Biol Fertil Soils (2001) 33:373–381
DOI 10.1007/s003740000336

ORIGINAL PAPER

Ana B. Menéndez · José M. Scervino
Alicia M. Godeas

Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina

Received: 3 February 2000 / Published online: 27 February 2001

Abstract The influence of tillage and monoculture on arbuscular mycorrhizae (AM) fungal species diversity in a field site of the Buenos Aires province (Argentina) was investigated through the morphological characterization of AM spores. Glomalean spores were isolated by wet sieving and decanting from three plots cropped either to wheat, barley or clover and from a grassland at the margins of the cultivated plots. Seventeen different Glomalean species were detected overall and seven of them were identified. Total species number as well as spore and species richness found in grassland and clover soil were higher than those found in soils planted either with barley or wheat. The most frequently occurring species in the site were *Glomus mosseae, Scutellospora pellucida, Glomus* sp. 7 and *Gigaspora* sp. 1. The first three were also the most dominant species and were found in the four types of analysed soils. In grassland soil and wheat, the dominant species was *Glomus* sp. 6. *S. pellucida* was dominant in barley, and in red clover the dominant species were *G. mosseae* and *S. pellucida*. Tillage and cereal monoculture negatively affected diversity of AM fungal species. Natural re-colonization of indigenous AM fungi was observed in cultivated soil with red clover for 3 years, suggesting that this host could be used as a cover crop to increase AM fungal inocula in disturbed soils. Arbuscular mycorrhizal populations were associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina.

Keywords Arbuscular mycorrhiza · *Glomus mosseae* · *Scutellospora pellucida* · Clover · Tillage

A.B. Menéndez (✉) · J.M. Scervino · A.M. Godeas
Departamento de Ciencias Biológicas,
Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Piso 4, Pab. II,
Ciudad Universitaria (1428), Buenos Aires, Argentina
e-mail: anamen@bg.fcen.uba.ar

Introduction

Most plant taxa have symbiotic associations with arbuscular mycorrhizae (AM) fungi, and soils bearing native or cultivated host plants harbour these fungi (Mosse 1973; Harley and Smith 1983). AM fungi contribute to soil structure and nutrient cycling (Miller and Jastrow 1992a, 1992b). However, agricultural practices like tillage, crop sequence, plant breeding, fertilizer and pesticide applications may alter AM fungal populations, species composition and root colonization (Schwab and Reeves 1981; Kurle and Pfleger 1994). In general, agricultural practices have included monoculture and conventional tillage. The latter, considered a disturbing practice, reduces the function of (AM) symbiosis through the breakdown of their hyphal network in the soil (Jasper et al. 1989).

Buenos Aires Province (307,562 km2) has a temperate climate and soil characteristics that favour the development of a high diversity of grasses. In this province, the agriculturally richest territory of Argentina, replacement of natural vegetation (prairies of natural grassland) by crop plants has increased during the last decades.

Although AM symbiosis in various grassland ecosystems has been extensively studied worldwide (Walker et al. 1982; Miller 1987; Bentivenga and Hetrick 1992; Sanders and Fitter 1992), nothing has been so far reported on indigenous AM fungi in the grassland prairies of Buenos Aires province.

This work aimed at studying the influence of tillage and monoculture on AM fungal species diversity in a field site, inferred from the morphological characterization of AM spores. We hypothesize that there must be a great diversity of AM fungal species associated with naturally occurring plant species, and that several decades of tillage and monoculture may have caused a negative effect on the composition of the AM fungal population.

The re-establishment of the original AM community, present before cultivation, might benefit the restoration of a functional and sustainable plant community applicable for multiple purposes included agricultural produc-

tion. However, a delay in the colonization of a site by mycorrhizal plant species might also result in a reduction in mycorrhizal fungal propagule reserves and, hence, the continued occupation of a site by nonmycotrophic species (Allen and Allen 1980, 1988; Miller 1987). Such sites are usually characterized by having low species richness and, often, poor productivity. We have personally observed that fallow sites in Buenos Aires province are rapidly occupied by several nonmycotrophic species like *Brassica* spp. On the other hand, it has been suggested that, in cases where AM fungal propagule densities are very low, the rate of restoration may be hastened by inoculation or manipulation of the mycorrhizal fungus population (Reeves et al. 1979; Janos 1980). Since clover is a kind of cover crop that improves plant vigour by increasing AM fungal inoculum potential (Kurle and Pfleger 1994), we additionally evaluated red clover (*Trifolium pratense*) as a cover crop for restoring the AM fungal population.

Materials and methods

Study site

This study was undertaken in a single field site (12 ha) located at the experimental station of INTA Castelar, Province of Buenos Aires, between December 1997 and February 1999. The field had been cropped to wheat and barley with conventional tillage during more than 40 years, without P fertilizer supply. In 1995, the field was divided into four plots (3 ha each), two of which were further seeded with clover for soil recuperation and no further tillage was applied to these plots. The remaining two plots continued being cropped, with conventional tillage, to barley or wheat, respectively. In 1995, soil chemical analysis at a depth of 20 cm showed the following composition: organic matter 3.5%, organic C 2.0%, N 0.16%, C/N 11.6, P 6.3 ppm, pH (H_2O) 5.9, pH (1 N KCl) 5.3.

Glomalean spores were studied in a grassland at the margins of the cultivated plots and in three plots cropped to wheat (plot 1), barley (plot 2) and clover (since 1995, plot 3), respectively. In the first two plots, sampling was done at harvesting. In the margins and in the plot cultivated with red clover (plot 3), well-developed, mature plants were chosen for sampling. The most important plants present in the grassland were: *Aloysia gratissima, Bromus unioloides, Hypochoeris chillensis, Paspalum dilatatum, P. notatum, Passiflora coerulea, Solidago chilensis* and *Wedelia glauca.*

Five soil samples were taken following a 30-m-long transect, from each of the 3 plots and the marginal grassland. Rhizospheric soil was removed at a depth of 20–30 cm, air dried, and kept in plastic bags at 4°C until use.

For assessing the structure of Glomalean communities, spores were isolated by wet sieving (smallest sieve 65 µm) and decanting (Gerdemann and Nicolson 1963) from three to five subsamples, each containing 150 g (air-dry weight) of soil. Spores were examined under binocular and light microscope, mounted in Melzer's reagent and grouped according to their size, colour and spore wall characteristics. Spores were described according to Walker (1983) and Morton (1988). Spore colour was determined according to Munsell colour table (Munsell Color Company 1954). Voucher specimens were preserved in lactophenol and deposited in the culture collection of the Department of Biological Sciences, School of Exact and Natural Sciences, University of Buenos Aires. Spores were photographed with a MC 100 Zeiss camera connected to an Axioskop Zeiss microscope.

With the data obtained we calculated: (1) spore density, measured as total number of spores occurring in 150 g (air-dry weight) of soil, (2) species richness, measured as the total number of different species occurring in 150 g (air-dry weight) of soil, (3) frequency of occurrence calculated as the percentage of samples from which a determined species was isolated, (4) the dominance coefficient as the ratio between the number of spores of a particular species to the total number of Glomalean spores (×100), and (5) the Shannon-Wiener diversity index (Krebs 1985)

Shannon-Wiener diversity index

$$\sum_{i=1}^{s} p_i \log_{10} p_i \qquad (1)$$

where S is the number of species and p_i is the proportion of the total sample belonging to the ith species. This function combines two components of diversity: S and evenness of allotment of individuals among the species.

Results

Seventeen different Glomalean species were detected overall, after more than 75 soil subsamples were analysed (Table 1). Only seven isolates were identified to the species level: *Acaulospora denticulata* Sieverding and Toro, *Glomus aggregatum* Schenk and Smith amend. Koske, *G. microaggregatum* Koske, Gemma and Olexia, *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, *Glomus coremioides* Berk. and Broome, *Scutellospora heterogama* (Nicol and Gerd) Walker and Sanders and *Scutellospora pellucida* Nicol and Schenk. Among the rest there were six *Glomus*, one *Scutellospora*, one *Gigaspora*, one *Acaulospora*, one *Entrophospora*, and one parasitic Glomalean species.

Fourteen species were found in both the grassland soil and in that cultivated with red clover (Table 2). The total number of species found in soil cultivated with barley and wheat was four and five, respectively. Soil re-vegetated with clover showed similar spore density and species richness as grassland soil, and both were higher than in soils planted either with barley or wheat (Fig. 1). Shannon-Wiener diversity index values were lower in

Table 1 Total arbuscular mycorrhizal fungi recovered from the site

	Frequency of occurrence (%)	Dominance (%)
Acaulospora denticulata	1.587	0.21
Entrophospora sp. 1	1.587	0.21
Gigaspora sp. 1	11.11	5.02
Glomus aggregatum	1.6	0.2
Glomus microaggregatum	3.2	2.7
Glomus mosseae	15.9	27.8
Glomus sp. 1	3.2	5.2
Glomus sp. 2	3.2	2.3
Glomus sp. 3	4.8	5.2
Glomus sp. 4	1.6	0.21
Glomus sp. 5	1.6	1.04
Glomus sp. 6	15.9	19.7
Glomus coremioides	1.6	0.21
Scutellospora heterogama	4.8	1.7
Scutellospora pellucida	19.04	21.8
Scutellospora sp. 1	3.2	1.04
Parasitic Glomalean	4.8	2.08

Table 2 Dominance (*x*) and frequency of occurrence (*y*) of arbuscular species in the rhizosphere of the grasses at the margins (*a*) and in the three plots cultivated to wheat (*b*), barley (*c*) and clover (*d*)

Species	a		b		c		d	
	x	y	x	y	x	y	x	y
Acaulospora denticulata	0	0	0	0	0	0	0.4	3.4
Entrophospora sp. 1	0.6	4	0	0	0	0	0	0
Gigaspora sp. 1	3.4	8	11	55	0		5.9	3.4
Glomus aggregatum	0	0	0	0	0	0	0.4	3.4
Glomus microaggregatum	6.3	4	0	0	4.2	9.1	0.4	3.4
Glomus mosseae	13	8	24	20	17	18	40.5	10
Glomus sp. 1	8	4	0	0	0	0	4.6	3.4
Glomus sp. 2	4	4	0	0	0	0	1.7	3.4
Glomus sp. 3	8	4	0		0		4.6	6.9
Glomus sp. 4	0.6	4	0	0	0	0	0	0
Glomus sp. 5	2.8	4	0	0	0	0	0	0
Glomus sp. 6	33.5	8	55	15	13	36	4.6	3.4
Glomus coremioides	9.1	8	0	0	0	0	0.4	3.4
Scutellospora heterogama	0	0	7.9	5	0		2.1	6.9
Scutellospora pellucida	8.5	12	2.6	5	70.8	18	29.8	17
Scutellospora sp. 1	0.6	4	0	0	0	0	2.1	6.9
Parasitic Glomalean	2.9	6.9	0	0	0	0	1.7	8

soil cultivated with barley and wheat than in grassland soil and in soil cultivated with red clover.

Overall, *Glomus* and *Scutellospora* were the most dominant and frequent genera (Table 1). *Glomus* species showed a frequency of 50.7% and a dominance of 64.5%, whereas for *Scutellospora*, these values were 26.9% and 24.5%, respectively. The most frequently occurring species were *Glomus mosseae* (27.8%), *Scutellospora pellucida* (21.7%), *Glomus* sp. 7 (19.7%) and *Gigaspora* sp. 1 (11.1%). The first three were also the most dominant species and were found in the four types of vegetation analysed.

In grassland soil, all 14 species showed similar frequency of occurrence. The dominant species was *Glomus* sp. 6 which comprised 33.5% of the spores (Table 2). In this soil, *Glomus* species accounted for 80% of Glomalean spores.

In wheat soil, spore dominance by *Glomus* sp. 6 ranked first with 55% dominance, followed by *Glomus mosseae* with 24% dominance. On the other hand, the most frequently recovered spore-forming species was *Gigaspora* sp. 1, which occurred in 55% of the soil samples examined. Another frequently observed spore-forming species was *Glomus mosseae* (20%). In this soil, *Glomus* species accounted for >78% of Glomalean spores, the rest of the spores belonging to the genera *Gigaspora* and *Scutellospora*.

In barley soil, the dominant species was *Scutellospora pellucida* (70.8%) and the most frequently recovered spore-forming species was *Glomus* sp. 6 (36%). The remaining two species found in this soil also belonged to the genera *Glomus* and *Scutellospora*. No *Acaulospora*, *Entrophospora* or *Sclerocystis* species were found in either of the former two soils.

In red clover, *Glomus mosseae* and *Scutellospora pellucida* were the dominant species with 40.7% and 29.8% dominance, respectively. This last species was also the most frequently recovered (30%). The remaining spores were distributed among the genera *Sclerocystis*, *Gigaspora* and *Acaulospora*.

Discussion

Seventeen different species of AM fungi have been detected in the single site analysed in this work. It is difficult to compare this result with those obtained by others, since most of the surveys of AM fungi have been carried out in sand dunes (Gemma et al. 1989; Friese and Koske 1991; Jun-ichi et al. 1994), in arid (Ba et al. 1996), forest (Sambandan et al. 1994) or tropical soils (Muthukumar et al. 1996). In addition, in many reports in which different types of mycorrhizae were recorded (Hayman 1975; Iabal et al 1975; Kruckelmann 1975; Hayman et al. 1976), the authors used a different taxonomy to identify species. There are, however, a few reports for crop soils in temperate climate, where the number of AM species recovered in a small area was similar to that recorded in this work. For example, 13 VA-mycorrhizal species were identified in a newly cleared woodland single site in northwest Florida and in three adjacent different grassland sites in Japan (Schenck and Kinloch 1980; Murakoshi et al. 1998).

Data show that agricultural practices applied to the field site under study affected the composition of the AM fungal population: there was a significant reduction in spore number, species diversity and Shannon-Wiener function values, in plots cropped with conventional tillage and cereal monoculture, compared to grassland soil. This last function showed a reduction in the number of species and evenness of allotment of individuals among the species in the first two plots compared to grassland soil. In addition, there was a change in species dominance and frequency of occurrence.

Although mechanisms have not been investigated here, one could speculate that tillage and, more likely,

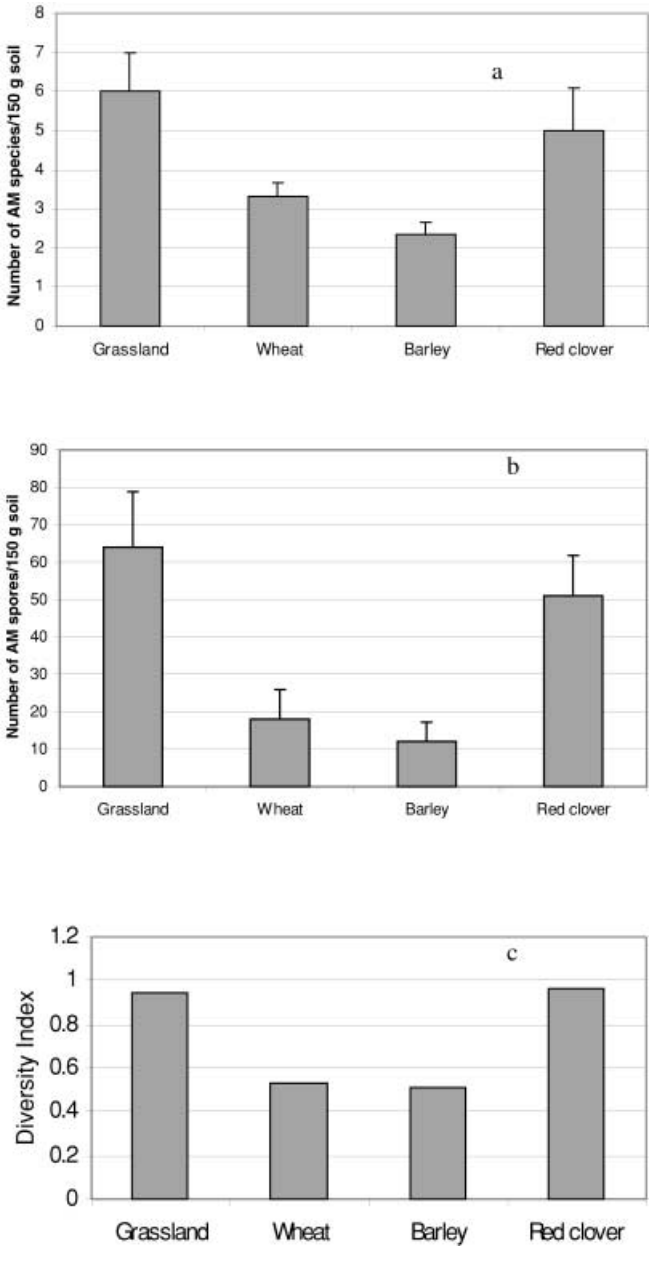

Fig. 1 Species richness (**a**) and spore densities (**b**), of arbuscular fungi in the site. Shannon-Wiener diversity index (**c**)

preferential host association, could have been involved in the differences in AM species composition observed between grassland and cropped soils in this work. It has been shown that preferential associations with a particular host may result in shifts of the AM species composition (Sanders and Fitter 1992; Kurle and Pfleger 1994). In turn, the most important effect of tillage on AM function is the disruption of extraradical hyphae (Evans and Miller 1990; Fairchild and Miller 1990). It has been suggested that AM fungal species, which grow and sporulate more rapidly, would be favoured in more intensive tillage systems, resulting in greater soil disturbance (Kurle and Pfleger 1994).

Tillage and cereal monoculture negatively affected species like *Acaulospora denticulata*, *Entrophospora* spp., *Glomus* sp. 1–5, *G. aggregatum*, *G. microaggregatum*, and *S. coremioides*. In contrast, the competitive ability of species like *Glomus mosseae*, *Glomus* sp. 6 and *S. pellucida* was improved. Such an improvement of their competitive ability might be related to several factors affecting the ability of AM fungi to colonize roots. Among these factors are external hyphae production, propagule size and timing as well as intensity of sporulation (Wilson and Tommerup 1992; Kurle and Pfleger 1994). In this study, the species that persisted after several decades of tillage were mainly those having the larger spores: i.e. *Glomus mosseae* and *S. pellucida*. These two species have been formerly recovered from soils cultivated with several crops (Schenck and Kinloch 1980). Daniels et al. (1981) showed that *G. mosseae* had both the largest spore size and the most rapid infection period compared with five other fungal isolates. In a study on AM fungal diversity of agricultural ecosystems using restriction fragment length polymorphism and sequence analysis of 18S rRNA gene fragments, diversity was dominated by *Glomus mosseae* and other closely related *Glomus* species (Husband et al. 1998). Although *Glomus* sp. 6 has comparatively smaller spores, its persistence in wheat and barley soils may be explained by the strong dominance of this species in grassland soil. Unfortunately, there are no data available on the extent of extraradical hyphae, propagule production or host preference, for most of the species detected in this work, which can be related to our results.

The present work has been focused only on spore-forming species. Since sporulation may be a very unreliable indication of root colonization in the field (Clapp et al. 1995), we are aware that data obtained on frequency and dominance may not necessarily be representative of the whole arbuscular mycorrhizal forming community.

Natural re-colonization of indigenous AM fungi was observed in soil cultivated with 3-year-old red clover as cover crop. During this period, it is likely that species present at low spore densities had been selectively enriched, thus resulting in the increase in their population size. The levels of spores, species richness and diversity, as well as the species dominance and frequency of occurrence, were similar in clover and grassland soil. These results suggest that the use of red clover as a cover crop could be a way to increase an AM fungal inoculum where it has decreased as a consequence of soil disturbance.

Description on the morphological features of the AM species occurring in the site

1. *Acaulospora denticulata* Sieverding and Toro (Fig. 2a, b).
2. *Entrophospora* spp. (Fig. 2c).
3. *Gigaspora* sp. 1. Spores formed singly in the soil, terminally on a bulbous base, white to pale yellow; glo-

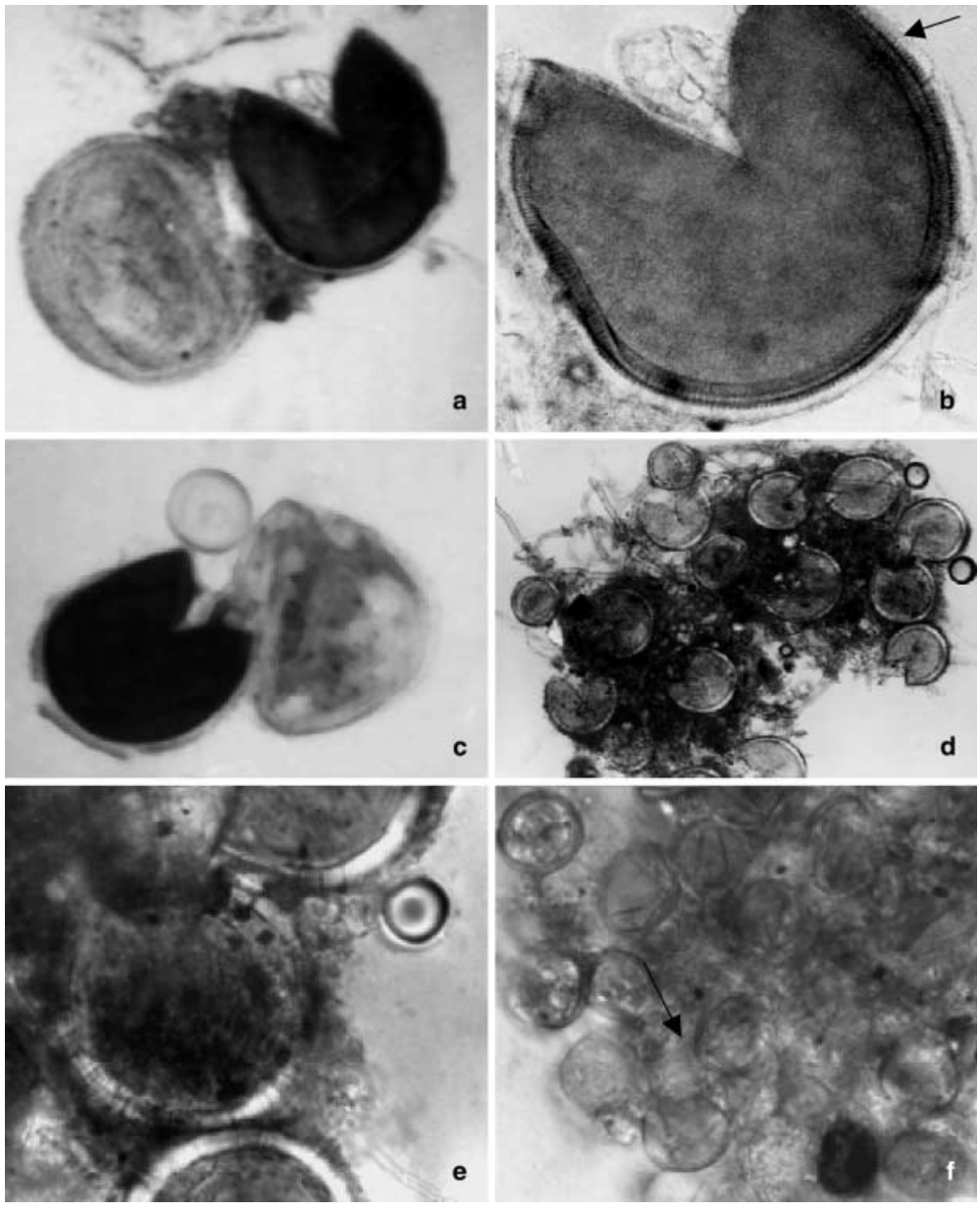

Fig. 2 **a**, **b** *Acaulospora denticulata*; **c** *Entrophospora* spp., **d**, **e** *Glomus aggregatum*; **f** *G. microaggregatum*

bose, ovoid to irregular, (230) 264 (320) µm diameter, turning slightly brownish yellow with Melzer. Spore wall hyaline, coriaceous, composed of four to nine laminae. Sporogenous base reddish brown, (31) 36.5 (41) µm diameter. Auxiliary cells were not found in soil. This species was associated with roots of clover, wheat, barley, and found in grassland.

4. *Glomus aggregatum* Schenk and Smith emend. Koske (Fig. 2d, e).
5. *Glomus microaggregatum* Koske, Gemma and Olexia (Fig. 2f).

   The former two species occasionally occur together and are easily separated by their smaller spores and lighter colour (Koske et al. 1986).
6. *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe.
7. *Glomus* sp. 1 (Fig. 3a, b). Spores produced singly in soil, reddish yellow, globose to subglobose, often parasitized and hence irregular in shape and opaque (184) 225 (260) µm diameter. Spore wall structure consisting of four walls (1–4) in two walls groups (A, B). Group A composed of a laminated (3–5), reddish yellow to light brown, 13- to 19-µm-thick wall 1. Group B composed of walls 2, 3 and 4. Walls 2 and 3 hyaline, rigid, 2,6-µm-thick each; wall 4 hyaline, 1.5- to 2-µm thick, membranous, flexible, papillate, slightly purple in Melzer's reagent. This species was associated with roots of clover and grasses. It resembles *G. gerdemannii* in the roughened, brittle external wall appearance, wall structure and the fact that *G. gerdemannii* has been observed to be colonized by other fungi fairly often (Rose et al. 1979). *G. gerdemannii*

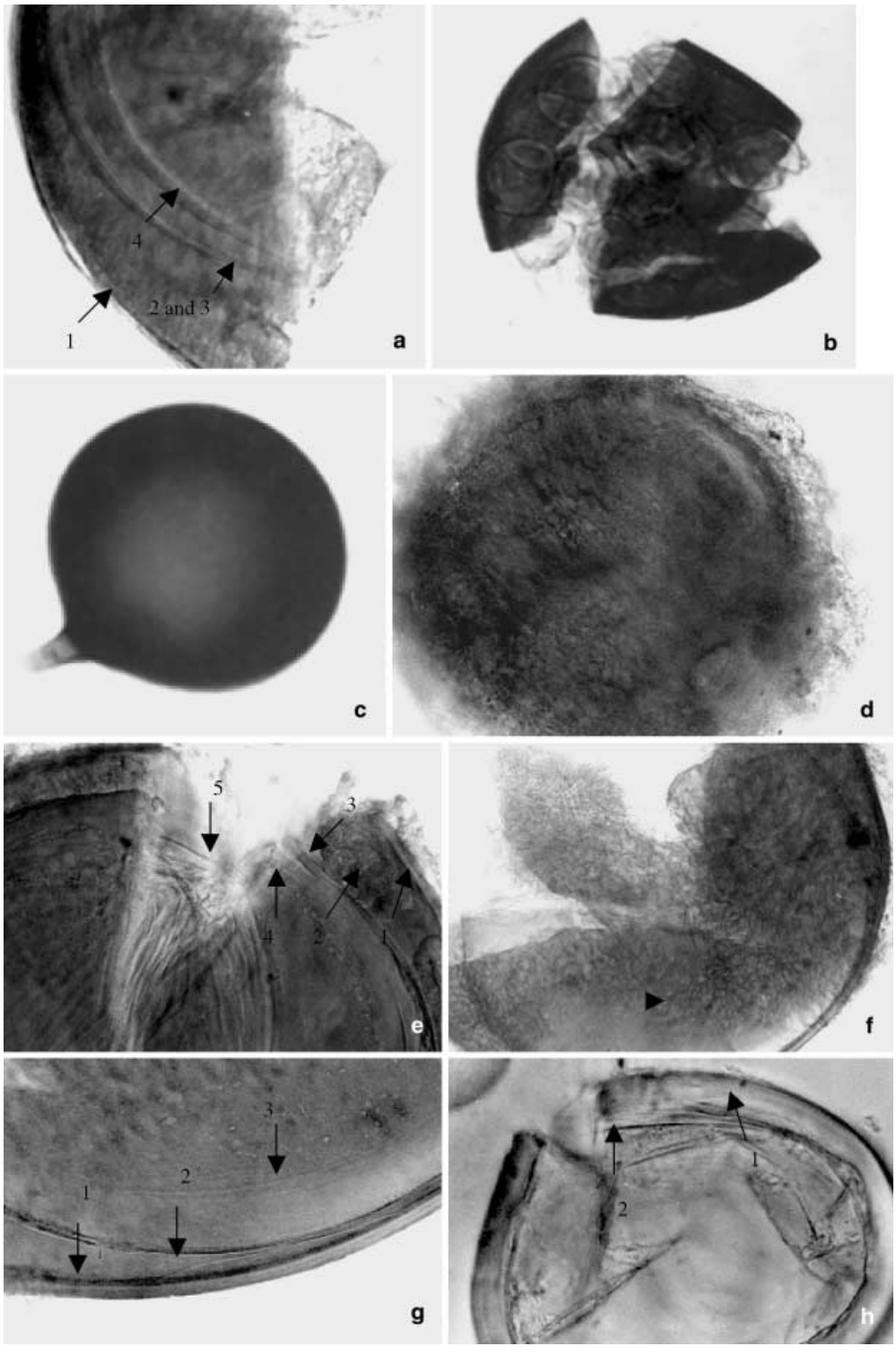

Fig. 3 **a**, **b** *Glomus* sp. 1. *Arrows* indicate four walls (1–4) and *arrowheads* parasitic spores. **c** *Glomus* sp. 2; **d**, **e** *Glomus* sp. 4. *Arrows* indicate five walls (1–5). **f**, **g** *Glomus* sp. 5. *Arrows* and *arrowheads* indicate three walls (1–3) and hyphal peridium, respectively. **h** Parasitic Glomalean. *Arrows* indicate two walls (1, 2)

has a five-layered spore wall, the outermost layer becoming roughened and cracked and flaking away in pieces soon after the spore has reached full size. The second layer inwardly degrades progressively to flake away as laminar amorphous pieces. The third, fourth and fifth inward layers are hyaline and persistent. It is possible that the outermost degrading layer observed in the spores of our material correspond to the second inward layer of *G. gerdemannii* spores. This species was found associated with roots of clover, wheat, barley, and in grassland soil.

8. *Glomus* sp. 2 (Fig. 3c). Chlamydospores produced singly in the soil, usually globose to ovoid, red, (153.9) 167.5 (208) µm diameter. Spore wall structure

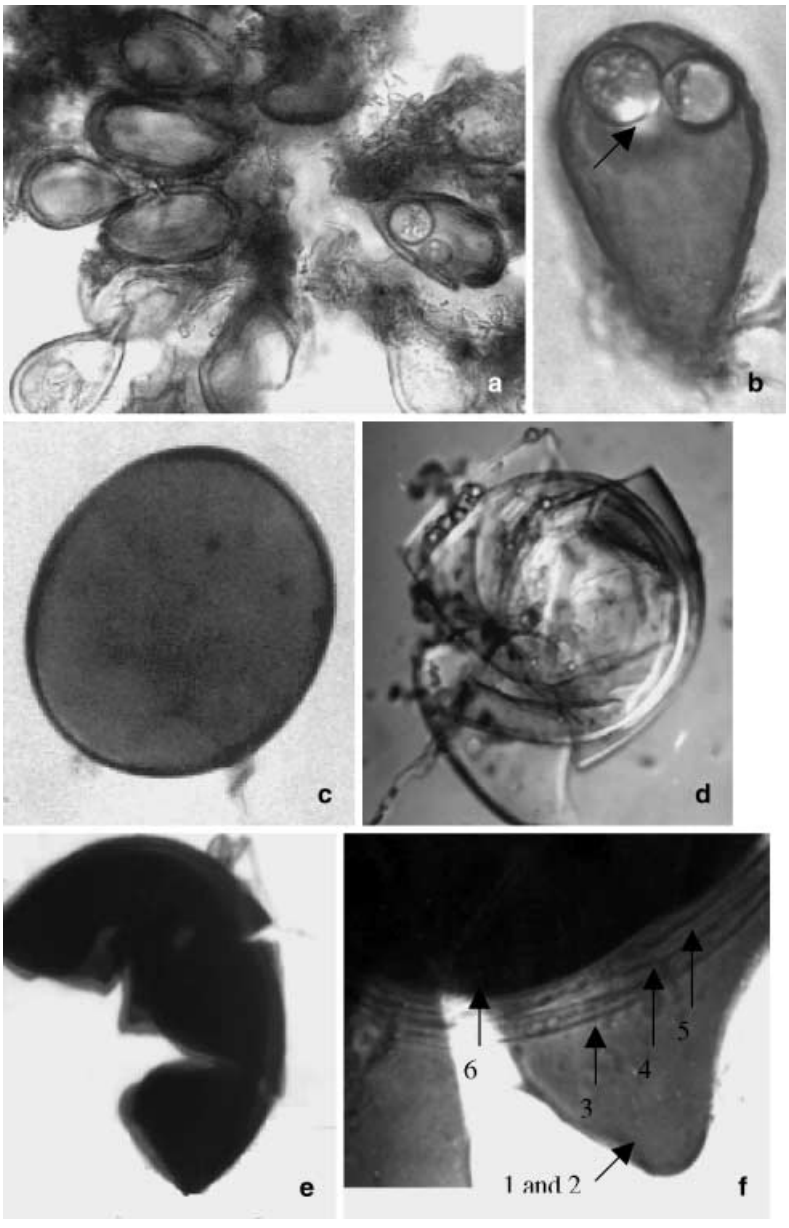

Fig. 4 **a**, **b** *Glomus coremioides*. *Arrows* indicate parasitic spores. **c** *Scutellospora heterogama*; **d** *Scutellospora pellucida* crushed spores; **e**, **f** *Scutellospora* sp. 1. *Arrows* indicate six walls (1–6)

consisting of two walls (1 and 2). Outer unit wall 2.08 µm thick, red, smooth. Inner wall very thin, 0.5–1 µm, hyaline. This species was found in grassland soil.

9. *Glomus* sp. 3. Chlamydospores formed singly in the soil, globose, ovoid to irregular in shape, pale yellow turning to reddish brown with age, (92) 118.6 (151) µm diameter. These spores were found associated with barley and grasses.
10. *Glomus* sp. 4 (Fig. 3d, e). Sporocarps reddish yellow, one spored, globose or subglobose 146 (193)×(130) 188 (234) µm diameter. Chlamydospores with five walls (1–5): inward, (1) a reddish yellow, outermost thick wall (wall 1), (2) a thick inner wall (wall 2) composed of amorphous material, (3) and (4) two hyaline, rigid walls (walls 3 and 4, respectively), and (5) an innermost, hyaline, membranous, wall (wall 5). These spores were found in the soil, associated with roots of clover and grasses. This species has the complex wall structure of *G. fistulosum*, *G. fragilistratum* and *G. gerdemannii* (Rose et al. 1979; Skou and Jakobsen 1989); however it differs in the sequence of the wall types.
11. *Glomus* sp.5 (Fig. 3f, g). Chlamydospores borne singly in the soil, globose to subglobose, reddish-brown, 160–180 µm diameter, covered by a hyphal mantle. Chlamydospore wall structure consisting of three separated walls (1, 2, 3). Wall 1 rigid, reddish brown, 4,6 µm thick; wall 2 hyaline, membranous, 3.5 µm thick; wall 3 hyaline, membranous 0.5 µm thick. This species was found in grassland soil.
12. *Glomus* sp. 6. Chlamydospores borne single in the soil, yellow to olive yellow, with guttulae, globose (99) 144 (172) µm diameter, subglobose 148×

160 µm to irregular. Spore wall formed by one laminated (four to six laminae) 2.7–5.2-µm-thick layer, not reacting with Melzer. Probably with an outermost evanescent wall. The septum is at a distance of 64 µm from the spore and its diameter is 13 µm. Oblique insertion. These spores have been found associated with the rhizosphere of clover, wheat, barley and grasses. It is probably *G. mosseae* but, because of the differences in spore characteristics with respect to *G. mosseae* as described above, until culture studies have been completed, we prefer to leave this as a different species.

13. Parasitic Glomalean (Fig. 3h). Chlamydospores produced inside other Glomalian spores, globose to subglobose 15.6–85 µm diameter, hyaline, sometimes with oil droplets. Spore wall composed of two walls (1 and 2). Wall 1 hyaline, 2–5 µm thick, laminated, turning purple with Melzer, sometimes surrounded by cytoplasmic debris from the host cell. Wall 2 membranous, hyaline and flexible. These spores were observed parasitizing *Glomus* sp. 1, *Gigaspora* sp. 1 and *S. coremioides.* (the presence of intact spores of these three species in soil allowed their identification). This species resembles *G. diaphanum* (Morton and Walker 1984) in size variability, colour (both remain hyaline throughout their life cycle) and spore-wall structure. Several AM species have been reported inside AM spores (Gerdemann and Trappe 1974; Mosse and Bowen 1968), but few have been identified to species.
14. *Glomus coremioides* Berk. and Broome (Fig. 4a, b). Sporocarps brown to dark brown, 250–330 µm diameter, subglobose. Chlamydospores (34.8) 39 (45)× (58) 66 (71) µm diameter, oblong, ellipsoid to clavate. Usually parasitized and old in appearance. This species was found associated with roots of clover and in grassland soil.
15. *Scutellospora heterogama* (Nicol and Gerd) Walker and Sanders (Fig. 4c).
16. *Scutellospora pellucida* Nicol. and Schenk (Fig. 4d).
17. *Scutellospora* sp. 1 (Fig. 4e, f). Spores borne singly in the soil, terminally on a bulbous base, reddish yellow, globose 191 µm diameter, subglobose or ovoid (161) 173 (185)×(192) 195 (230) µm diameter. Bulbous base concolorous with the spore wall, 27.2 µm. Spore wall structure consisting of six walls (walls 1–6) in two groups (A and B). Group A composed of two walls, 1 and 2. Wall 1 ornamented with long projections, firmly attached to wall 2. Wall 2 coloured and laminated (2–3). Group B composed of three hyaline, flexible walls and an innermost coloured, laminated (2–3), rigid wall. No walls reacted with Melzer. Germination shield was not observed. These spores were found in the rhizosphere of clover and in grassland soil. The description of this species did not match with any so far described *Scutellospora* species, therefore this is possibly a new species.

Acknowledgements We wish to thank Ing. Héctor Saione from INTA Castelar for helping us during sampling, Dr E. Wright for the language review of the manuscript and the referees for their valuable suggestions. This work was supported by the following projects: Pict 01–04510 (Agencia Nacional de Promoción Científica y Tecnológica), PIP 812 (CONICET) and TY02 (UBA).

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