

CONTRIBUTION TO THE STUDY OF ALKALOPHILIC AND ALKALI-TOLERANT ASCOMYCOTA FROM ARGENTINA

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Abstract. Eliades, L. A., M. N. Cabello & C. E. Voget. 2006. Contribution to the study of alkalophilic and alkali-tolerant Ascomycota from Argentina. *Darwiniana* 44(1): 64-73.

Several species of alkalophilic and alkali-tolerant soil Ascomycota were isolated and reported here, namely: *Emericellopsis minima*; *Melanospora zamiae*; *Neosartorya stramenia*; *Neurospora tetrasperma*; *Talaromyces flavus* var. *flavus*; *Talaromyces trachyspermus*; *Talaromyces stipitatus* and *Westerdykella dispersa*. *Neosartorya stramenia*, *Neurospora tetrasperma* and *Westerdykella dispersa* were recorded for the first time for Argentina.

Keywords. Soil fungi, Ascomycota, alkalophilic, alkali-tolerant, Argentina.

Resumen. Eliades, L. A., M. N. Cabello & C. E. Voget. 2006. Contribución al estudio de Ascomycota alcalofílicos y alcalino-tolerantes de Argentina. *Darwiniana* 44(1): 64-73.

Se informa sobre 8 especies de Ascomycota alcalofílicos y alcalino-tolerantes de suelo: *Emericellopsis minima*; *Melanospora zamiae*; *Neosartorya stramenia*; *Neurospora tetrasperma*; *Talaromyces flavus* var. *flavus*; *Talaromyces trachyspermus*; *Talaromyces stipitatus* y *Westerdykella dispersa*. *Neosartorya stramenia*, *Neurospora tetrasperma* y *Westerdykella dispersa* son nuevos registros para Argentina.

Palabras clave. Hongos del suelo, Ascomycota, alcalofílico, alcalino-tolerante, Argentina.

INTRODUCTION

Fungi are often dominant members of soil microbiota, especially in acidic environments, and may operate over a wider pH range than that of many heterotrophic bacteria (Gadd 2004). While there are numerous reports on fungi isolated from acidic to neutral soils, there are only a few reports about fungi from alkaline soils. Alkaline soils were examined for their fungal biota by Rai et al., (1971) and Nagai et al., (1995,1998). Soils with large amounts of calcium carbonate were studied by Vardavakis (1990) in Greece, whereas in Argentina, Cabello & Arambarri (2002) and Eliades et al. (2004) have reported fungi from alkaline soils. In all these contributions only a few Ascomycota have been cited.

The native dry forest dominated by *Celtis tala* and *Scutia buxifolia* is the main woodland com-

munity of the eastern plain called Pampa in Buenos Aires Province, Argentina. Woody ranges are located almost parallel to the coast of the La Plata River. These trees grow in soils with calcareous structure of biological origin (mollusk shells) classified as Rendoll (Sánchez et al., 1976). Between these woody ranges, salt meadow covers the inter-ranges; the alluvial soils with contrasting structures and high sodium content are characterized as Natracuallf, whereas the moist meadow with defective drain and hydromorphic, non-alkaline soils is described as Argialbol.

The aim of the present work was to characterize and illustrate morphological features of alkalophilic and alkali-tolerant soil Ascomycota at two different depths in each soil: Rendoll, Natracuallf and Argialbol by using soil washing methods for isolation. The ability of these fungi to grow at different pH was investigated.

MATERIALS AND METHODS

Study area, types of soil

The study area was a dry forest dominated by *Celtis tala* and *Scutia buxifolia* 20 km south east from Magdalena city (35° 11' S, 57° 17' W) in the Province of Buenos Aires, Argentina. The climatic characteristics of this forest have been described by Arturi et al. (1996).

Table 1 shows the main characteristics of the three types of soils sampled.

Fungal isolation

Ascomycota were isolated from soil collected from the described area by the washing method (Parkinson & Williams, 1961) on malt extract agar [MA: 10g of malt extract powder (Difco), 2.5g of peptone, 20g of agar, 1 L of distilled water] which initial pH was adjusted after autoclaving with buffers according to Nagai et al. (1998) to the values 8, 9, 10, and 11. Twenty plates were prepared for each pH and four soil particles transferred. After incubation for 5-7 days at 24° C, the growing fungi were observed under a light microscope. Eight species of Ascomycota were isolated from alkaline pH.

Growth rates of isolates at various pH

One strain was selected at random as a representative from each species. The strains were inoculated onto malt extract agar MA which initial pH was adjusted after autoclaving to 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0. Growth patterns of the different species were obtained by measuring twice the colony diameter of the representative strains after incubation for one to two weeks.

Strains that could not grow at pH 10 were considered as alkalophobic; those that grew at pH 10 as alkali-tolerant; and those that could grow at pH over 10, but not at pH 5, as alkalophilic.

RESULTS

Table 1 shows the characteristics of the profiles of the 3 soils sampled. All of them presented silt to clayey silty texture. The pH ranges varied from acid soils (Argialbol) to alkaline (Rendoll and Natracualf). In Natracualf soil, the increment of pH was due to the elevated contents of sodium at the two depths sampled (5.39 and 13.9 meq/100 g⁻¹, respectively), while in Rendoll, the increment of pH was due to the high contents of calcium carbonate (48 and 94%, respectively).

Table 1. Physical and chemical properties of the forest soils used in this study.

Soil	Argialbol		Natracualf		Rendoll	
Horizons	A1	A2	B1	B21t	A1	AC
deep (cm)	0-20	20-23	0-7	7-36	0-20	20-35
Clay %	34,96	18,94	13,52	35,95	9,95	15,56
Silt%	55,27	70,9	65,42	46,01	38,79	16,77
Sand%	9,77	12,16	21,06	19,84	51,26	67,67
Total C (%)	7,89	1,64	1,71	0,66	6,06	2,24
Organic matter (%)	13,6	2,83	2,95	1,14	10,44	3,86
Total N (%)	0,88	0,16	nd	nd	0,66	0,19
C/N	9	9,9	nd	nd	9,1	11,4
Ca (meq/100 g -1)	11,9	5,1	4,79	nd	nd	nd
Mg (meq/100 g -1)	5	2,9	8,83	nd	nd	nd
K (meq/100 g -1)	0,32	0,13	1,6	2,56	0,9	0,5
Na (meq/100 g -1)	0,65	0,37	5,39	13,9	0,7	0,5
CaCO ₃ (%)	nd	nd	nd	0,3	48	94
pH (H ₂ O)	6	7,1	9.6	10	7,4	7,8

Table 2. Species of Ascomycota isolated from the three soils at two depths and various pH.

Soils	Argialbol		Natrualf		Rendoll		pH isolation
Deep	sup	20cm	sup	20cm	sup	20cm	
Ascomycota species							
Bionectriaceae							
<i>Emericellopsis minima</i>	x						11
Sporormiaceae							
<i>Westerdikella dispersa</i>	x						8-9
Trichocomaceae							
<i>Neosartorya stramenia</i>			x			x	6- 8-11
<i>Talaromyces flavus</i> var <i>flavus</i>	x	x	x	x	x	x	6- 7-8-9-11
<i>Talaromyces stipitatus</i>	x	x	x			x	6-7-8
<i>Talaromyces trachyspermus</i>	x						7-8
Sordariaceae							
<i>Neurospora tetrasperma</i>			x				6
Ceratostomataceae							
<i>Melanospora zamiae</i>					x		10

Table 2 shows 8 species of Ascomycota isolated from the 3 different soil types at two depths and various pH.

Taxonomy

BIONECTRIACEAE

- 1. *Emericellopsis minima*** Stolk, Trans. Brit. Mycol. Soc. 38: 419. 1955. (Fig. 1A-B). For synonyms see Domsch et al. (1993).

Observations. During development of the colonies of *E. minima*, the first observed phase of its cycle was the anamorph belonging to *Acremonium* sp., then the culture developed ascomata. Our specimen agrees with the original description and with that by Wright et al., (1971) from Argentina.

Material examined

ARGENTINA. **Buenos Aires.** *Magdalena*, IX-2003, *Eliades s.n.* LPS culture N° 839 isolated from superficial horizons from Argialbol at pH 11.

TRICHOCOMACEAE

- 2. *Neosartorya stramenia*** (Novak & Raper) Malloch & Cain, Can. J. Bot. 50: 2622. 1972. (Fig. 1C).

Aspergillus stramenius Novak & Raper, in Raper & Fennell, The genus *Aspergillus*: p 260. 1965 (nom. holom.).

Colonies on MEA attaining a diameter of 20-23 mm in 7 days, velvety. Teleomorph: *Ascomata* scattered on the basal mycelium, non-ostiolate, globose to subglobose, 30-150 µm in diameter. *Asci* 8 spored, globose to subglobose or pyriform, 12-17 x 10-12 µm, evanescent at maturity. *Ascospores* hyaline to pale yellow, lenticular 6-7 x 3-5 µm, with two equatorial crests which are somewhat widely separated, with convex surfaces finely roughened (spinulose under SEM).

The anamorph showed the characteristics of the genus *Aspergillus* Micheli ex Link. *Conidial heads* loosely columnar, 60-100 µm long. *Conidiophores* 40-120 x 3-7 µm., smooth and fairly thick walled; vesicles flask-shaped, 8-14 µm diam, *Aspergillia* uniseriate; phialides cylindrical, 4,5-6 x 2-3 µm,

covering the upper one-half to two-thirds of the vesicle. *Conidia* hyaline to pale-colored, globose to subglobose, 2-3 µm diam, delicately roughened.

Observations. This species is recorded for the first time in Argentina.

Material examined

ARGENTINA. **Buenos Aires: Magdalena**, II-2005 Eliades. LPS culture N° 833 isolated from Rendoll at 20cm deep horizon and Natracualf soils, at pH 6, 8 and 11.

3. *Talaromyces flavus* (Klockner) Stolk & Samson var. *flavus*, Stud. Mycol. p 2. 1972. (Fig. 1D).

Gymnoascus flavus Klöcker, Hedwigia 41: 80. 1902. For more synonyms see Stolk & Samson 1972.

Anamorph: *Penicillium vermiculatum* Dangeard, Botaniste 10: 23. 1907.

Observations. Our specimens are in agreement with those described from Argentina by Bertoni et al. (1973).

Material examined

ARGENTINA. **Buenos Aires: Magdalena**, IX-2003, *Eliades s.n.* LPS culture N° 838 from Argialbol superficial pH 7; LPS culture N° 832 from Argialbol, 20 cm deep, pH 9.

4. *Talaromyces stipitatus* (Thom) C. R. Benjamin, Mycologia 47: 684. 1955. (Fig. 1E).

Penicillium stipitatus Thom, Mycologia 27: 138, 1935. (Diagnosis Latina status perfecti).

Anamorph: *Penicillium stipitatum* Thom ex C. W. Emmons, Mycologia 27: 138. 1935.

Observation. Our species agree with that described by Stolk & Samson (1972). This fungus was described in our country by Wright et al., (1971).

Material examined

ARGENTINA. **Buenos Aires: Magdalena**, IX-2003; *Eliades s.n.* LPS culture N° 836 from Natra-

cualf superficial isolated at pH 6; LPS culture N° 835 from Natracualf superficial pH 8; LPS culture N° 840 isolated from Argialbol superficial pH 7.

5. *Talaromyces trachyspermus* (Shear) Stolk & Samson, Stud. Mycol. 2: 32. 1972. (Fig. 1 F).

Arachniotus trachyspermus Shear, Science N.Y. II 16 :138. 1902

Talaromyces spiculispurus (Lehman) Benjamin, Mycologia 47: 683. 1955.

Anamorph: *Penicillium spiculispurum* Lehman, Mycologia 12: 268. 1920.

Observation. This species has been previously cited by Godeas (1972).

Material examined

ARGENTINA. **Buenos Aires: Magdalena**, IX-2003 *Eliades s.n.* LPS culture N° 831 isolated from superficial horizon from Argialbol at pH 7.

SPORORMIACEAE

6. *Westerdykella dispersa* Clum, Mycologia 47: 899, 1955. (Fig. 2A-B).

Mycelia homotallic, profusely branched, the hyphae 1-6 (µm in diameter; Teleomorph: ascomata 60-580 (µm in diameter; *asci* globose, 10-14.5 µm in diameter. *Ascospores* 2.0-3.0 x 2.5-5 µm, hyaline when young, later becoming light brown.

Anamorph: the conidial state corresponds with the species of Fungi Imperfecti *Phoma* Saccardo. *Pycnidia* varying from globose to irregularly-elongated in shape, glabrous; the lower portion colorless and the walls of the cells around the slightly papillate ostiole light brown, 26-75 x 26-158 µm . *Conidiophores* simple, extremely short. *Conidia* hyaline, unicellular, oblong, 1.5-3.5 x 2.6-5 µm.

Observation. This is the first record from our country.

Material examined

ARGENTINA. **Buenos Aires: Magdalena**. IX-2003, *Eliades s.n.* LPS culture N° 830 isolated from Argialbol superficial horizon at pH 8; LPS culture N° 834, Argialbol superficial horizon, pH 9.

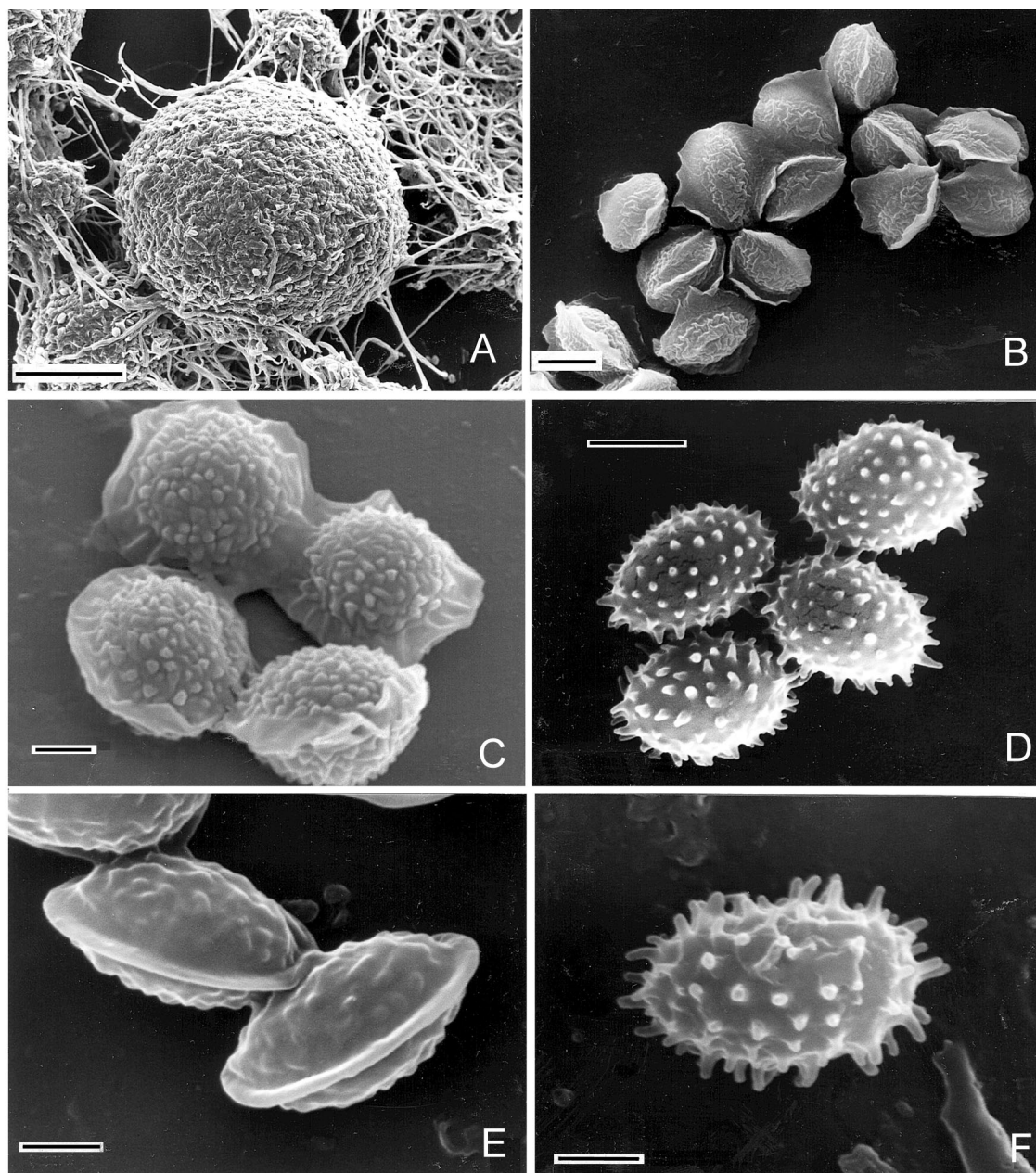


Fig. 1. A-B, *Emericellopsis minima*, A, ascomata (SEM). Scale bar = 5 μ m. B, ascospores (SEM). Scale bar = 2 μ m. C, ascospores of *Neosartorya stramenia* (SEM). Scale bar = 1 μ m. D, ascospores of *Talaromyces flavus* var. *flavus* (SEM). Scale bar = 2 μ m. E, ascospores of *Talaromyces stipitatus* (SEM). Scale bar = 1 μ m. F, ascospores of *Talaromyces trachyspermus* (SEM). Scale bar = 1 μ m.

CERATOSTOMATACEAE

7. *Melanospora zamiae* Corda, Icones Fungorum I: 27. 1837. (Fig. 2C-D).

For synonyms see Calviello 1973.

Observation. During development of the colonies of *M. zamiae*, both stages of the life cycle - anamorphic and teleomorphic- appeared at the same time. The anamorphic state belonging to the mitosporic genus *Acremonium*. *M. zamiae* was

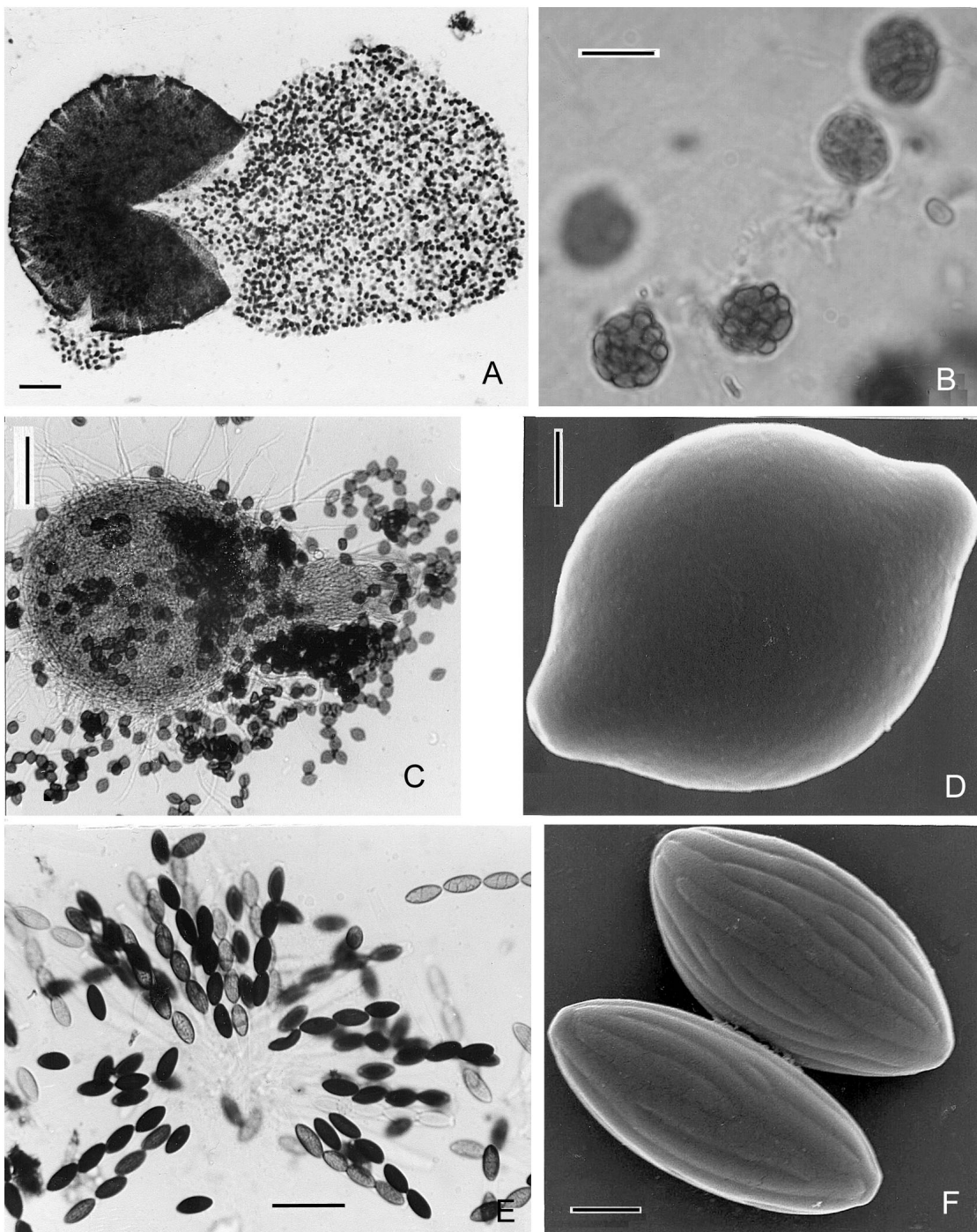


Fig. 2. A-B, *Westerdykella dispersa*. A, ascomata. Scale bar = 100 µm. B, asci with ascospores. Scale bar = 5 µm. C-D, *Melanospora zamiae*. C, ascomata. Scale bar = 100 µm. D, ascospore (SEM). Scale bar = 2 µm. E-F, *Neurospora tetrasperma*. E, asci with ascospores. Scale bar = 100 µm. F, ascospores (SEM). Scale bar = 5 µm.

isolated and described in our country by Calviello (1973).

Material examined

ARGENTINA. **Buenos Aires:** *Magdalena*, VI-2004, *Eliades s.n.* LPS culture N° 841, isolated from the calcareous soil Rendoll at pH 10.

SORDARIACEAE

8. *Neurospora tetrasperma* Shear & Dodge, J. Agric. Res. 34: 1027 (1927). (Fig. 2 E-F).

For synonyms see García et al. (2004).

Colonies brown or orange. Teleomorph: *Ascomata* ostiolated, 180-570 µm. *Asci* 4- spored. *Ascospores* ellipsoidal or elongated, 25-46 x 13-25 µm; *wall* with 16-22 sometimes branched ribs and ornate epispore, one germ pore at each end. Anamorph: *Chrysonilia tetrasperma* (Shear & Dodge) Arx (García et al., 2004). *Conidia* subglobose to obovoid, smooth, 5-10 (-15) x 8-10 µm, orange in mass.

Observation. This is the first record from our country.

Material examined

ARGENTINA **Buenos Aires:** *Magdalena*, XI-2004, *Eliades s.n.* LPS culture N° 837 isolated from alkaline soil Natracualf at pH 6.

Growth patterns of the isolates

Fig. 3 shows the growth patterns of 8 Ascomycota isolated. Members of the Family *Trichocomaceae*, namely *Neosartorya stramenia*, *Talaromyces flavus* var *flavus*, *T. stipitatus* and *T. trachyspermus* showed different patterns of growth. It was found that *N. stramenia* could grow at all the pH tested. All the species of *Talaromyces* developed mycelial growth up to pH 8 and 9.

Although we were able to isolate *T. flavus* var. *flavus* from soil particles at pH 9 and 11, this species did not show the same ability to keep growing at these pH after isolation.

Emericellopsis minima isolated from Argialbol at pH 11 was able to grow in all the pH tested. The restricted development observed corresponds to the intrinsic characteristics of the species. *Westerdykella dispersa* showed optimal growth from pH 5 to pH 8, while *Melanospora zamiae*, isolated from Rendoll at pH 10, showed optimal growth from pH 5 to 10 and a decrease in mycelial development at pH 11. *Neurospora tetrasperma* increased its growth when the pH in the culture medium was increased.

It is important to point out that all the Ascomycota tested developed ascomata in various culture media and at the pH tested.

DISCUSSION

The distribution of Ascomycota in soils is generally low when compared with other fungal groups (Cabello et al., 2003). This distribution can be higher and can reach up to 49% of the total taxa isolated if the species belong to fungal communities developed on organic debris (Van Ryckegem & Verbeken, 2005).

In the present work, 8 species of Ascomycota, isolated from soils with silt to clayey silty texture and from pH 6 to pH 11, are reported. All the isolated Ascomycota are either alkali-tolerant or alkalophilic species according to Nagai et al. (1998). In previous works about alkaline soils, the richness of isolated species of Ascomycota has been much lower (Cabello & Arambarri, 2002; Vardavakis, 1990). *Neosartorya stramenia*, *Neurospora tetrasperma* and *Westerdykella dispersa* are reported for the first time in Argentina.

Emericellopsis minima, *Neosartorya stramenia* and *Melanospora zamiae* were isolated at pH 10 to 11. These species are alkalophilic according to the pH isolation medium. Of these three species, *E. minima* was always found associated with its anamorph *Acremonium* sp, which is the first phase of the cycle of the fungus that appears in the culture medium. It developed ascomata after a few days.

Besides *Acremonium*, *M. zamiae* also showed its anamorphic stages in *Proteophiala* sp. (Ciferri, 1957) and *Phialophora* sp. (Ito & Yokoyama, 1987) The presence of the complete cycle (anamorph + teleomorph) is a relevant fact and the

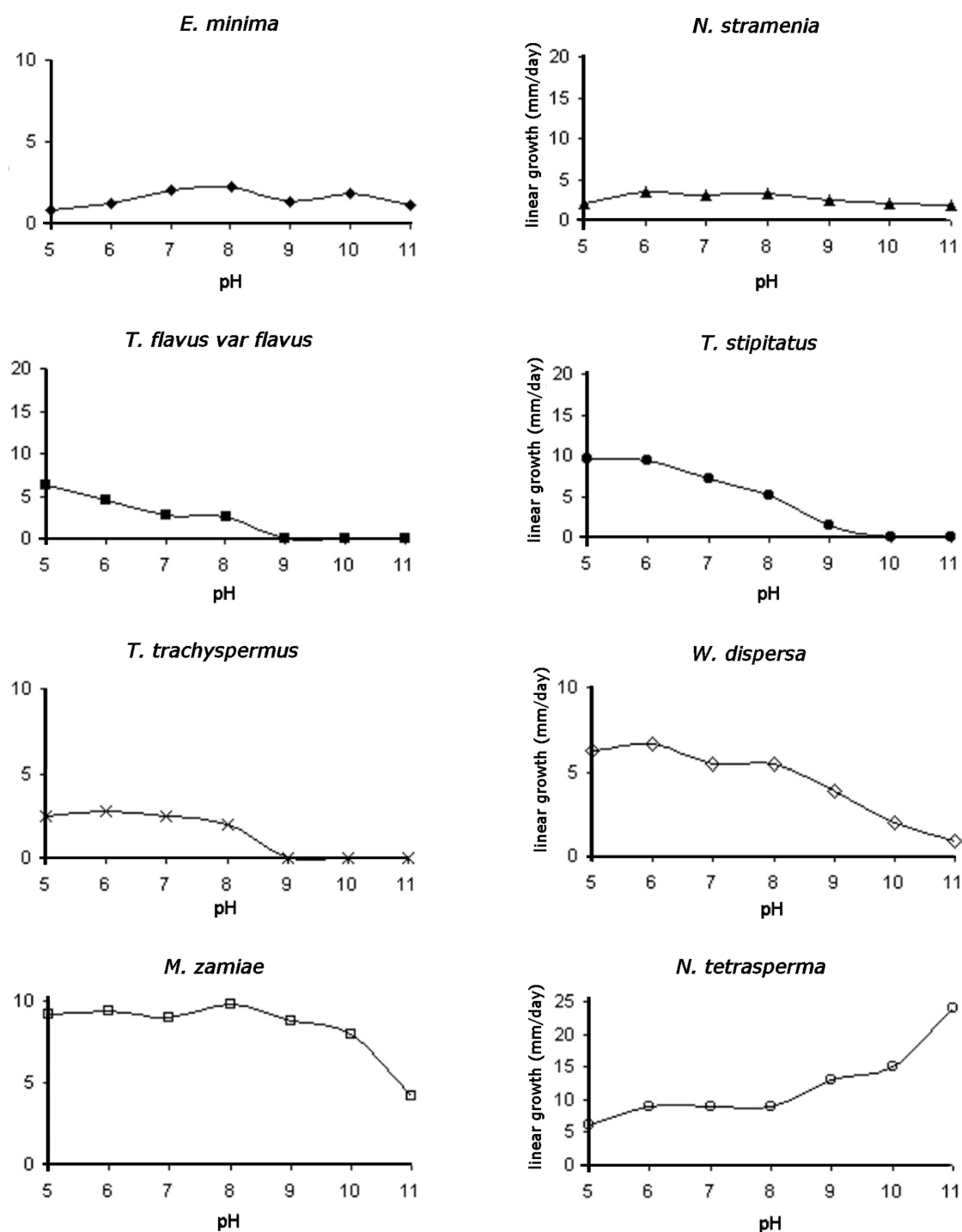


Fig. 3. Growth patterns of 8 Ascomycota isolated.

appearance of the anamorphic phase is consistent with the classification of the genus *Acremonium*

as alkali-tolerant (Nagai et al., 1998; Cabello & Arambarri, 2002).

Among the members of the family *Trichocomaceae*, only *Neosartorya stramenia* can be considered an alkalophilic species because it can keep growing up to pH 11. The low values (measured in mm/day) correspond to intrinsic characteristics of the species (Yaguchi et al., 1994). Its anamorphic stage is found in *Aspergillus stramentinus*. Cabello & Arambarri (2002) have previously observed that the species of the genus *Aspergillus* predominated in alkaline isolation media. Due to the fact that there are few reports on *N. stramenia* in the literature, this species is probably a rare fungus (Raper & Fennell, 1965; Yaguchi et al., 1994).

Other members of the family *Trichocomaceae* are the three species of *Talaromyces*.

All the species here described belonging to this genus are homothallic, and they have their conidial states in the genus *Penicillium*. Among all the isolated Ascomycota, *Talaromyces* species were the most sensitive to high pH, although we were able to isolate them at pH 6 to pH 11. Their anamorphs belonging to *Penicillium* preferred acid pH (Gams, 1992). Although some members from the genus *Talaromyces* reported here were isolated at pH 11, they can not be strictly considered as alkaline-tolerant since the growth patterns showed that they were not able to grow at high pH. The fact that they were isolated at a high pH could be due that these species were over-represented in the soil.

Although *Neurospora tetrasperma* has been frequently isolated from burnt vegetation (Jacobson et al., 2004), in this study this species was isolated from the alkaline Natracuall soil. Taking into account the great mycelial development at pH 11, we can state that this species preferred alkaline conditions.

The Ascomycota isolated from these soils at different pH ranges were different from these species reported in other soils in Argentina (Cabello et al., 2003). The soil fungal Ascomycota need further analysis and physiological studies in order to know how Ascomycota fungal strains live in alkaline conditions.

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