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SHORT NOTE

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Spore ontogeny of the arbuscular mycorrhizal fungus *Archaeospora trappei* (Ames & Linderman) Morton & Redecker (*Archaeosporaceae*)

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Abstract Arbuscular mycorrhizal (AM) fungi in the genus *Archaeospora* (family *Archaeosporaceae*) contain both monomorphic and dimorphic species. The synanamorphism is often hard to discern without ontogenetic observations. Here, the spore ontogeny of *Ar. trappei* is reported from single species pot culture studies. The sporogenous hypha swelled up to a terminal sporiferous saccule and produced a lateral spore primordium on its neck. The saccule expanded fully before the spore primordium emerged. The saccule transferred its contents into the expanding spore and collapsed while wall differentiation continued inside the spore. The spore wall of *Ar. trappei* differentiated sequentially, in discrete steps, as in *Acaulosporaceae* members. In contrast, *Ar. trappei* produced a simplified spore wall in which the components differed in chemical and physical characteristics from those of the *Acaulosporaceae* members. Ontogenetic studies confirmed *Ar. trappei* to be monomorphic and producing acaulosporoid spores. The fungus is a new record to New Zealand.

Keywords AM fungi · Taxonomy · Spore ontogeny · *Archaeospora* · Myrtaceae

Introduction

During a survey of arbuscular mycorrhizal (AM) fungi, *Archaeospora trappei* was isolated abundantly from pot cultures containing soils collected under native vegetation in the North Island of New Zealand. Ames and Linderman (1976) originally described this AM fungal species as *Acaulospora trappei* (*Acaulosporaceae*) until Morton and Redecker (2001) sequenced its small subunit rRNA gene and transferred it to a new genus, *Archaeospora*, under a new family, *Archaeosporaceae*, assigning *Ar. trappei* to be its type species. The order *Glomales* (*Zygomycotina*) constituted this and other AM fungi (Morton and Benny 1990), until Schüssler et al. (2001) proposed a new, small subunit rRNA sequence-based taxonomic scheme. Schüssler et al. (2001) have placed *Ar. trappei* under a new order, *Archaeosporales*, which is one of four orders representing the new AM fungal phylum, *Glomeromycota*. The taxonomic criteria proposed by Morton and Redecker (2001) for the family *Archaeosporaceae* remain unchanged. The ability of this fungus to form AM associations with higher plants has been documented (Gazey et al. 1993; Morton and Redecker 2001).

It has been claimed that explicit usage of molecular data is vital to define the evolution, systematics and identification of AM fungal species (Morton and Redecker 2001; Redecker et al. 2000; Schüssler et al. 2001, and references therein). However, developmentally defined spore morphological data may still be a requirement, because they provide a means to record the whole range of morphological characteristics that are usually missing in most original descriptions (Dalpé and Declerck 2002; Wu and Sylvia 1993; Wu et al. 1995). The enormous genetic diversity among the AM fungi and associated taxonomic variation evidenced by Schüssler et al. (2001) substantiate the fact that complete sets of spore morphological data will have a considerable influence on the emerging molecular taxonomy and its stability.

Formulation of new taxonomic characters and concepts using spore developmental morphological criteria have been attempted, but have been limited to a small number of species [e.g. four species of *Acaulospora* (Dalpé and Declerck 2002; Schultz et al. 1999; Stürmer and Morton 1999a); one species of *Entrophospora* (Stürmer and Morton 1999a); all five known species of *Gigaspora* (Bentivenga and Morton 1995); seven species of *Glomus*

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(Morton 1996; Stürmer and Morton 1997; Kennedy et al. 1999); and eight species of *Scutellospora* (Franke and Morton 1994; Morton 1995; Stürmer and Morton 1999b)]. Even in their solely molecular signature sequence-based taxonomic revision, Morton and Redecker (2001) have sought spore ontogenetic information to reveal the synanamorphic nature of *Ar. leptoticha* (Schenck & Smith) Morton & Redecker and *Ar. gerdemannii* (Rose, Daniels & Trappe) Morton & Redecker, indicating the need for thorough morphological evaluation of *Archaeospora* spp. for correct identification. Apart from spore and root mycorrhizal morphological accounts, the monomorphic nature of *Ar. trappei* has not been established by ontogenetic evidence (Morton and Redecker 2001). In this paper, the early events of spore development and subsequent wall differentiation in *Ar. trappei* are documented with a view to expanding the taxonomic knowledge of this species, which shows wide distribution in New Zealand native bush soils.

Materials and methods

Soil samples, including roots from the surrounding vegetation, were collected from root zones of *Metrosideros excelsa* Sol. Ex Gaertn. (Pohutukawa) (*Myrtaceae*) in the coastal vegetation between Coromandel and Colville townships (Coromandel Peninsula; 36°41′S, 175°32′E), and from root zones of *Metrosideros robusta* A. Cunn. (Northern rata) which is patchily distributed in the lower Orongorongo Valley bush of the Rimutaka Forest Park (41°17′S, 174°56′E). Both sites are situated in the North Island of New Zealand. Root zones surrounding ten trees of each species were sampled. Eight, 15-cm-deep soil samples were collected from each tree at a radius of 1–3 m from the tree trunk. The soils from Coromandel Peninsula and Rimutaka Forest Park, respectively, showed the following properties: pH (1:2.5 soil:water), 5.2–6.9 and 5.0–7.6; cation exchange capacity, 21.1 and 14.9 mE/100 g; Bray-2 available P, 21.3 and 33.0 μg P/g; phosphate retention, 3.15% and 1.5%. The vegetation of the lower Orongorongo Valley bush is documented in Campbell (1984), while that of the Coromandel sampling area was dominated by *M. excelsa*, with sporadic occurrence of understorey shrubs of *Pittosporum* sp and *Hebe* sp.

The AM fungi were "trapped" in pot cultures from freshly collected untreated soils. Initially, thoroughly mixed soil samples from each site with particles of <2 mm were transferred to 250 ml plastic pots, seeded either with *M. excelsa*, *M. robusta*, or *Sorghum bicolor* and allowed to grow for 16 weeks in a greenhouse maintained at 14–21°C with a pot level photosynthetic photon flux density of 800 µmol m^{-2} s^{-1} for 16 h. The pot cultures that showed AM root colonisation were wet-sieved (Kormanik and McGraw 1982), and spores of *Ar. trappei* were collected in vials containing deionised distilled water. Eight vials were prepared, each containing about 200 spores, and stored at 4°C until further use.

After about 2 weeks, the stored spores were examined in water under a stereomicroscope and any contaminated spores were discarded (J.B. Morton, personal communication). Spores in each vial were placed directly on the root systems of a bundle of four, 2-week-old *Sorghum sudanense* seedlings that had already been raised in acid-washed, sterilised quartz sand (Morton et al. 1993). The inoculated *S. sudanense* seedlings were raised in 150 ml pots containing a 1:1 (v/v) mixture of acid-washed, autoclaved quartz sand and steamed field soil of <2 mm particle size. No pH adjustments were necessary, as the pH of the mixture did not differ substantially from that of the original field soils. These single species pot cultures were grown for 16 weeks in the greenhouse under environmental conditions similar to those specified above. They received daily sprinkles of tap water only.

Roots in the single species pot cultures were examined for AM colonisation at 6 weeks after transplanting. Those pot cultures that contained more than 20% root colonisation were retained and others discarded. Watering ceased at the end of 20 weeks and pot contents were air-dried, chopped, mixed with steamed soils of the respective study sites, and the monospecific AM isolates re-cultured in association with *S. sudanense*. Re-culturing was repeated for a second time. Each of the first and second monospecific pot culture cycles comprised 12 pots. Spores from two pot cultures of the second monospecific culture cycle were harvested at 8, 10, 12, 16 and 18 weeks after seed germination. Pot contents were wet-sieved, about 200 spores collected in deionised distilled water, and examined under a stereomicroscope equipped with a two-branched fibre optic illuminator with a light temperature of 3,200°K.

Spore colour was determined using the colour chart of Brundrett et al. (1996), in which colour is expressed in proportion to cyan, yellow and magenta (CYM). For colour determination, spores and the colour chart were simultaneously illuminated with the fibre optic light source. Intact and squashed spores were permanently fixed on microscopic slides either in a mounting medium containing 1:1 polyvinyl alcohol lactoglycerine (PVLG) and Melzer's reagent, or PVLG alone (Koske and Tessier 1983). The sub-cellular organisation at different stages of spore development was examined and photographed using an Olympus photomicroscope equipped with differential interference contrast optics. The terminology of wall types follows those of Franke and Morton (1994), Morton (1985), Walker (1983) and Walker and Vestberg (1998). Spore slides have been deposited with Oregon State University (OSC) (Corvallis, Ore.) and the Plant Diseases Division Herbarium (PDD) (Mount Albert, Auckland, New Zealand).

Results

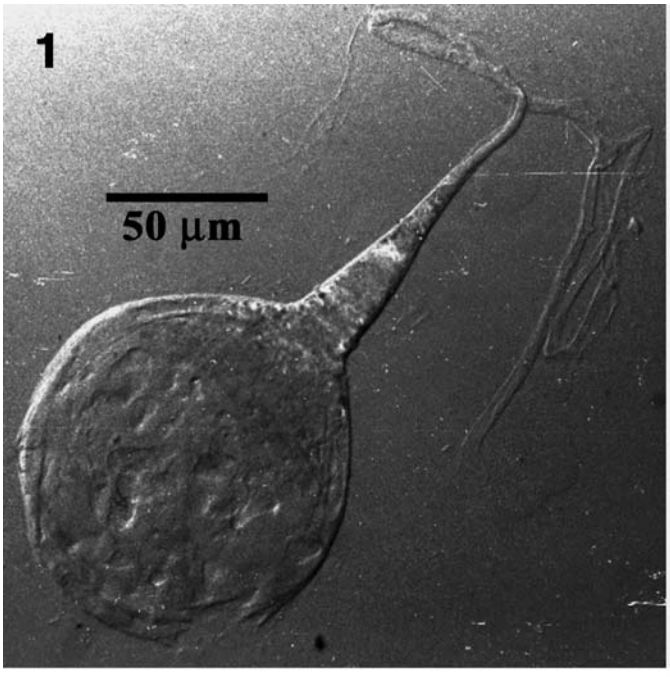
Ar. trappei sporulated profusely in association with all three host plant species—*M. excelsa*, *M. robusta*, and *S. bicolor*—originally used to trap the fungus. The spore and saccule dimensions are listed in Table 1, and they did not differ significantly among the hosts used. At least one of the two monospecific pot cultures harvested at different times of the second pot culture cycle yielded more than 20 spores. The majority of collections yielded hundreds of spores, facilitating observation and capturing features of ontogenetic progression. Pot cultures of the final harvest, i.e. 18 weeks after seed germination, also yielded spores of different stages of development, but the frequency of juvenile spores was substantially less than that of matured spores (data not shown).

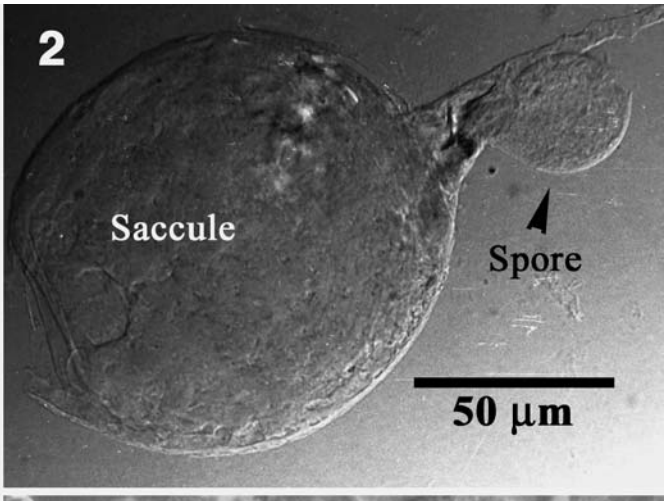
The diagnostic morphological characteristics of the species when spores were fully developed, along with previously reported quantitative data, are summarised in Table 1. The sporogenous hyphal tip expanded and ended in a single terminal "sporiferous saccule" or "saccule" (Fig. 1). The saccule contents were opaque, granular and whitish. When saccule expansion apparently ceased, a spore primordium emerged, laterally on the neck of the sporogenous hypha, at a distance from the base of the saccule (Fig. 2). The distance between the spore and saccule is given in Table 1. As the spore grew in size, the saccule "pushed" its contents through a common connection; the movement of contents could be easily visualised under a stereomicroscope by its relative position in the channel between the spore and the saccule. When the

Table 1 Comparison of essential diagnostic characteristics of fully developed spores of *Archaeospora trappei* from previous descriptions with those raised in single species pot cultures with *Sorghum sudanense* in the present study

	Present study	Original description[a]	Redescription[b]
Colour of spore	Hyaline	Hyaline	Hyaline to creamy white
Colour of saccule	Hyaline	Hyaline	Hyaline
Diameter of saccule (μm; mean of n=85)	68–101; mostly globose	50–82×42–72; sub-globose to ellipsoid or obovoid	40–48×50–72; mostly oblong, rarely globose
Diameter of spore (μm; mean of n=125)	82–95; mostly globose	42–99×42–70 globose to ellipsoid or obovoid	40–80 (mean 58); globose, sub-globose or irregular
Distance between spore and saccule (μm)	17–37.5	Not mentioned	10–30
Spore wall	3 layers; hyaline	Defined as 1.2–2.3 μm thick, single layer	3 layers; hyaline
L1			
Thickness (μm)	1.0–2.0	Not applicable	1.0–1.2
Wall type and physical appearance	Evanescent or flaky		Semi-flexible
Reaction with Melzer's reagent	None		
L2			
Thickness (μm)	0.5–1.0	Not applicable	<0.5–0.7
Wall type and physical appearance	Unit		Semi-flexible
Reaction with Melzer's reagent	None		
L3			
Thickness (μm)	1.5–3.0	Not applicable	1.3–2.5
Wall type and physical appearance	Coriaceous		Semi-flexible
Reaction with Melzer's reagent	None		

[a] Ames and Linderman (1976)
[b] Morton and Redecker (2001)





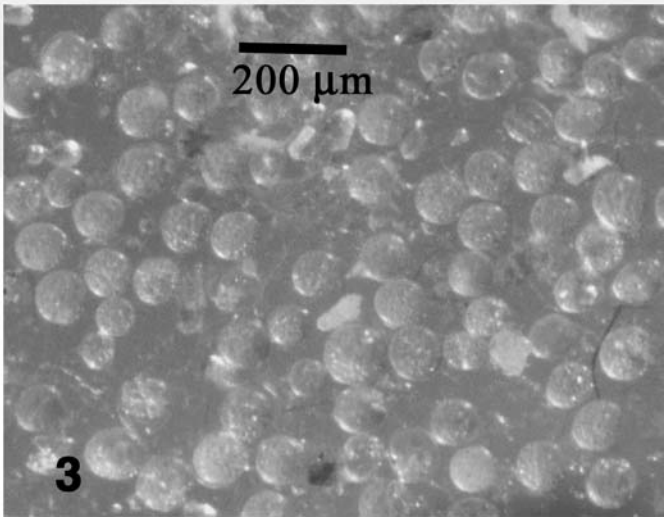
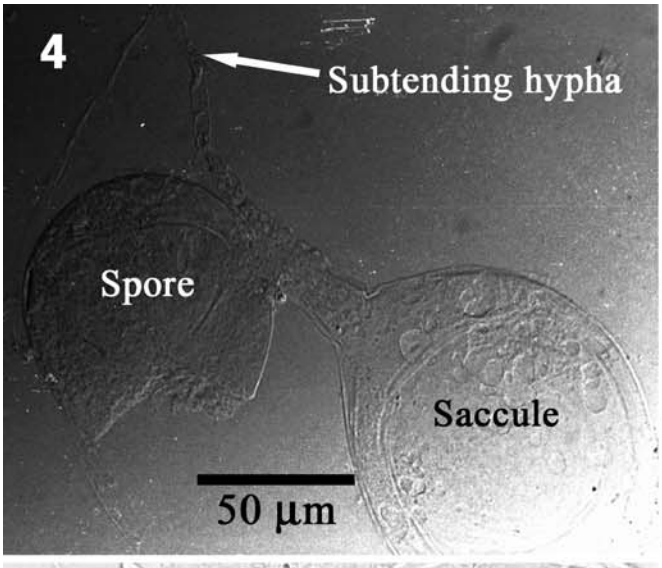
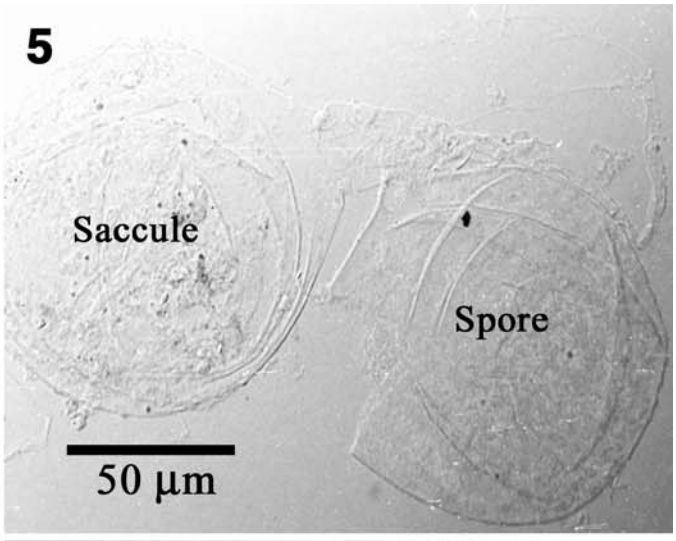


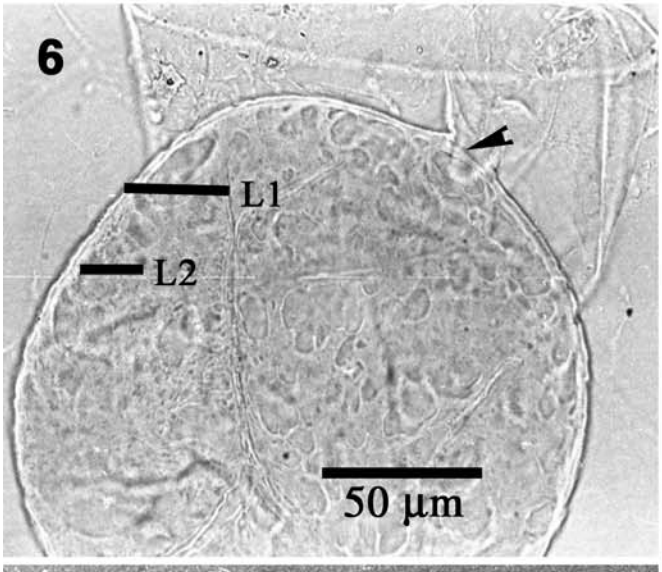
Fig. 1 Fully formed sporiferous saccule (or saccule)

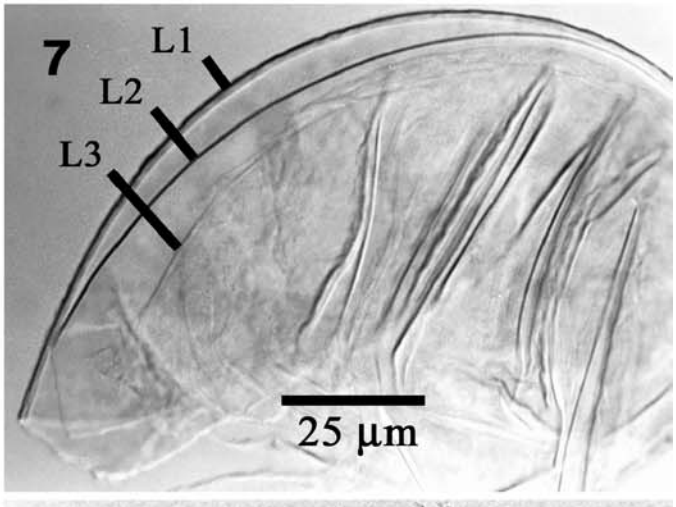
Fig. 2 Developing spore on the saccule neck

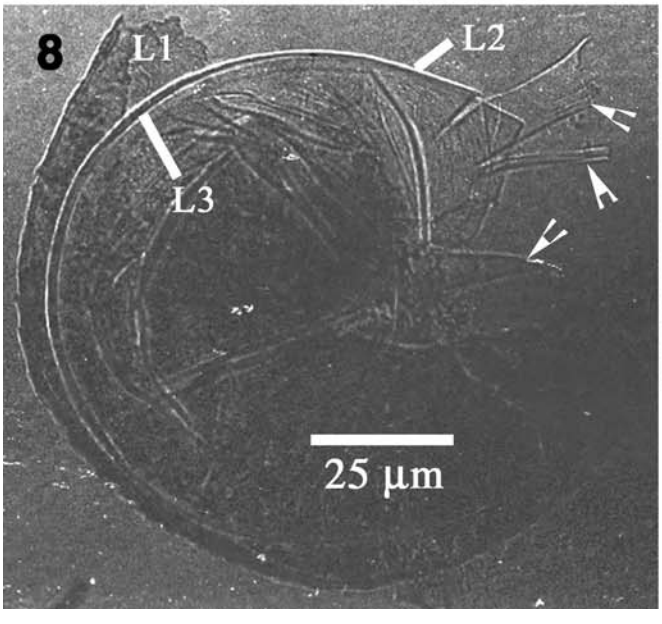
Fig. 3 Collection of intact spores at different stages of development

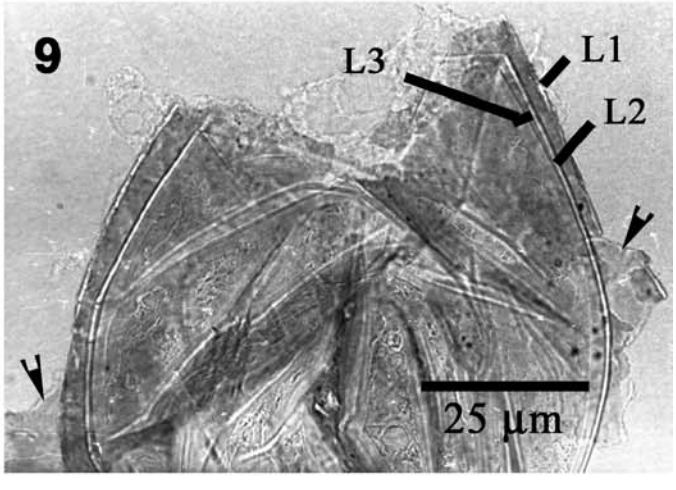

4
Subtending hypha
Spore
Saccule
50 µm


5
Saccule
Spore
50 µm


6
L1
L2
50 µm


7
L1
L2
L3
25 µm


8
L1
L2
L3
25 µm


9
L3
L1
L2
25 µm

spore was fully expanded, it was filled with the hyaline contents of the saccule, leaving it transparent. The contents appeared globular and glistening in the spore (Fig. 3), as opposed to their granular and opaque appearance in the saccule. The spore did not attach itself to the sporogenous hypha through any pedicel, but remained sessile from juvenile through to full development (Figs. 2, 4, 5). The saccule and spore shared a common wall when the spore was still developing (Figs. 2, 4, 5, 6). The common wall was hyaline, 0.5–1.0 μm thick on the saccule and <0.5 μm thick in the developing spore. As the spore expanded in volume, the outermost layer shared with the saccule, denoted L1 of the spore wall, thickened to 1.0–2.0 μm. At the next stage, an internal hyaline layer developed, occluding the connection between the spore and the saccule (Fig. 6). The L1 adhered tightly at the early phase of development (Fig. 6), but appeared to lose its integrity with spore development and became loosely adhering to L2 (Fig. 7). Similarly, L1 of some spores sloughed partially even before spore wall differentiation was completed. The L1 persisted in about 50% of the fully developed spores examined. As L2 did not attach itself firmly on to L1, and did not form a thickened ring around the point of saccule attachment, cicatrixes were not visible in these spores when the L1 had sloughed off. The L2 of the spore wall of *Ar. trappei* never thickened to >1.0 μm, was permanent and pigmentation was absent. The physical appearance of L2 resembled a unit wall after some differentiation (sensu Walker 1983; Figs. 7, 8).

The developing spore produced a third, final layer inside the spore that emerged as a thin membrane (Fig. 7). It gradually thickened to 3.0 μm towards maturity (cf. Figs. 7, 8, 9). The L3 was extensively folding upon crushing, but non-elastic, perhaps transforming into a "coriaceous" wall (Fig. 9). Thus, the spore wall is comprised of three components (sensu Walker and Vestberg 1998), and their gradual development of different qualitative physical properties was apparent with maturity (e.g. evanescent L1, unit L2, coriaceous L3; (Figs. 8, 9; Table 1). None of the wall components was dextrinoid with Melzer's reagent.

Fig. 4 Thickening of juvenile spore wall with increasing spore size

Fig. 5 Thickening of juvenile spore wall with increasing spore size

Fig. 6 Synthesis of the second layer (L2) resulting closure of the pore between saccule and the spore (*arrow*)

Fig. 7 Origin of the third layer (L3) as a thin, folding membrane

Fig. 8 The gradual thickening of L3, and the "unit" wall appearance (*arrows*) of fully differentiated L2

Fig. 9 Spore showing all three fully differentiated wall layers with thickened, "coriaceous" appearance and folding in L3, and sloughing of L1 (*arrows*)

Specimens examined

PDD 72203–72205 assigned as *Acaulospora trappei*; OSC 76194–76199 assigned as *Acaulospora trappei*.

Discussion

The single species pot cultures produced only acaulosporoid saccule and spore primordia and continued to differentiate as shown in Figs. 1–9, confirming that *Ar. trappei* could be monomorphic in origin. Morphological evidence to substantiate formation of a pedicel between the spore and the saccule since youth, which facilitated proper identification of the species, was lacking. *Ar. trappei* has not previously been reported in New Zealand (Hall 1977; Mosse and Bowen 1968), nor was it found in any previous herbarium collections in PDD, Auckland. Thus, the present account extends the geographic distribution of the species further and adds a new record to New Zealand. *Ar. trappei* sporulated abundantly when associated with dicot as well as monocot plant species indicating that it may not show substantial host preferences in completing its life cycle. Results also indicated that freshly collected spores may be able to colonise *S. sudanense* when used in numbers as high as 200 spores per pot, without requiring prolonged storage. This lack of dormancy for spore germination in the basionym, *Acaulospora trappei*, has been documented by Gazey et al. (1993).

The diameter of spores and saccules, and wall thicknesses measured for *Ar. trappei* in this study showed slightly higher values than those reported by Ames and Linderman (1976) and Morton and Redecker (2001). Similar differences have been found to occur between AM fungal isolates of different geographic origin (Franke and Morton 1994; Morton 1995; Schultz et al. 1999), perhaps due to factors governed by type of host plant and soil, and substrate and experimental conditions. As in most developmentally defined *Acaulospora* spp., the saccules were always found to be fully inflated before formation of the spore primordia on the sporogenous hyphal necks. To date, the only exception to this trait is the simultaneous development of spore and saccule in *Acaulospora rehmii* (Dalpé and Declerck 2002). As the spore grew in volume, a gradual movement of saccule contents was evident in *Ar. trappei*, whereas Dalpé and Declerck (2002) could not discern such cytoplasmic movement in *A. rehmii* in root organ cultures. The saccule may act merely as a reservoir in both developmental modes, and further inference on its functional significance from ontogenetic studies becomes impossible.

The wall components (sensu Walker and Vestberg 1998) developed in a succession, where full differentiation of the outer layers appeared to be the precursor to the origin of the subsequent inner layer. This observation is in agreement with findings on other developmentally defined AM species (Bentivenga and Morton 1995; Dalpé and Declerck 2002; Franke and Morton 1994; Kennedy et

al. 1999; Morton 1995, 1996; Morton and Redecker 2001; Schultz et al. 1999; Stürmer and Morton 1997, 1999a, 1999b). Wall differentiation proceeded in discrete phases, as in *Acaulospora* spp. (Dalpé and Declerck 2002; Schultz et al. 1999; Stürmer and Morton 1999a), but differed from the latter by the absence of tightly adhering components (or wall "groups"; sensu Walker 1983) and inner flexible layers (sensu Morton 1995). The wall components in *Ar. trappei* were single, did not show any integration into groups and adhered loosely when fully differentiated.

The physical characteristics of L1 resembled the definitions given to an evanescent wall (sensu Walker 1983). L1 may differ in chemical composition from similar surface layers in other *Archaeospora* spp., *Paraglomus* spp. and certain *Glomus* spp. in that it is neither dextrinoid with Melzer's reagent nor mucilagenous (Pfeiffer et al. 1996; Kennedy et al. 1999). The lack of dextrinoid reaction on the surface layer resembles *Acaulospora* spp. Nevertheless, L1 was thicker in *Ar. trappei* than in some *Acaulospora* spp. (Schultz et al. 1999; Stürmer and Morton 1999a), retained even in a considerable number of older spores, and can hence be considered semi-permanent.

Morton and Redecker (2001) designated the inner layers of *Ar. trappei* as semi-flexible. However, from origin through to full differentiation, the physical characteristics of L2 in the present study resembled that of a unit wall (Figs. 6, 7, 8, 9; sensu Walker 1983). Similarly, even though it folded extensively on crushing, whether or not the L3 is semi-flexible as mentioned by Morton and Redecker (2001) was impossible to determine in the present study. The L3 originated as a thin membrane (Fig. 7) and progressively thickened to 1.5–3.0 μm with spore age. Therefore the definition "coriaceous" (Walker 1983) appeared to be more appropriate in defining the physical nature of this layer in *Ar. trappei*.

The endosporic development of the L2 of the spore wall is shared commonly by spores of *Ar. trappei* and *Acaulospora* spp. (Dalpé and Declerck 2002; Schultz et al. 1999; Stürmer and Morton 1999a). The surface layer (L1) and the inner L2 are tightly adherent in the *Acaulospora* spp. and are therefore components of a single entity, namely the spore wall. The outer layer (L1) decomposes rapidly in *Acaulospora* spp.; the L2 thickens by lamina formation and renders mechanical support to the matured spore. Similarly, the non-adherent (or sometimes loosely adherent) L1 in *Ar. trappei* does not appear to be the major structural component of the spore wall as it sloughs off with age in at least one-half of the spore population. Instead, the L2 appears to provide structure and mechanical support to the spores of *Ar. trappei*, particularly at maturity.

Apart from major differences in physico-chemical characteristics, the simplistic wall organisation is unique to *Ar. trappei*. According to Morton and Redecker (2001), *Ar. trappei* is an ancestral clade, in which only the acaulosporoid nature of spore formation is shared commonly by *Archaeosporaceae* and *Acaulosporaceae*. The relatively more complex subcellular organisation in members of the latter family is claimed to be evolutionarily more advanced than the simplified wall organisation in the former (Schüssler et al. 2001). According to molecular analysis, such contrasting phenotypic variation has been found to underpin greater genetic variability (Morton and Redecker 2001). For an asexually reproducing group of organisms, even subtle variation exhibited in the mode of spore formation, (i.e. whether acaulosporoid or glomoid), and simplicity in wall differentiation as illustrated in the present study, bear taxonomic significance.

Recent taxonomic revisions have proven molecular analyses to be indispensable in recognising phylogenetic relationships among the AM fungi. For example, Schwarzott et al. (2001) argue that future emendation of *Archaeospora* is necessary as molecular evidence suggests paraphyly in the genus. Furthermore, Schüssler et al. (2001) have shown that the AM fungi themselves are a somewhat ancient, monophyletic group that has been brought under a new phylum, *Glomeromycota*, by these authors. The molecular information could be used as a framework for more insightful re-evaluation of the morphological variation of existing species and discovery of new species. In this respect, ontogenetic and subcellular morphology of AM spores add a new dimension to systematics and identification. They furnish observable characters that signify differences between taxa. Unambiguous identification of taxa into species is becoming ever more important in experiments and diversity studies as many more scientific investigations of the role of these fungi in ecosystem processes await. A standard system of morphological characterisation with emphasis on spore developmental criteria will strengthen the molecular findings, at the same time encouraging researchers who have no ready access to molecular tools to correctly classify AM fungi and document their diversity in different parts of the world.

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