Two new species of Arthopyrenia from Italy

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Abstract: Arthopyrenia tuscanensis Coppins & S. Ravera and A. coppinsii S. Ravera are described as new to science. They share the characteristic tadpole-shaped ascospores with a conical lower cell, but are otherwise comparable to A. salicis A. Massal.

Key words: Arthopyrenia, Arthopyreniaceae, Italy, Naetrocymbe, Naetrocymbaceae, new species

Introduction

Arthopyrenia A. Massal. 1852 is a genus of pyrenocarpous lichens belonging to the family Arthopyreniaceae Walt. Watson 1929 placed by Eriksson *et al.* (2003) in "Dothideomycetes *et Chaetothyriomycetes* incertae sedis".

The genus, as defined by many authors such as Zahlbruckner (1921, 1926), was an assemblage of lichenized and non-lichenized ascomycetes with perithecioid ascomata and bitunicate asci. The family has been recently re-arranged by Harris (1995). The nonlichen forming species characterized by short-celled paraphyses with refractive bodies near the septa, obpyriform asci with a distinctive apical region lacking a nasse and short rod-shaped microconidia, have been included in the new family Naetrocymbaceae Höhnel ex R.C. Harris. Consequent from this has been the recombination of a few common species, such as Naetrocymbe punctiformis (Pers.) R.C. Harris, and a narrower re-definition of the Arthopyreniaceae. Aptroot (1998, 2002), however, disagreed with the importance of the hamathecial tissues as a valuable character in Arthopyrenia s. lat. as reported by Harris (1973, 1975, 1995), Tucker & Harris (1980) and Coppins (1988), and considered subdivision at the generic level unnecessary. In Aptroot's opinion, this cosmopolitan genus should be considered in its original wide concept i.e. characterized by branched pseudoparaphyses, which may disappear, and sole-shaped ascospores. Nevertheless, Eriksson *et al.* (2003) have accepted the *taxa* proposed by Harris (1995).

During a survey of Italian *Arthopyrenia*, two new species characterized by 2-celled, more or less tadpole-shaped ascospores have been discovered and are described below. Specimens of these two species have been collected in Italy more than once over a period of several years.

Material and Methods

The study is based primarily on specimens from Herbarium lichenum—Herbarium Universitatis Senensis (SIENA), and on collections from Tuscany made by B. J. Coppins, P. W. James and F. Rose in 1985 deposited in the Herbarium of the Royal Botanic Garden Edinburgh (E).

The morphology of the specimens was examined by standard microscopic techniques. Hand-cut sections and squash preparations were examined in water and in 10% aqueous solution of KOH; detailed observations of asci were made on sections of perithecia mounted in toluidine blue O (TBO) (0.05%) and gently squashed. Measurements of hamathecial filaments and ascospores (50 from each species) were made at × 1000 magnification, those of entire asci were made at × 500 magnification.

The phytoclimatic areas and the altitudinal zones in Italy were obtained from ITALIC (Nimis 2003).

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The Species

Arthopyrenia tuscanensis Coppins & S. Ravera, sp. nov.

Species corticola, thallus endophloeodes. Ascomata perithecioidea, subglobosa, $0.09 - 0.23 \times 0.09 -$ 0.11 mm; involucrellum crassum, clypeatum, 20-25 µm latum, textura intricata; excipulum angustatum; sine pseudoparaphysibus distinguibilibus, sed periphyses ramosae, $5-10 \times 1-1.5 \,\mu\text{m}$. Asci fissitunicati, obclavati-obpyriformes, $38-55 \times 12-14 \,\mu\text{m}$, octospori; endotunica apicem versus sensim incrassata, poro in sectione optica rotundato; parietes omnium cellularum substantia 'toluidine blue' adiuvante colore blue reagentes, structura obconica in tholo asci eadem substantia adiuvante intensius reagens sed indistincta est. Ascospores hyalinae, laeves, sine epispora, 11.7- $14.8 \times 3.6-4.9 \,\mu\text{m}$, bicellulares, eguttulatae, ad septum valde constrictae; cellula superior brevis, latitudine cellulam inferiorem superans; cellula inferna conica, elongata.

Typus: Italy, Tuscany, Siena, between Gaiole and Radda in Chianti, on twigs of *Quercus pubescens* L., 350 m alt., 17 April 1996, *S. Loppi, E. Putortì & C. Signorini*, 2039 (Herbarium lichenum—SIENA holotypus).

(Figs 1A–E)

Thallus endophloeodal, inconspicuous, probably non-lichenized.

Ascomata perithecial, $0.09-0.23 \times 0.09-$ 0.11 mm, black, subglobose, circular or ellipsoid, numerous, scattered, superficial. Ascomatal wall of textura intricata, black, not continuous below the hamathecium; involucrellum dark reddish brown, c. 20-25 µm thick, intermixed with dark hyphae c. $3 \,\mu m$ thick, amorphous pigment localized in the cell wall; excipulum c. 7-10 µm and brown, at the base hyaline, very thin to $5 \,\mu m$ thick and scarcely discernible; the wall pigment remains brown in K. Hamathecium, paraphyses absent, periphysoids branched, $5-10 \times 1-1.5 \,\mu\text{m}$ wide, septate. Asci bitunicate, arising from the subhymenial zone at the base of the centrum, inclined towards the ostiole, obclavate-obpyriform, $38-55 \times$ 12–14 µm, dehiscence typically fissitunicate; endotunica gradually thickened to the apex, ocular chamber rounded, obconical structure in tholus reacting to toluidine blue (tholus type "2", following Grube, 1993). Ascospores $11.7-14.8 \times 3.6-4.9 \,\mu m$ [average (\pm SD): 12·8(\pm 0.8) × 4·1(\pm 0·4),

length/breadth ratio $3 \cdot 2(\pm 0 \cdot 4)$ (n=50)], 8 per ascus, colourless, 1-septate, nonguttulate, constricted at the septum with a short upper cell $5 \cdot 0 - 6 \cdot 6 \times 3 \cdot 3 - 4 \cdot 9 \mu m$ [average (\pm standard deviation): $5 \cdot 6(\pm 0 \cdot 4) \times 4 \cdot 1(\pm 0 \cdot 4)$, length/breadth ratio $3 \cdot 2(\pm 0 \cdot 4)$ (n=50)] and an elongate pointed lower cell (tadpole-shaped) $5 \cdot 8 - 8 \cdot 0 \times 2 \cdot 7 - 4 \cdot 4 \mu m$ [average (\pm SD): $6 \cdot 6(\pm 0 \cdot 5) \times 3 \cdot 5(\pm 0 \cdot 4)$, length/breadth ratio $1 \cdot 9(\pm 0 \cdot 2)$ (n=50)], markedly unequal, mature spores often showing a slight median constriction in the lower cell; perispore indistinct.

Pycnidia not observed.

Etymology. The epithet "tuscanensis" refers to the Italian administrative region ("Toscana", Tuscany) from where all the specimens were collected.

Ecology and habitat. The new species grows on smooth bark of young deciduous trees and on twigs, accompanied by *Arthonia punctiformis* Ach., *Mycomicrothelia confusa* D. Hawksw. and *Naetrocymbe punctiformis* (Pers.) R.C. Harris.

Distribution. Known from the submediterranean belt of the humid submediterranean phytoclimatic region in Tuscany (Fig. 2).

Notes. Arthopyrenia tuscanensis characteristically lacks pseudoparaphyses, a feature shared only with mature ascomata of Arthopyrenia salicis A. Massal. among European species of the genus. Nevertheless, these two species differ in several respects (Table 1). In practice, A. tuscanensis can be easily distinguished in squash preparations by its characteristic ascospores with a conical lower cell, a feature not shared by A. salicis (Coppins 1988; fig. 1D & E).

Additional specimens examined. Italy: Tuscany: Grosseto, 44 km ENE of Grosseto, Mount Amiata, 42°54'N 11°40'E, 1100 m alt., on bark of young Castanea sativa L., in old chestnut wood, 1985, B. J. Coppins 12325, P. W. James & F. Rose (E); Lucca, road between Bagni di Lucca and Montefegatesi, on bark of young Castanea sativa L., 1985, B. J. Coppins 12161, P. W. James & F. Rose (E).

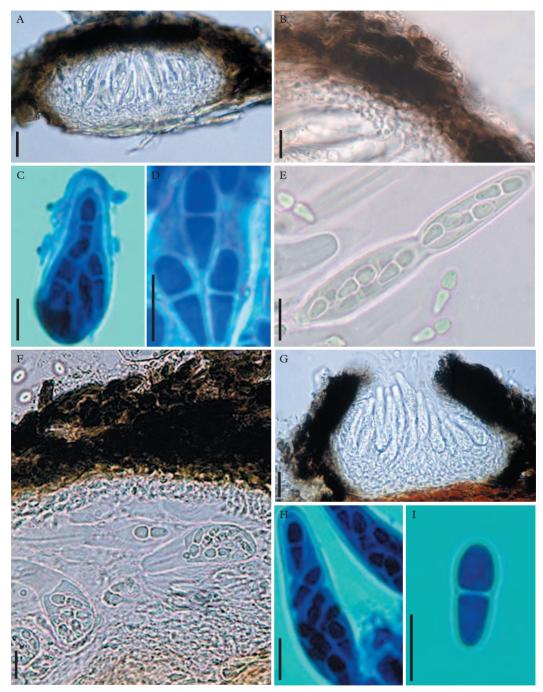


FIG. 1. Arthopyrenia tuscanensis (A–E) and Arthopyrenia coppinsii (F–I). A & G, vertical sections of perithecia in 10% KOH; B & F, showing hamathecium and K – excipulum and involucrellum (in KOH); C & H, bitunicate asci and tholus in 0.05% TBO; E, showing fissitunicate discharge and tadpole-shaped ascospores (in KOH); D & I, ascospores (in TBO). Scales: A, G=20 µm; B–F, H & I=10 µm.



FIG. 2. Distribution of Arthopyrenia tuscanensis (\blacksquare) and A. coppinsii (\bullet) in Italy.

Arthopyrenia coppinsii S. Ravera sp. nov.

Species corticola, Arthopyrenia tuscanensi similis, a qua imprimis differt pseudoparaphysibus praesentibus et ascosporis latioris. Ascomata perithecioidea, sub-globosa, 0.21×0.11 mm; involucrellum 13–20 µm latum, textura intricata; pseudoparaphyses non persistentes, periphyses ramose, $5-10 \times 1-1.5$ µm. Asci fissitunicati, obclavates-obpyriformes, $37-45 \times 12-15$ µm, octospori; endotunica apicem versus sensim incrassata, poro in sectione optica rotundato, structura obconica in tholo 'toluidine blue' reagens. Ascospores hyalinae, $8.8-10.9 \times 3.0-4.5$ µm, 1-septate, septo \pm mediano; cellula supera cellula inferna latior, cellula inferna leviter angularis.

Typus: Italy, Tuscany, Siena, Campo alla Badia, between Gaiole in Chianti and San Gusmè, on bark of *Quercus* sp., 600 m alt., 13 April 1999, *S. Loppi*, 2386 (Herbarium lichenum—SIENA—holotypus).

(Figs 1F-I)

Thallus endophloeodal, inconspicuous, probably non-lichenized.

Ascomata perithecial, 0.21×0.11 mm, black, subglobose, circular or ellipsoid, scattered, superficial, many with depressed

ostioles. Ascomatal wall of textura intricata, black, not continuous below the hamathecium; involucrellum dark brown, c. 13-20 µm thick, intermixed with dark hyphae c. $2.5-2.7 \,\mu\text{m}$ thick, amorphous pigment localized in the cell wall; excipulum brown, $5-7 \,\mu\text{m}$ thick, at the base hyaline; the wall pigment remains brown in K. Hamathecium with pseudoparaphyses dissolving and leaving only fragments (to 1 µm wide) embedded in gel; periphysoids $5-15 \times 1-2 \,\mu m$, branched and septate. Asci $37-45 \times 12-$ 15 µm, obclavate-obpyriform, fissitunicate, endotunica thickened to the apex, ocular chamber rounded, obconical structure in tholus reacting to toluidine blue. Ascospores $8 \cdot 8 - 10 \cdot 9 \times 3 \cdot 0 - 4 \cdot 5 \text{ } \text{um}$ [average (± SD): $10.1(\pm 0.6) \times 3.7(\pm 0.4)$, length/breadth ratio $2.8(\pm 0.2)$ (n=50)], 8 per ascus, colourless, 1-septate, non-guttulate, constricted at the septum with a larger upper cell $3 \cdot 3 - 5 \cdot 4 \times 3 \cdot 3 - 4 \cdot 8 \mu m$ [average (\pm SD): $4 \cdot 4(\pm 0 \cdot 4) \times 3 \cdot 7(\pm 0 \cdot 4)$] and a pointed lower cell $4 \cdot 3 - 5 \cdot 9 \times 2 \cdot 5 - 3 \cdot 7 \mu m$ [average $(\pm SD): 5.1(\pm 0.4) \times 3.1(\pm 0.3)$, length/ breadth ratios $1.6(\pm 0.2)$ (n=50)]; perispore indistinct but immature spores from broken asci have a broad halo of ascoplasm. Pvcnidia not observed.

Etymology. This species is dedicated to Brian Coppins, Royal Botanic Garden Edinburgh, for his contributions to the taxonomy of microlichens.

Ecology. Arthopyrenia coppinsii seems to prefer well-preserved woodland and appears to be more hygrophytic than A. tuscanensis.

Habitat. This species, accompanied also by Arthonia excipienda (Nyl.) Leight. on Fraxinus excelsior L. (Brunialti et al. 2001), has been collected on trees where species belonging to Lobarion pulmonariae Ochsner 1928 [Degelia spp., Fuscopannaria ignobilis (Anzi) P.M. Jørg., Pannaria conoplea (Ach.) Bory, Lobaria spp., Collema furfuraceum (Arnold) Du Rietz, Collema subflaccidum Degel., Leptogium brebissonii Mont.] occur with a high cover value (Brunialti et al. 1999; S. Loppi, data not published).

Character	<i>A. salicis</i> (source: Purvis <i>et al.</i> 1992)	A. tuscanensis	A. coppinsii
Ascomata (mm)	$0.15-0.3 \times 0.15-0.2$	$0.09-0.23 \times 0.09-0.11$	0.21×0.11
Pseudoparaphyses	Absent or gelatinized	Absent	Gelatinized
Periphysoids size (µm)	$7-15 \times 0.15-0.2$	$5-10 \times 1-1.5$,	$5-15 \times 1-2,$
and shape	unbranched, 0- to 2-septate	branched and septate	branched and septate
Asci size (µm) and shape	$33-45 \times 14-20$, obpyriform	38–55 × 12–14, obclavate-obpyriform	$37-45 \times 12-15$, obclavate-obpyriform
Ascospores size (µm) and shape	(12–)14–17(–21) \times 4–5.5, 1-septate, guttulate, clavate to ellipsoid, lower cell often with a median constriction, apices blunt	11.7–14.8 × 3.6–4.9, 1-septate, non-guttulate, tadpole-shaped, lower cell often with a median constriction and with apex strongly pointed	$8\cdot8-10\cdot9 \times 3-4\cdot5$, 1-septate, non-guttulate, clavate, lower cell lacks a median constriction, lower apex \pm pointed

TABLE 1. Characters distinguishing Arthopyrenia salicis, A. tuscanensis and A. coppinsii

Distribution. Known from the submediterranean belt of the humid submediterranean phytoclimatic Region, in Liguria and Tuscany (Fig. 2).

Notes. The size of ascomata, asci, periphysoids and ascospores suggest affinities to Arthopyrenia salicis, with which it has been confused by Italian lichenologists (Putorti & Loppi 1999; Brunialti et al. 2001). Arthopyrenia coppinsii can be distinguished from A. salicis by the usually non-guttulate ascospores each with a pointed lower cell that lacks a median constriction, a smaller and more globose upper cell and the initial presence of slender and branched pseudoparaphyses. Furthermore, it seems to have a different habitat ecology being found in species-rich communities on rough bark.

Additional specimen examined. Italy: Ligury: Genova, Rezzoaglio, Villanoce, on bark of old Fraxinus excelsior L. along a stream, 850–900 m alt., 2000, R. Benesperi, G. Brunialti, P. Giordani & S. Ravera [2888] (Herbarium lichenum—RO).

Discussion

The two new species are very similar with very small ascomata and are likely to be confused, although differences in morphoanatomical characters can be used to separate them and to distinguish them from *Anisomeridium ranunculosporum* (Coppins & P. James) Coppins, another species with similar ascospores.

Arthopyrenia tuscanensis is characterized by ascospores with a conical lower cell, almost twice as long as wide (l/b ratio 1.9), often showing a slight median constriction, whereas ascospores of A. coppinsii are relatively shorter and more ovoid (l/b ratio 1.6) with the lower cell never showing a constriction. Furthermore, the asci of A. tuscanensis are longer and proportionally narrower than the asci of A. coppinsii and the two species have different hamathecial characteristics.

Tadpole-shaped ascospores are a diagnostic feature of *Anisomeridium ranunculosporum* but that species is easily distinguished from these two *Arthopyrenia* species by its welllichenized thallus (with *Trentepohlia*), K+ greenish involucrellum, larger, guttulate ascospores, and usual presence of macroconidia (Coppins 1988).

The two new species are similar to *Arthopyrenia salicis* A. Massal., and all three are typical of the genus with regard to their excipulum and asci (according to: Grube & Hafellner 1990; Grube 1993; Harris 1995). However, they have periphysoids, lack pseudoparaphyses at maturity and seem to be facultatively lichenized. *Arthopyrenia salicis* has been included in the genus *Arthopyrenia* as a "temporary expedient"

(Harris 1995): the same solution being adopted here for the two new species.

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