

Two new lignicolous species of *Nitschkia* from Argentina

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Abstract: Two species of *Nitschkia* are described from bark and wood of a legume shrub, native to the semiarid regions of Argentina. *Nitschkia campylospora* is characterized by asci with a variable number of ascospores, mainly 16, and curved ascospores; hairy ascomata and large ascospores are two distinct features of *Nitschkia pilosa*. The new species are compared with most similar species. A key to *Nitschkia* species is provided with the inclusion of comments on those from Argentina and neighboring countries.

Key words: Munk pores, Nitschkiaceae, systematics

INTRODUCTION

During biodiversity studies on micromycetes growing on bark and decorticated wood of a native legume shrub from Argentina, *Geoffroea decorticans* (Gill. ex Hook & Arn.) Burk., two species belonging to *Nitschkia* G.H.Oth ex Karst. were found. *Nitschkia* is characterized by turbinate ascomata that become cupulate upon collapse, a coriaceous peridium composed of brown, thick-walled, angular cells with Munk pores, unitunicate evanescent asci lacking an apical apparatus, and diverse ascospores, both in number and form.

The two species differ from the hitherto published species and here are described as new. A key to species of *Nitschkia* is provided. Some additional information on species from continental southern (semi-arid and subtropical) South America is included.

MATERIALS AND METHODS

Ascomata were mounted in water, in 3% KOH, in 1% aqueous phloxine and in lactophenol. Ascomata were hand-sectioned with a razor blade. Measurements (at least 30 of each character) were made of material in water. Asci were ob-

served with 1% Aq. Congo Red, Janus Green, Melzer's Reagent and 5% calcofluor (Romero and Minter 1988). Images were taken using bright field (BF), fluorescence (FL) and phase contrast (PC) with a Zeiss Axiolab microscope. The photographic plates were produced electronically with Adobe Photoshop 7.0.

Nitschkia campylospora Bianchinotti sp. nov.

FIGS. 1–10

Ascomata superficialia, dense gregaria vel caespitosa, nigra, subglobosa, sicca cupulato collapsa, tuberculata minutissime rugulosis, tomento castaneo iridescenti preadita, non-ostiolata. Asci clavati, multispori, (4–)16(–25) sporati. Ascosporae allantoideae, curvata vel lunata, (8.5–)9.5–13 × (1.5–)2–2.5 μm, hyalinae, laeves, uniseptatae, guttulatae, in asci irregulariter dispositae. In ligno decorticato emortuis.

Specimens examined. ARGENTINA. BUENOS AIRES: Partido Coronel Rosales, Villa Maio, Ruta Provincial 229 km 9, on decorticated wood of *Geoffroea decorticans*, 30 May 1990, *Bianchinotti 115* (HOLOTYPE: BBB); same locality and substratum, 12 Oct 2002, *Bianchinotti 171, 172* (BBB), *173* (ISOTYPE: F).

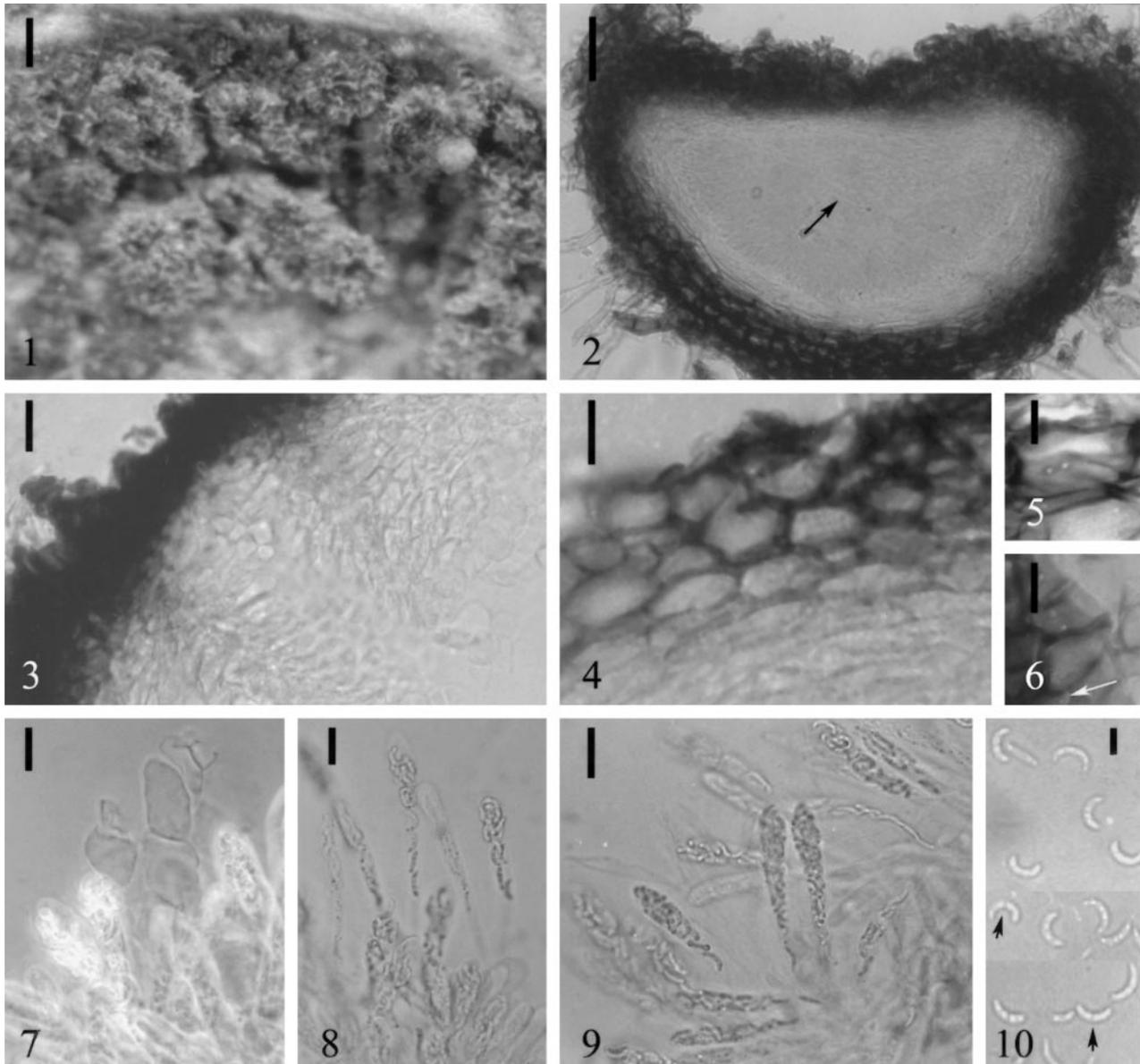
Ascomata superficial on decorticated wood, gregarious, subglobose, cup-shaped when collapsing, 200–400 μm diam, non papillate, non ostiolate, surface minutely warted, black. Ascomata surrounded by an iridescent light brown tomentum, composed of undulating, richly branched, thick-walled, dark hyphae, 7–12 μm diam; subiculum more abundant in immature ascomata. Peridium pseudoparenchymatous comprising two layers; external layer of large, polyhedral, thick-walled, heavily pigmented, dark brown cells possessing Munk pores. Inner layer comprising pale, thin-walled cells; cells surrounding the interior of the locule tangentially compressed, with acute ends. Quëllkörper short and dome-like. Asci clavate, more than 100 μm long, unitunicate, thin-walled, evanescent, with (4–)16(–25) ascospores irregularly disposed in each ascus; apex simple. Ascospores allantoid, strongly curved, (8.5–)9.5–13 × (1.5–)2–2.5 μm, hyaline, smooth, 1-septate, with two large guttules in each cell.

Anamorph. None known.

Etymology. From Greek χαμπυλοζ: curved, referring to shape of ascospores.

Nitschkia pilosa Bianchinotti sp. nov. FIGS. 11–20

Ascomata superficialia, sparse gregaria, subglobosa vel



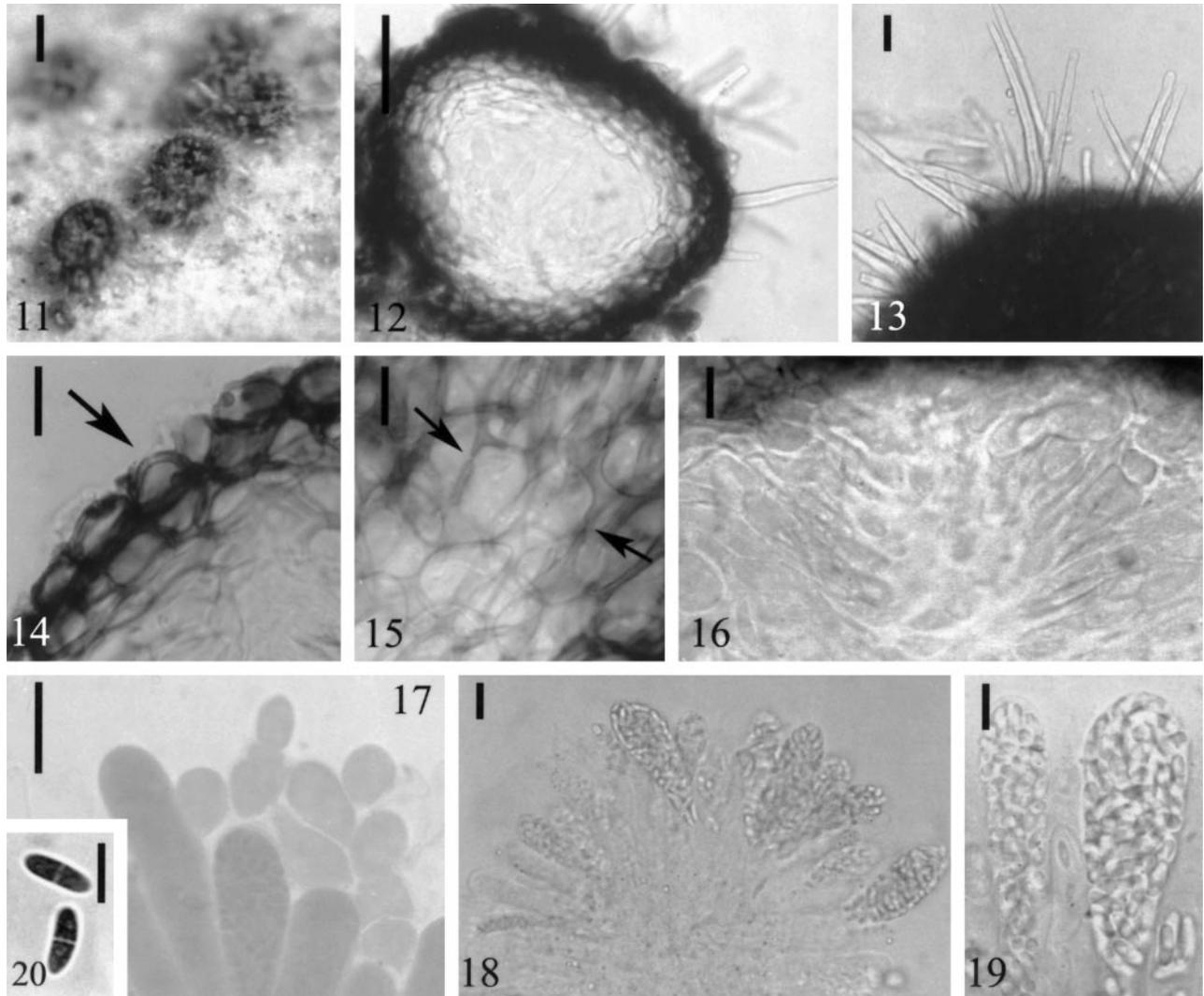
FIGS. 1–10. *Nitschkea campylospora*. 1. Ascomata on the substratum. 2. Longitudinal section through ascoma showing Quëllkörper (arrow). 3. Detail of Quëllkörper zone. 4. Longitudinal section through ascomatal wall. 5, 6. Munk pores. Arrow points to a typical pore surrounded by a ring-shaped thickening of the wall. 7. Cellular remnants among immature asci (PC). 8, 9. Mature asci with irregular number of ascospores. 10. Ascospores. Note the median septum (arrows). Scale bars: 1 = 100 μm , 2–3 = 25 μm , 4–6 = 10 μm , 7–9 = 20 μm , 10 = 10 μm .

turbinata, 125–380 μm diametro, sicca cupulato collapsa, pilis hyalinis septatis ornata, fusco-atra vel atra. Asci oblongo clavati, 75–120 \times 15–35 μm , stipitati, apice simplici, ca 64-spore. Ascosporeae suballantoidea vel allantoidea, (6.5–)9.4–11(–12) \times (2.5–)3–4(–5) μm , hyalinae, laeves, uniseptatae. In cortice *Geoffroea decorticans* (Hook. & Arn) Burk. Argentina.

Specimens examined. ARGENTINA. BUENOS AIRES: Partido Coronel Rosales, crossroads between Ruta Nacional 3 and Ruta Provincial 229, bark of *Geoffroea decorticans*, 22 May 1991, *Bianchinotti 118* (HOLOTYPE: BBB); same collecting information, *Bianchinotti 116, 117* (ISOTYPES: F);

Partido Bahía Blanca, Campo Tarantino, 38°40'S, 62°18', bark of *Geoffroea decorticans*, 1 Sep 1986, *Bianchinotti 7, 50, 52* (BBB); same locality and substratum, 25 Dec 1986, *Bianchinotti 109, 110* (BBB); 10 April 1987, *Bianchinotti 86* (BBB); 7 Jul 1987, *Bianchinotti 83, 96, 97, 98, 99* (BBB).

Ascomata superficial on inner bark, scattered to gregarious, subglobose to turbinate, collabent on drying, 125–380 μm diam, with a short papilla and a well developed sterile base, non ostiolate, surface minutely warted, hairy, dark brown to black. Hairs subcylindrical, septate, to 150 μm long, 4–10 μm at base,



FIGS. 11–20. *Nitschkia pilosa*. 11. Ascomata on the substratum. 12. Longitudinal section through ascoma. 13. Ascomatal hairs. 14. Longitudinal section through ascomatal wall. Arrow indicates a Munk pore in the exterior wall. 15. Tangential section through ascomatal wall showing Munk pores connecting cells (arrows). 16. Detail of Quëllkorper zone. 17. Immature asci. Note cellular remnants among them. 18. Young and mature asci. 19. Detail of mature asci. 20. Mature ascospores stained with Aq. Phloxine. Scale bars: 11 = 200 μm , 12 = 50 μm , 13 = 20 μm , 14–20 = 10 μm .

thick-walled, subhyaline to pale brown; thinner and paler towards the acute or rounded ends, base cylindrical to subglobose. Peridium ca 30 μm wide except up to 80 μm wide in the basal portion; composed of two regions, external region composed of 5–7 layers of large, 7–15 μm diam, polyhedral, thick-walled, heavily pigmented, dark brown cells; cells paler and thinner-walled toward the interior, organized in more or less vertical rows in the basal portion, with simple Munk pores. Internal region composed of 3–4 layers of thin-walled, hyaline, tangentially compressed cells with acute ends, 2–3 μm wide. Quëllkorper short and dome-like. Asci oblong to clavate, 75–120 \times 15–35 μm , unitunicate, thin-walled, evanescent, 64-spored, apex simple lacking any visible apparatus. Ascospores

subballantoid to allantoid, (6.5–)9.5–11(–12) \times (2.5–)3–4(–5) μm , hyaline, smooth, 1-septate.

Etymology. From Latin *pilus* (hair), referring to those that cover the ascomata.

Anamorph. None known.

DISCUSSION

Several researchers (Fitzpatrick 1923, 1924, Nannfeldt 1975a, b, Sivanesan 1974, Subramanian and Sekar 1990) have studied members of Nitschkiaceae. A close relationship between this family and Lasiosphaeriaceae has been postulated several times (Arx 1981, Barr 1990, Carroll and Munk 1964, Sivanesan 1974, 1975, 1978). The number of genera in the fam-

ily and the criteria for their separation greatly vary, but the broader generic concept adopted by Nannfeldt (1975b) is preferred until molecular studies allow more accurate decisions.

Most *Nitschkia* species are saprobic on branches or decorticated wood, and a few are hyperparasitic on fungi. Typically their small nonostiolate ascomata look like sessile apothecia when dry, and are commonly surrounded by a subiculum. The tomentum in *N. campylospora* is typical of the genus, but ascumatal hairs as those found in *N. pilosa* have not been reported. These hairs are similar to those described in some species of *Lasiosphaeria* Ces. & De Not. (e.g., *L. strigosa* [Albert. & Schw.] Sacc. and *L. stuppea* Ellis. & Ev., as illustrated by Seaver 1912) and resemble those described in *Nitschkiopsis* Nann. & R.Sant., a lichenicolous member of Nitschkiaceae (Nannfeldt 1975b).

The ascumatal peridium is pseudoparenchymatous, composed of two distinct layers. One striking feature is the presence of Munk pores in the cells of the outer layer. Typically one per cell, they are ca 1 μm diam and surrounded by a ring-shaped thickening of the wall. They are not always obvious, and their number varies depending upon the species and developmental stage (Nannfeldt 1975b). In *N. campylospora*, the conspicuous pores are typical of the genus and two per cell frequently can be observed. In *N. pilosa*, ascumata of different ages show pores that are simpler, appearing merely as circular thinning of the wall. One or more pores could be observed both in the common wall between adjacent cells and in the exterior wall as well. They resemble the simple pores described in *Lasiobertia* Sivan., a genus similar to *Oxydothis* Penz. & Sacc., with a rather uncertain taxonomic position in the opinion of Eriksson and Santesson (1986), that was considered a member of the Lasiosphaeriaceae by Barr (1990) and later transferred to the Hyponectriaceae by Hyde (1993).

Peridial pores were considered for a long time unique to members of "Coronophorales", but Cannon (1995) clearly showed that they are more widespread among ascomycetes than previously thought. A few more examples can be added to those mentioned by Cannon (1995)—*Lasiobertia africana* Sivan., *Lasiosphaeriella dennisii* Sivan. (Sivanesan 1975), *Lasiosphaeriopsis salisburyi* D. Hawksw. & Sivan. (Hawksworth 1980), *L. stereocaulicola* (Lindsay) O. E. Erikss. & R. Sant. (Eriksson and Santesson 1986), and *Melanospora sphaerodermoides* Grove (Shoemaker and Smith 1970).

The function of Munk pores is unknown. A hypothetical explanation is that they could help in transporting moisture to the interior of the ascoma, which is diminished by the heavily pigmented and thick-

ened walls, thus enhancing the discharge of ascospores from the entirely closed ascumata. Observations made after wetting ascumata of the two species described here were similar to those reported by Nannfeldt (1975b) for *N. parasitans*, in which spores were extruded in a slimy mass from the top of ascumata that had been kept in a moist chamber. It is thought that the cell mass known as Quëllkörper, which may become swollen, plays an important role in ascumata discharge. In addition, Vujanovic (2002) recently has reported the important increase of Quëllkörper cells size in water.

Asci in the two species described here are typical of the family, being long claviform, unitunicate, thin-walled and evanescent. No evidence of any thickening or apical structures could be found in bright field or phase contrast observations made with Congo Red, Janus Green or fluorescence microscopy using calcofluor. True paraphyses are lacking. Instead, cellular remnants corresponding to the "restes des cellules nourricières" described by Parguey-Leduc (1966) were observed among immature asci.

Asci in most *Nitschkia* species are octosporous; rarely they are 4-spored and in a few they are polysporous. The mostly 16-spored asci of *Nitschkia campylospora* and the particular shape of the ascospores distinguish it from other species. Although variation in number of ascospores in asci of the same ascumata has been reported previously in *Nitschkia affinis* (H. & P. Syd.) Nannf. and in *N. leonensis* (Sivan.) Nannf., the most frequently observed numbers of ascospores in both species are 32 and 25. The ascospores strongly resemble those of *Acanthonitschkea argentinensis* Speg., which is characterized by dark bristles on the subiculum and ascumata, 8-spored asci, and smaller ascospores (6–8 \times 2 μm) (Spegazzini 1908).

Asci in *N. pilosa* and *N. molnarii* Funk are both 64-spored, but the latter has glabrous ascumata and smaller ascospores (Funk 1979). In addition, ascospores of *N. pilosa* are much wider (up to 5 μm) than those in all other polysporous species described to date.

An unidentified stromatic coelomycete producing hyaline microconidia in phialidic conidiogenous cells occasionally was observed growing close to the ascumata of *N. campylospora*. Funk (1979) considered a pycnidial fungus with Munk pores in the outer wall cells to be the anamorphic state of *N. molnarii*. Vujanovic (2002) found similar pycnidia in the same pustule as *N. parasitans* var. *mijuskovicii* Vujanovic. This circumstantial evidence would suggest the pycnidial states belong in the respective life cycles. However, because no *Nitschkia* species has been grown in

27. Asci (4-) 16 (-25) spored. Ascospores allantoid, strongly curved, $(8.5-9.5-13 \times (1.5-2-2.5 \mu\text{m}))$ 1. *N. campylospora*
- 27'. Asci mostly 32-spored. Ascospores otherwise 28
28. Ascospores suballantoid, hyaline, $7-9 \times 1.5 \mu\text{m}$ *N. callista* (Berk. & Curt.) Nannf.
- 28'. Ascospores ovoid, fusiform to ellipsoidal, hyaline or colored, wider 29
29. Ascospores ovoid, hyaline, $5-6 \times 3 \mu\text{m}$ *N. multiasca* (Pat. & Gaill.) Nannf.
- 29'. Ascospores ellipsoidal, fusiform or ovoid, hyaline becoming light brown, $6-10 \times 3-4 \mu\text{m}$ *N. variabilis* Romero & Samuels Argentina. Described from decorticated wood of *Eucalyptus viminalis*. Known only from type locality (Romero and Samuels 1991).
30. Ascumata glabrous, seated on a pseudoparenchymatous subiculum 31
- 30'. Ascumata hairy, subiculum absent. Ascospores suballantoid to allantoid, $(6.5-9.5-11(-12) \times (2.5-3-4(-5) \mu\text{m}))$ 2. *N. pilosa*
31. Asci (25-) 32-spored. Ascospores subcylindrical to ellipsoidal, $5.5-7 \times 1-1.5 \mu\text{m}$ *N. affinis* (H. & P. Syd.) Nannf.
- 31'. Asci 64-spored. Ascospores allantoid, $6-8 \times 1.5-2 \mu\text{m}$ *N. molnarii* Funk

Excluded: *Nitschkia archeri* (Berk.) Subram. & Sekar and *N. didyma* (Speg.) Subramanian & Sekar. Type material of both species were first examined by Fitzpatrick (1923) and then by Nannfeldt (1975b) who considered them as belonging to *Thaxteria* Sacc. because of their large ostiolate ascumata, which not become characteristically cupulate, the asci with truncate tips with thickened walls and small rings and the broadly subcylindrical, geniculate rather allantoid, 3-septate, dark brown ascospores.

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