in Argentina and other countries, while other species as *E. comosa* has been only found in Argentina.

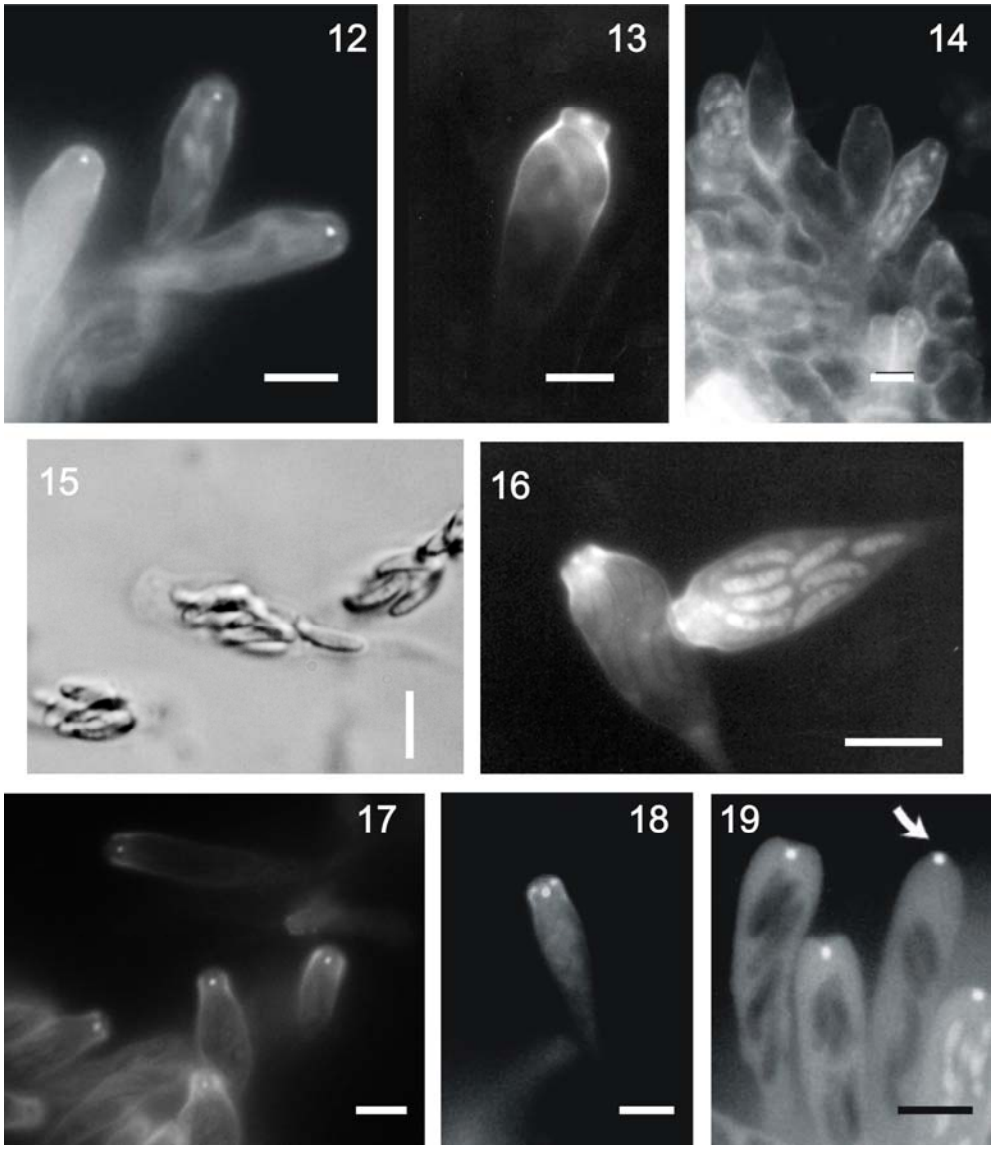
Specimens examined: see Table 1.

Type 3 (Fig. 19)

These asci are clavate, with a thick-walled apical region, without a channel, and with a very small apical ring.



Figs 1-11. Examples of **Type 1** asci: **1-2**. *Eutypella andicola* (Holotype LPS 2124). **3**. *Diatrype enteroxantha* (BAFC 34555). **4-6**. *Eutypella leprosa* (BAFC 34626). **7-8**. *Cryptosphaeria sulcata* (Holotype BAFC 50923). **9-10**. *Eutypella ruficarnis* (BAFC 342625). **11**. *Diatrype chlorosarcha* (CUP 1060).



Figs 12-19. Examples of **Type 2** and **Type 3** asci: **Type 2**: **12**. *Eutypella alsophila* (Lectotype PC-Mont.73). **13**. *Eutypella comosa* (BAFC 34603). **14**. *Eutypella gliricidiae* (Holotype, S-Rehm) **15-16**. *Eutypella scoparia* (BAFC 34604). **17-18**. *Eutypella kochiana* (Holotype S-Rehm). **Type 3**: **19**. *Diatrype phaselina* (BAFC 32456). Bars: 3-12, 19 = 10 μm; 1-2, 13-18 = 5 μm.

Using reflected light fluorescence attachment for calcofluor preparations, the thickened apical wall of unopened asci fluoresced strongly. A ring-like

apical structure fluoresced more strongly than the surrounding wall material. Eventually, the ascal apex collapses resembling a truncate apex.

Observations: Rappaz (1987) described *Diatrype phaselina* (Mont.) Rappaz. Among the materials studied by him there was a material identified as *Eutypa bambusina* Penz. & Sacc. (LIL). We have studied a duplicate of this material in the present work. So we have assumed that *D. phaselina* (Rappaz, *op. cit.*) has ascus morphology similar to type 3. An Argentine specimen has also been identified as *D. phaselina*.

Specimens examined: see Table 1.

Phylogenetic analysis

The phylogenetic analysis using equally weighted characters (NONA) yielded 361 parsimonious trees; all trees of length (L) = 85, consistency index (Ci) = 0.52 and retention index (Ri) = 0.68. Figure 20 (TreeBase) shows one of the most parsimonious trees with optimised characters on branches. A consensus tree of all shortest trees was also calculated.

When using different concavities with implied weight analyses (Pee Wee) we found similar results as in NONA, though K = 3 showed the most divergent result, and will be discussed herein. The K = 3 analysis found 21 equally parsimonious trees of length (L) = 95, consistency index (Ci) = 0.47, retention index (Ri) = 0.65 and fit = 262.3.

Figure 21 (TreeBase) shows a comparison between the consensus trees from the shortest trees found using unweighted and weighted analyses and bootstrap values are shown above the branches with Bremer support below.

The species of the *Diatrypaceae* included in the analysis, are strongly supported as monophyletic (100% bootstrap value and 12 informative synapomorphies) with *Kretzchmaria clavus* and *K. sigmoidea* as outgroup taxa. In the consensus tree of both analyses, only three clades are recognized. Clade 1: *Diatrype spilomea*, *Diatrype stigmoides* (C1); Clade 2: *Diatrype* sp., *Cryptosphaeria sulcata*, *Eutypella canodisca* (C2) and Clade 3: *Echinomyces obesa*, *Eutypella arecae*, *Eutypella comosa, Eutypella gliricidiae*, *Eutypella scoparia* (C3). Clade 1 is supported with 67% and Clade 2 with 62% bootstrap values only when using unweighted characters. Because of their low support in analyses using weighted and unweighted characters, we do not consider them to be phylogenetic informative, only one synapomorphy support the clades. Clade 3 includes 5 taxa with type 2 asci, and is strongly supported with 75% and 76% bootstrap values in the unweighted and weighted character analyses, respectively. Clade 1 is supported with 67% and Clade 2 with 62% bootstrap values only when using unweighted characters.

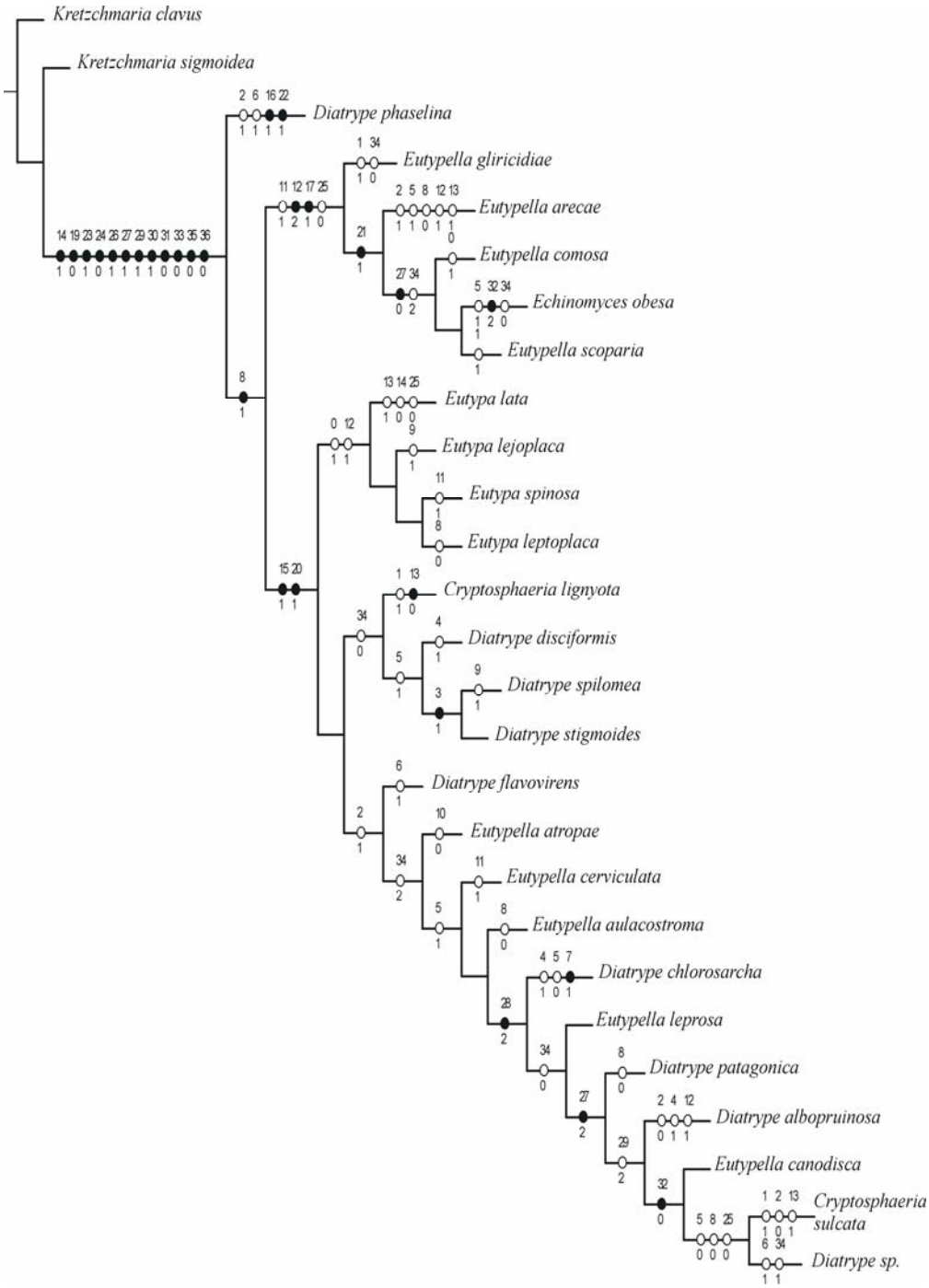


Fig. 20. One of the most parsimonious trees with optimised characters on branches.

Our results suggest only Clade 3 represents a monophyletic group characterized by two synapomorphies, one of them represents the Type 2 ascus. None of the clades represents the generic concept used in the family.

When constraining the trees to make two monophyletic groups representing species only with Type 1 and Type 2 ascus, because Type 3 is represented by *Diatrype phaselina* 313 trees of length L = 85 were found. If the traditional genera were constrained, 583 trees of length L = 100 were obtained, 15 steps longer than the unconstrained search.

Discussion

Few cladistic studies in fungi have been based on morphological characters (Schumacher, 1990; Reynolds, 1991; Pitt, 1995; Villegas *et al*., 1999), probably because of the difficulty of finding enough characters to be of use. In this work, we conducted a morphological analysis to clarify the phylogenetic relationships within the octosporous genera of *Diatrypaceae*.

The cladistic analyses using morphological characters supported the monophyly of the family *Diatrypaceae*. Our results are agree with Acero *et al*. (2004) used molecular data to elucidate that the limits of genera as traditionally placed in the *Diatrypaceae*, do not reflect the natural relationships. Our analyses suggest that the morphology of the ascus better explains the phylogenetic relationships among the species of *Diatrypaceae* than stromatal characters.

Clade C3 was a unique monophyletic, well supported group, and it includes species with Type 2 asci. We recognize the following species as belonging to this clade: *Eutypella alsophila*, *E. arecae*, *E. comosa, E. curvispora*, *E. gliricidiae*, *E. kochiana, E. scoparia* and *Echinomyces obesa*. Acero *et al*. (2004) described the Clade "9 Group" (not supported) containing most of the species from genus *Eutypella*; within this group, with three groups of species. One of them includes *Eutypella alsophila*, *Eutypella kochiana* and *Eutypella scoparia*, with high bootstrap values. Acero *et al*. (2004) result agrees with this arrangement of species based on the morphology of ascus, supporting the monophyly of Clade 3.

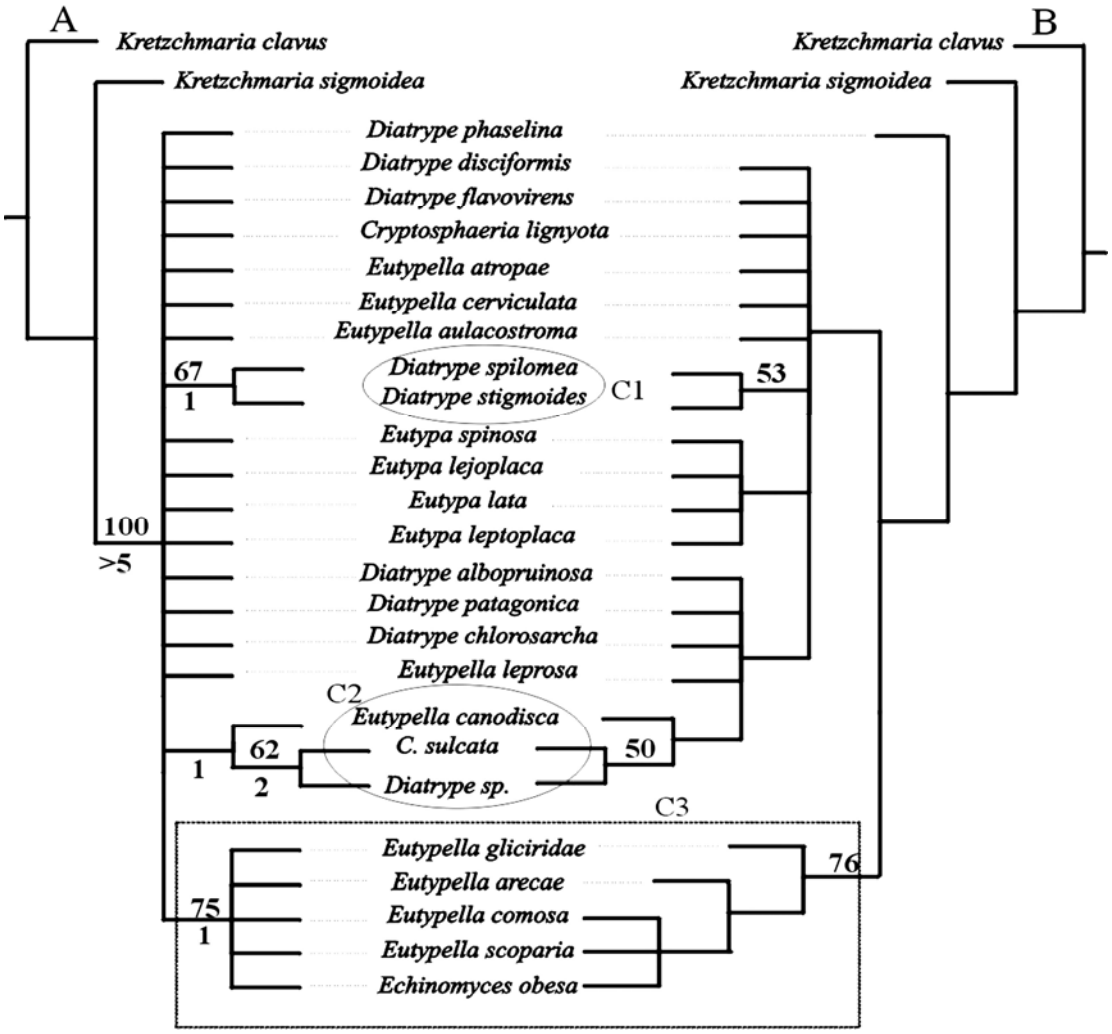


Fig. 21. Consensus Trees NONA (A) and PEEWEE (B) with bootstrap values above branches and Bremer support, below (C.= *Cryptosphaeria*; C1: clade 1; C2: clade 2; C3: clade 3).

Species with Type 2 asci were previously suggested intuitively as a "group" by other taxonomists. Berlese (1968) erected the genera *Peroneutypa* and *Peroneutypella*, to comprise species with small, clavate asci and perithecia with long necks. Using molecular data of Acero *et al.* 2004 and our

morphological evidences we conclude that species with Type 2 asci, should be placed in a monophyletic group and included in the same genus. The generic names *Eutypella, Eutypa, Diatrype* and *Cryptosphaeria* are not available because the type species of each genus has Type 1 asci. Rappaz (1987) proposed *Peroneutypa bellula* (Dezm.) Berl., as the type species of the genus *Peroneutypa* Berl., 1902 and it was listed as a synonym of *Eutypella scoparia*. Based on this consideration, *Peroneutypa* Berl., being the oldest available generic name, is resurrected to accommodate these seven species. The following new combinations are proposed:

Peroneutypa alsophila (Mont.) Carmarán & A.I. Romero, **comb. nov**.
Mycobank 500707

≡ *Spaheria alsophila* Mont. in Durieu, Expl. Sci. Algérie 1(12): 462. 1849
≡ *Eutypa alsophila* (Mont.) Sacc., Syll. Fung. 1:169.1882
≡ *Eutypella alsophila* (Mont.) Berl., Icon fung. 3:58.1902
Specimen examined: ARGELIA, "nº 73 (79?), in caul. *Salsolae oppositif*. Mostagamen",without other data. As *Sphaeria alsophila* (PC-Mont 73 **Lectotype**).

Peroneutypa arecae (H. & P. Syd.) Carmarán & A.I. Romero, **comb. nov.**
Mycobank 500708

≡ *Peroneutypella arecae* H. & P. Syd., Philip. J. Sci. 9: 163. 1914.
≡ *Eutypella arecae* (H. & P. Syd) F. Rappaz, Mycol. Helvetica 2 (3): 533. 1987
Specimen examined: see materials and methods.

Peroneutypa comosa (Speg.) Carmarán & A.I. Romero, **comb. nov.**
Mycobank 500709

≡ *Eutypa comosa* Speg., Anal. Soc. ci. Argent. 12: 104. 1881
≡ *Peroneutypella comosa* (Speg.) Berl., Icon. Fung. 3: 85. 1902
= *Peroneutypa portoriciensis* Petr., Ann. Mycol. 21: 306. 1923. *Syn. nov*.
= *Eutypella portoriciensis* (Petr.) Rappaz, Mycol. Helvetica 2 (3): 543. 1987.
Specimen examined: ARGENTINA, Buenos Aires, Flores, on Celtis tala, Aug-1880, C. Spegazzini (LPS 2080 **Lectotype**);Buenos Aires, Isla Martín García, on fallen branch, November 1992, C. C. Carmarán (BAFC 34605), Misiones, Parque Nacional Iguazú, Sendero Macuco, on fallen branch, April 1993, C. C. Carmarán (BAFC 34609), Entre Ríos, Concordia, on fallen branch, November 1992, C. C. Carmarán (BAFC S/N). Porto Rico, Acrood, old log, 17 December 1915, Fink (W 00268: Herb. Petr. 34313, Porto Ric. Fung. 930 **Isotype**).

Peroneutypa curvispora (Starb.) Carmarán & A.I. Romero, **comb. nov.**
Mycobank 500710

≡ *Cryptosphaeria curvispora* Starb., Arkiv Bot. 5: 28. 1905
≡ *Eutypella curvispora* (Starb.) Rappaz, Mycol. Helvetica 2 (3): 542. 1987
Specimen examined: ?, sub. *Cryptosphaeria curvispora* "M.s. 58" (S-Starb **Neotype).**

Peroneutypa gliricidiae (Rehm) Carmarán & A.I. Romero, **comb. nov.**
MB 500711

≡ *Eutypella gliricidiae* Rehm., Philip. J. Sci. 8:189.1913

= *Eutypella erythrinae* Kar & Maity, Trans Br. mycol. Soc. 55:1. 1970

Specimen examined: PHILIPPINES, Luzon, Los Banos, on Gliricidia maculatum, 27 September 1912, Baker 80 (Rehm **Lectotype**).

Peroneutypa kochiana (Rehm) Carmarán & A.I. Romero, **comb. nov.**
MB 500712

≡ *Eutypella kochiana* Rehm., Annals mycol. 11(5): 400. 1913

Specimen examined: RUSSIA, Tiflis, Géorgie, on Kochia sp., January 1913, Newodowski (S-Rehm ex Herb. Mycol. Hort. Bot. Tifilis, **holotype**).

Peroneutypa scoparia (Schwein: Fr.) Carmarán & A.I. Romero, **comb. nov.**
MB 500713

≡ *Sphaeria scoparia* Schwein: Fr., Syn. Fung. Carol. Sup.: 37. 1882, Sys. Mycol. 2: 379. 1823

Complete list of synonyms Rappaz, 1987

Specimen examined: ARGENTINA, Buenos Aires, Montes Largos, on fallen branches of *Sambucus australis* Cham. & Schlecht., 1881, C. Spegazzini, *Eutypa tuyutensis* Speg. (LPS 2063 **holotype);** Buenos Aires, Isla Martín García, on fallen branch, September-1992, C.C.Carmarán (BAFC 34 604); Buenos Aires, Lomas de Zamora, Santa Catalina, April-1990, C.C.Carmarán, (BAFC 32.454); Buenos Aires, Ensenada, Punta Lara, on fallen branch, February-1992, C. C. Carmarán (BAFC 34 655). Entre Ríos, Concordia, Arroyo Ayuí, on fallen branch, October 1992, C.C.Carmarán (BAFC 34629); Misiones, Cainguas, Parque Provincial Salto Encantado, on fallen branch, April 1993, C.C.Carmarán (BAFC 34 611). Misiones, Parque Nacional Iguazú, on fallen branch, January 1995, C.C.Carmarán, (BAFC 34557); Misiones, del Dorado, on fallen branch, Febraury 1992, C. C. Carmarán, (BAFC 34 657). Jujuy, route Nac. 9, at 47 km from Salta, city, on fallen branch, July 1994, C.C.Carmarán, (BAFC 34 554). CHILE, on dead branches of *Aristotelia maequi* L'Hérit, 1910, *Peroneutypa valdiviana* Speg. (LPS 2069 **holotype).** PARAGUAY, Posta Cué, on dead roots of *Citrus aurantium* L., September-1883, *Eutypa auranticola* Speg. (LPS 2082 **holotype**); on *Citrus aurantium* L., 1921, *Eutypella pusilla* Speg, (LPS 2118 **holotype**).

Peroneutypa obesa (Syd.) Carmarán & A.I. Romero, **comb. nov.**
MB 500714

≡ *Peroneutypella obesa* Syd., in de Wildeman, Annal. Mus. Congo Belge, Bot. Ser. 5, 3(1): 16. 1909.

≡ *Echinomyces obesa* (Syd.) Rappaz, Mycol. Helvetica 2 (3): 548. 1987

Specimen examined: ARGENTINA, Buenos Aires, Lomas de Zamora, Santa Catalina, on fallen branch, November 1992, C.C. Carmarán (BAFC 51139). ZAIRE, Kisantu, "ad corticen arboris", 1906, Vanderryst *Peroneutypella obesa* (S-Syd, **holotype**).

In the same sense, the genus *Echinomyces* Rappaz is considered synonym of *Peroneutypa* Berl.

Species with Type 1 asci (Figs 1-11) in most of the trees (Fig. 20) appear as a single clade, but this grouping does not have the support in any of the phylogenetic analyses. Nevertheless, our results suggest the species with Type 1 asci are related and belong to a unique clade, so these species could conform only one genus.

These new taxonomic arrangements, based on phylogenetic evidence, are proposed here.

Key to species of *Peroneutypa* Berl.

1. Interior of the stroma slightly or well-developed 2
1. Interior of the stroma not developed 5

2. Interior of the stroma well-developed, white to light brown. Necks prominent, spiny or bristly appearance to the stromatic surface. Black line present. Spores 3-7 µm long, strongly curved..... ***P. obesa***
2. Interior of the stroma slightly developed; if it is well developed then the colour is dark brown to almost black 3

3. Necks very long, prominent, over 1 mm long. Stroma effuse extended plates or discrete, with many perithecia. Apical apparatus J^+ o J^- ..... ***P. comosa***
3. Necks not prominent; never as long as 1 mm long. Stroma with few perithecial 4

4. Apical apparatus J^+ ..... ***P. alsophila***
4. Apical apparatus J^- ..... ***P. arecae***

5. Ascospores strongly curved ..... ***P. curvispora***
5. Ascospores allantoid 6
6. Stroma extended, forming an stromatic layer within the substratum. Necks long, slightly prominent, emerging in clusters, frequently through a superficial stromatic disc. Spore-bearing part of the ascus not over 18 µm long, apical wall above the ascospores easily visible, straight (truncate) apex ..... ***P. scoparia***
6. Combination of stromatic features differing from above. Spore-bearing part of the ascus over 18 µm long; if it is shorter then the apical wall above the ascospores difficult to observe and concave 7

7. Apical apparatus very visible o conspicuous, J+. Asci 18-28 µm long..... ***P. kochiana***
7. Apical apparatus inconspicuous, hard to observe, J-. Asci 10-16 µm long..... ***P. gliricidiae***

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