

## NEW RECORDS OF ARTHONIALES (LICHENIZED FUNGI) FROM IRAN

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Three species of *Arthoniales* are reported from Iran (Golestan province) for the first time: *Opegrapha niveoatra* (Borrer) J.R. Laundon (Opegraphaceae), *Pachnolepia pruinata* (Torss.) Frisch & G. Thor (Arthoniaceae) and *Enterographa elaborata* (Lyell ex Leight.) Coppins & P. James (Roccellaceae). They grow on the bark of trees in broad-leaf forests and woodland (Alangdarreh, Shastkolateh, and Bandargaz forests) in Golestan Province. The morphological characteristics, images, habitat, and geographic distribution of these species are discussed.

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گزارش‌های جدید از راسته *Arthoniales* (قارچ‌های گل‌سنگی شده) از ایران

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سه گونه از راسته *Arthoniales* شامل: *Opegrapha niveoatra* (Borrer) J.R. Laundon (Opegraphaceae), *Pachnolepia pruinata* (Torss.) Frisch & G. Thor (Arthoniaceae) و *Enterographa elaborata* (Lyell ex Leight.) Coppins & P. James (Roccellaceae) برای اولین بار از ایران (استان گلستان) گزارش می‌شوند. این گونه‌ها روی پوست درختان در جنگل‌های پهن برگ (جنگل‌های النگ‌دره، شصت کلاته و بندرگز) در استان گلستان رویش دارند. ویژگی‌های ریخت‌شناسی، تصاویر، رویشگاه و پراکنش جغرافیایی این گونه‌ها مورد بحث قرار گرفته است.

### INTRODUCTION

The Arthoniales are one of the largest groups of lichenized fungi worldwide. They belong to the Pezizomycotina and are currently classified in the Arthoniomycetes (Hibbett & al. 2007; Spatafora & al. 2006). This order comprises ca. 55 genera and 1200 species (Kirk & al. 2008), which are arranged in seven families: Andreiomycetaceae, Arthoniaceae, Chrysotrichaceae, Lecanographaceae, Opegraphaceae, Roccellaceae and Roccellographaceae (Ertz & Tehler 2011, Frisch & al. 2014, Hodkinson 2013, Lücking &

al. 2017). More than half of the species are attributed to the traditional genera *Arthonia* and *Opegrapha* with ca. 400 and 300 species, respectively (Kirk & al. 2001).

The Arthoniales are distributed worldwide, from arctic-alpine regions to the tropics and from arid ecosystems to humid forests. They grow on various substrates including shaded rock, bark, wood, bryophytes, and even living leaves. They may also live on other lichens, both autotrophic and parasitic (Frisch & al. 2014). This order is thought to be originally lichenized (Hafellner 2009; Nelsen & al. 2009) with

several independent reductions to the non-lichenized, lichenicolous, or parasitic state. Photobionts in most of the species are of the trentepohlioid type (Coppins & Aptroot 2009, Fletcher & Purvis 2009). The highest numbers of species are found in subtropical coastal habitats with a Mediterranean or desert-type climate (Aptroot & Sparrius 2008, Follmann & Werner 2003, Tehler 1983, 1990). Five families of Arthoniales occur in Iran: Arthoniaceae with 4 genera and 19 species, Roccellaceae with 2 genera and 2 species, Opegraphaceae with 2 genera and 4 species, Chrysotrichaceae with 1 genus and 2 species, and Lecanographaceae with 1 species (Kazemi & Ghahremaninejad 2008, Seaward & al. 2004, 2008, Sohrabi & al. 2006, Younesi & al. 2015). They are mostly reported from Mazandaran, Golestan, Zanjan, Ilam, and West Azerbaijan provinces. All species reported so far from Iran grow on tree bark.

Below we present three species recently discovered for the first time in Iran.

## MATERIAL AND METHODS

The examined specimens were collected in Golestan province on three sampling sites: Alangdarreh, five km SW of Gorgan city; along the road of the Naharkhoran Forest, Shastkolatch forest, W of Gorgan city; Bandargaz woodland, W of Bandargaz city. Their altitudinal range is 300-900 m above sea level. The most important trees in these forests are *Acer cappadocicum* Gled, *Quercus castaneifolia* C.A.M., *Alnus subcordata* C.A.M., *Parrotia persica* C.A.M., *Zelkova carpinifolia* (Pall.) Dipp., *Carpinus betulus* L., *Fagus orientalis* Lipsky, *Cupressus sempervirens* L., *Tilia platyphyllos* Scop., *Pterocarya fraxinifolia* Land. (Spach.) (Sagheb-Talebi & al. 2003).

The specimens were identified by usual lichenological methods (Smith & al. 2009). The morphology was studied on the base of hand-cut sections of specimens and by a Luxeo 4D stereomicroscope and a Zeiss compound microscope. For the chemical tests, the used reagents are 10% KOH (K), sodium hypochlorite (C), paraphenylenediamine in ethanol (Pd) and Lugol's solution (I) and some samples were analyzed with thin layer chromatography (TLC) using solvent systems A (toluene, dioxane, acetic acid), B (hexane, diethyl ether, formic acid), and C (toluene, acetic acid), (Orange & al. 2010). Images

were captured with a stereomicroscope connected to a Luxeo 4D camera with Pixelpro software. Determination of the taxa was accomplished using the keys in Cannon & al. (2020), Wieczorek (2018), and Smith & al. (2009). Furthermore, the identified species were compared with the herbarium specimens in B (acronym according to Thiers 2016).

The species names were updated in accordance with the Index Fungorum (<http://www.speciesfungorum.org/names/names.asp>). The voucher specimens are deposited in the lichen herbarium of the Research Institute of Forest and Rangelands Tehran, Iran (TARI).

## RESULTS

Three species of *Arthoniales* not previously reported from Iran were identified in the Hyrcanian Forest in Golestan province. They are presented below with pertinent specimens, short descriptions of morphology and chemistry of Iranian specimens, notes on ecology and distribution, and differences from confusable species.

***Opegrapha niveoatra*** (Borrer) J.R. Laundon

*Examined specimen:* Golestan: Gorgan, Naharkhoran road, Alangdarreh forest, Doberar forest; 36.79501° N, 054.45652° E; 354 m; Kazemi 7756 (TARI).

Thallus crustose, thin, often smooth, sometimes cracked, with more or less distinct prothallus, white to gray, cream-grayish, cream-olivaceous. Apothecia lirellate, slightly prominent, scattered or contiguous, simple, straight, curved or stellate, sessile, 0.4-1.3 × 0.1-0.2 mm, disc split-shaped, black, epruinose (Fig. 1A). Asci clavate to cylindrical, 8-spored, 75-90 × 11-14 μm (Fig. 1B). Ascospores quite elongated, acicular to fusiform, slightly curved, hyaline, with thick outer walls and thin septa, 18-29 × 2.2-4 μm, 4-7(-8) septate (Fig. 1C). Pycnidia often present, black, immersed or subimmersed, conidia worm-like, curved, 4-9 × 0.9-1.4 μm.

**Chemistry:** thallus K-, C-, KC-, P-.

**Habitat:** On bark of *Parrotia persica* trunk in parkland.

**Geographical distribution:** Europe (Torrente & al. 1989), North America (Fryday & al. 2001), North Africa (Egea 1996) and Asia (Yazici & Aptroot 2007).

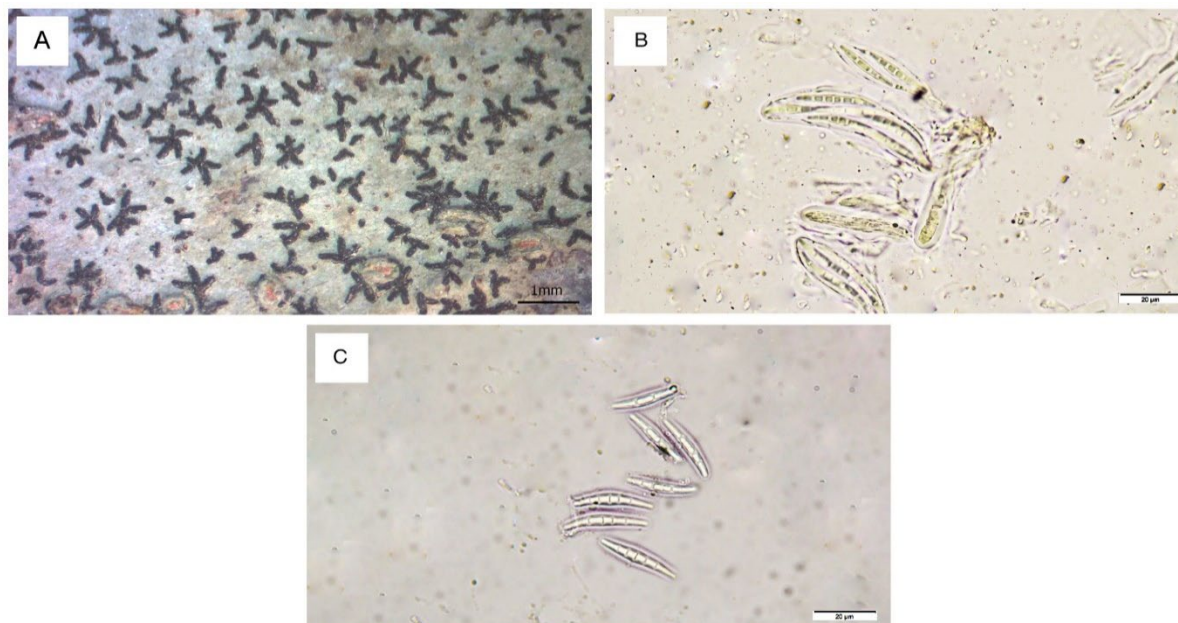


Fig. 1. *Opegrapha niveoatra* (TARI, Kazemi 7756). A, thallus and apothecia; B, asci; C, ascospores.

**Notes:** *Opegrapha niveoatra* is morphologically similar to *O. vulgata* and *O. vermicellifera*. All three species have 4-7 septate ascospores with a length of 15-30 µm and grow on moderately acidophilic tree bark in light forest. *Opegrapha niveoatra* and *O. vulgata* differ from *O. vermicellifera* by the non-pruinose and inconspicuous, often scarce pycnidia, whereas apothecia are generally present. *Opegrapha vermicellifera* on the contrary has whitish pruinose pycnidia that are very conspicuous, semiglobose, and abundant, while apothecia are often absent. *O. vulgata* differs from *O. niveoatra* and *O. vermicellifera* by its long conidia (9-15 µm in length instead of 4-9 × 0.9-1.4 µm), (Smith & al. 2009).

***Pachnolepia pruinata*** (Torss.) Frisch & G. Thor

**Examined specimens:** Golestan: Gorgan, Alangdarreh forest, around checkpoint, 36.77601° N, 054.44816° E, 404 m, S. Kazemi, 7769 (TARI); Gorgan, Yalou village, Shastkolateh forest, 36.78286° N, 054.36492° E, 273 m, S. Kazemi, 7694 (TARI). Gorgan, Shastkolateh forest, forest park, 36.790120° N, 54.415677° E, 445 m, S. Kazemi, 7905 (TARI). Gorgan, Gorgan, Naharkhoran road, Delbar mountain, 36.746164° N, 54.494024° E, 844 m, S. Kazemi, 8772 (TARI).

Thallus crustose, matt or powdery, rimose-cracked, to 1 mm thick, sometimes warty, without soredia, pale grayish to white or pale brown, pruinose, pruina pale grey-bluish. Apothecia immersed, variable in shape, angular to shortly branched, substellate to stellate,

sometimes rounded, up to 1 mm wide; disc brown to black, pale to dark brown, pale gray by white pruina of calcium oxalate (Fig. 2A); epithecium red-brown; hymenium colourless to pale brown, 45-65 µm tall; hypothecium hyaline; paraphysoids branched and anastomosed. Asci *Arthonia*-type, clavate, 8-spored. Ascospores colorless, transversely 3-5-septate, cylindric-obovoid, 14-21 × 5-8 µm, upper cell not enlarged (Fig. 2B). Pycnidia not seen.

**Chemistry:** Thallus and apothecia C+ red, K-, Pd-. TLC: arthoniaic acid.

**Habitat:** Corticolous on *Quercus castaneifolia* and *Acer cappadocicum*, in Shastkolateh deciduous forest, Alangdarreh Forests, and Naharkhoran Forest Park.

**Geographical distribution:** North America, Mediterranean parts of Europe, North Africa, and Asia (Ismailov & al. 2017).

**Notes:** *Pachnolepia pruinata* is similar to *Arthonia* species by the *Arthonia*-type asci and transversely septate ascospores, and differs by the pruinose apothecia and the C+ red reaction of thallus and apothecia. *Pachnolepia pruinata* looks also like *Lecanographa lyncea*. However, the latter species does not react with C.

***Enterographa elaborata*** (Lyell ex Leight.) Coppins & P. James

**Examined specimen:** Golestan: Bandargaz, around Bolbol spring; 36.705833° N, 53.865277° E; S. Kazemi 9010 (TARI).



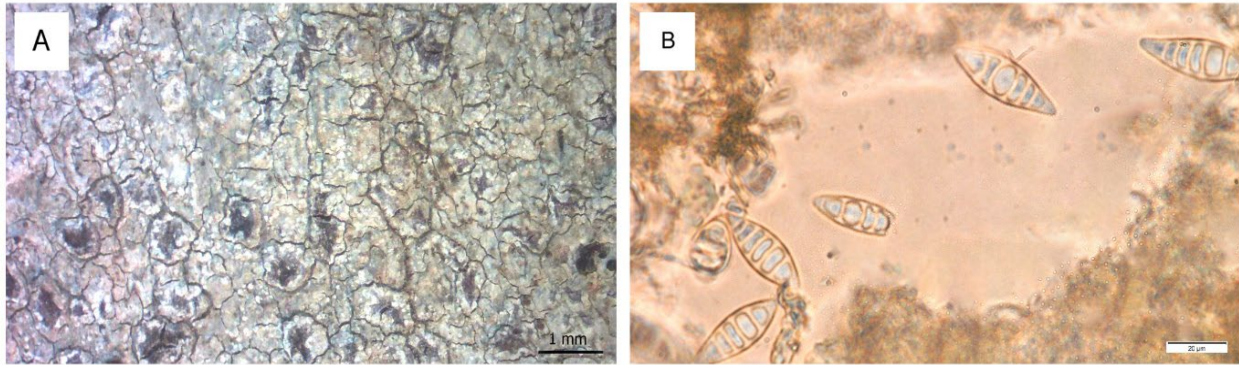


Fig. 2. *Pachnolepia pruinata* (TARI, Kazemi 7769). A, thallus and apothecia; B, ascospores.

Thallus crustose, pale pink-brown to pale brown or creamy, smooth and shiny, with dark prothallus. Apothecia lirelliform, often branched or stellate, obvious, serpentine, brown-red to brown-black, immersed in thallus,  $0.2-4 \times 0.09-0.16$  mm length (Fig. 3A); paraphyses  $0.7-1 \mu\text{m}$  wide. Asci 8-spored, cylindrical to cylindrical-clavate, fissitunicate. Ascospores hyaline, fusiform to often acicular, straight or often curved with a thin episporium,  $32-63 \times 2.5-4.2 \mu\text{m}$ , transversely 5-17-septate (Fig. 3B). Pycnidia rare, immersed, dark brown,  $0.1$  mm wide; conidia hyaline, thread-like,  $12-17 \times 0.7 \mu\text{m}$ .

**Chemistry:** thallus K- or K+ pale yellow, C-, KC-, Pd+ yellow-orange.

**Habitat:** on smooth-barked *Zelkova carpinifolia* and *Pterocarya* in woodland.

**Geographical distribution:** *Enterographa elaborata* is an internationally rare lichen of humid, temperate, relic woodlands. It is found in humid ravines in western Europe from Denmark and Ireland to the Iberian Peninsula and Macaronesia, in the Mediterranean and warm temperate rainforests by the Black Sea (Cannon & al. 2021, Sanderson 2021) and in SW Asia (Iran, Georgia).

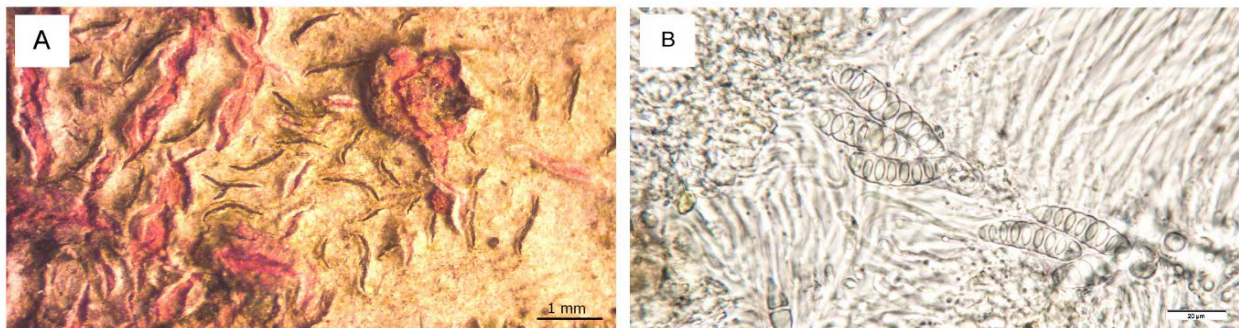


Fig. 3. *Enterographa elaborata* (TARI, Kazemi 9010). A, thallus and apothecia; B, ascospores.

**Notes:** This species was already collected and identified in 2007, in Golestan; Gonbad-e-Kavus district; along the road from Khan Bebin to Shirabad waterfall;  $36.9648^\circ$  N,  $55.0300^\circ$  E; 120 m; H. Sipman, M. Sohrabi, U. Søchting & R. Zare 55198 (IRAN 13002 L, B 60 0175314), but so far not published. *E. elaborata* resembles *E. crassa* (DC.) Fée and *E. hutchinsiae* (Leight.) A. Massal., both much more common in Europe. *E. elaborata* differs by the P+ red spot test, which is negative in the mentioned species, Morphologically it differs from both by the longer, regularly forked lirellae and its ascospore size is larger

(*E. hutchinsiae* (22-)  $24-28$  (-32)  $\times 4-5 \mu\text{m}$ , *E. crassa*  $28-38 \times 4-6 \mu\text{m}$ ) (Cannon & al. 2021).

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