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Seven species of *Graphis* from Portugal reported new to Europe

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Abstract: Seven *Graphis* species are reported as new to Europe from the Serra de Sintra and the Planalto das Cezaredas, two regions of Portugal where graphidoid *Graphidaceae* are abundant and diverse. The following species were identified: *Graphis cincta*, *G. crebra*, *G. handelii*, *G. leptospora*, *G. lineola*, *G. plumierae*, and *G. verminosa*. Many of these share two characters: a densely inspersed hymenium and the presence of norstictic acid in the thallus. A key to the *Graphis* species known from Europe is provided. The possible reasons for the high, previously unknown, diversity of graphidoid *Graphidaceae* in this region are discussed. Global warming might be a contributory factor.

Key words: climate change, global warming, *Graphidaceae*, graphidoid, introduced species, lichens, Planalto das Cezaredas, Serra de Sintra

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Introduction

Graphis is the type genus of the family *Graphidaceae*. The family is, in its present delimitation, the second largest lichenized family of the Ascomycota, with over 2500 accepted species and a predicted number of nearly 5000 species worldwide (Lücking *et al.* 2014). The genus *Graphis* is one of the largest lichen genera, with over 370 species accepted in the most recent world key (Lücking *et al.* 2009; Barcenas Peña *et al.* 2014), to which about ten newly discovered species are being added every year. The family and its type genus are predominantly tropical.

Europe is relatively poor in *Graphidaceae*, with only a few members of the *Fissurinoideae* (Rivas Plata *et al.* 2012), which are mostly restricted to the Azores (Aptroot *et al.* 2010), a handful of *Gomphilloideae* (as extra-tropical foliicolous lichens are not diverse and most species of this subfamily are obligately foliicolous; Smith *et al.* 2009), less than ten *Thelotrematеae* (also mostly restricted to the Azores; Purvis *et al.* 1995; Aptroot 2010), ten species of *Diploschistes* and split-offs (Lumbsch 1989), and only around ten *Graphideae*, including one *Leiorreuma*, one *Glyphis*, three species of *Phaeographis*, and a handful of *Graphis* species (Lücking *et al.* 2009; Smith *et al.* 2009; Neuwirth & Aptroot 2011). The total number of *Graphidaceae* known from Europe is thus less than 40 (hence 1–2% of the world total).

The European species of the *Graphis scripta* (L.) Ach. complex have been the subject of a recent study (Neuwirth & Aptroot 2011) showing that up to four morphologically distinguishable species can be found to occur together. A subsequent phylogenetic study (Kraichak *et al.* 2015) showed that even more species occur within this group and that the morphological distinction can be challenging, especially due to the common occurrence of young thalli without fully developed characters. In addition to the four members of this group, only three other species of the genus *Graphis* s. str. are known from Europe, viz. *G. elegans* (Borrer ex Sm.) Ach. with striate labiae and transversely septate ascospores, *G. inustuloides* Lücking (Lücking & McCune 2012, also variously known as *Graphina anguina* auct. *non* Müll. Arg., *Graphis britannica* Staiger, and *G. inustula* Nyl. *non* Stirt.) with erumpent, simple labiae, discs that become open and

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muriform ascospores, and *Graphis ruiziana* (Fée) A. Massal. [= *Graphina ruiziana* (Fée) Müll. Arg.] with superficial, simple labiae with closed discs and muriform ascospores. Two European species that are still generally cited as *Graphis* or *Graphina* species, viz. *Graphina pauciloculata* Coppins & P. James and *Graphis alboscripta* Coppins & P. James (Benfield *et al.* 2009), do not belong to the genus *Graphis* s. str. The position of the first is still unknown (Lücking *et al.* 2009), though it is possibly a *Glyphis*; the second is a *Fissurina*.

Over the last few years, the first author studied the graphidoid *Graphidaceae* in two regions of Portugal where they are relatively abundant and diverse, viz. the Serra de Sintra and the Planalto das Cezaredas.

The Serra de Sintra is a granitic mountain range in western Portugal (Mesquita *et al.* 2005), very close to the capital Lisbon, with the highest point 529 m, near the town of Sintra. Its climate is temperate with much oceanic influence and hence it has a higher rainfall than other areas close to Lisbon. It also has a unique vegetation with *c.* 900 indigenous plant species of which 10% are endemic. Its forests, very rich in mediterranean-atlantic species, constitute the transition zone between the north and south of the country (Castro *et al.* 2001). Several species of *Quercus*, namely *Q. faginea*, *Q. pyrenaica*, *Q. roble* and *Q. suber*, are locally dominant (Proença 2009).

The Planalto das Cezaredas is located in the centre of the western region of Portugal, covering four counties (Gomes 2012): Bombarral, Lourinhã, Óbidos and Peniche. The highest elevation of the plateau is 164 m and the lowest is 32 m. As for the climate, it is in a transition between the west coastal maritime climate region (a narrow temperature range, with frequent fogs during summer mornings) and the maritime climate region of the Atlantic seaboard (moderate summers with an average maximum temperature of between 23–29 °C in the hottest month, and moderate winters with an average minimum temperature of between 4–6 °C in the coldest month). The flora of the region is characterized by the existence of large patches of *Quercus coccifera* and several other oak species, pines (*Pinus pinea* and *P. sylvestris*), and different types of shrubs (Carvalho 1997), but the ancient flora has been progressively destroyed in favour of agriculture or substituted by other trees, mainly *Eucalyptus*.

During the study of graphidoid *Graphidaceae* in the regions of Serra de Sintra and Planalto das Cezaredas, the following species were found that were already known to occur in Portugal (Poelt & Vězda 1981; Jones 2002; Carballal *et al.* 2004): *Glyphis cicatricosa* Ach., *Graphis elegans*, *G. inustuloides*, *G. scripta* complex (not studied further here), *Leiorreuma lyellii* (Sm.) Staiger, *Phaeographis dendritica* (Ach.) Müll. Arg., and *P. inusta* (Ach.) Müll. Arg. Only two additional graphidoid *Graphidaceae*, *Graphis ruiziana* and *Phaeographis smithii* (Leight.) B. de Lesd., are known from Europe; both occur in the Iberian Peninsula but the latter is not yet known from Portugal (López de Silanes & Álvarez 2003). Neither species was found by the first author in the two areas investigated.

Besides those species that were already known from Portugal, many *Graphis* specimens were found that clearly do not belong to any species known from Europe. Most notably, many specimens had a clearly inspersed hymenium, a character so far unknown in European *Graphis* species. The majority also had a thallus that reacts KOH+ yellow turning red forming needle-shaped crystals in microscopic view and containing norstictic acid, a character otherwise known in European species only from *G. elegans*. A detailed study revealed at least seven different additional species of *Graphis*. This doubles the number of *Graphis* species known from Europe, even if all phylogenetic microspecies in the *G. scripta* complex (Kraichak *et al.* 2015) are accepted. All newly reported species are described and keyed out below. All specimens cited here have been assigned to species that were previously described, but never recorded from Europe. Some additional specimens remain unidentified at the moment and await further study.

Material and Methods

Identification and descriptive work was carried out in Amadora, and in Soest using an Olympus SZX7

stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water, in which all measurements were taken. Some of the specimens were tested in the field for the reaction with KOH (10–15%), but for all specimens the reaction to KOH was analyzed under the compound microscope, using a thin section of the thallus mounted in water with a drop of KOH added. Lirella morph terminology follows Lücking *et al.* (2009). All specimens mentioned were collected either in 2014 or at the beginning of 2015, and will be preserved in LISU with some duplicates in ABL. The chemistry of some specimens was investigated by thin-layer chromatography (TLC) using solvent A (Orange *et al.* 2001).

The Species

Graphis cincta (Pers.) Aptroot

Aptroot in Archer, *Fl. Australia* **57**: 651 (2009).—*Opegrapha cincta* Pers., *Ann. Wetter. Ges.* **2**: 15 (1811); type: Dominican Republic, *s. col.* (L—holotype).

(Fig. 1A)

Thallus KOH+ yellow to red (TLC: norstictic acid). *Lirellae lineola*-morph, no striation, excipulum laterally carbonized, disc not pruinose, hymenium densely inspersed.

Ascospores 5–11-septate, 20·5–45·0 × 6·5–8·5 µm.

Notes. The thalli of these specimens are all very similar, except maybe for the colour: white in 1825 and 6547, greenish grey in 6191 and 6594 and in between (i.e. whitish with a shade of grey and/or light green) for 6582. The latter also exhibits some pruinosity. The lirella morphology is also similar for all specimens, but short (up to 2·5 mm) in 6191 and 6194, to more frequently elongate (up to 5 mm) in the other three specimens, sparsely branched, mostly erumpent with a basal to lateral thalline margin in all cases. The labiae are entire and the disc concealed, though some of the lirellae show part of the disc, especially in specimens 1825 and 6547.

Specimens examined. **Portugal:** Serra da Sintra, Sítio de Santa Eufémia, *Quercus* forest, 439–467 m, 38°47'08"N, 9°23'07"W, 2014, *Zaca* 1825; 38°47'15"N, 9°23'09"W, 2014, *Zaca* 6191; 38°47'12"N, 9°23'07"W, 2014, *Zaca* 6548; 38°47'15"N, 9°23'05"W, 2014, *Zaca* 6594; 38°47'15"N, 9°23'06"W, 2014, *Zaca* 6582.

Graphis crebra Vain.

Hedwigia **38:** 256 (1899); type: Guadeloupe, Gourbeyre, *P. Duss* 541 (TUR-V 27617—holotype).

(Fig. 1B)

Thallus KOH+ yellow to red. *Lirellae lineola*-morph, no striation, excipulum laterally carbonized, disc pruinose, hymenium inspersed.

Ascospores 6–11-septate, (28·9–)32·2–40·5 (–42·3) × (7·0–)7·5–8·2(–8·8) µm, (3·5–)4·2–5·2(–5·5) times as long as wide (n = 30).

Specimen examined. **Portugal:** Serra da Sintra, public park at Sintra, wooded park mainly with *Quercus* and *Platanus*, 278 m, 38°47'44"N, 9°22'48"W, 2014, *Zaca* 6647.

Graphis handelii Zahlbr.

Zahlbr. in Handel-Mazzetti, *Symb. Sinic.* **3**: 44 (1930); type: China, Tschangscha, *Handel-Mazzetti* 11403 & *Niutoutschou, Handel-Mazzetti* 12788 (W—syntypes).

(Fig. 1C)

Thallus KOH+ yellow to red. *Lirellae handelii*-morph, no striation, excipulum completely carbonized, hymenium inspersed.

Ascospores mostly 5-septate, (14·9–)17–22 (–24·4) × (5·7–)6·4–7·4(–7·9) µm, (2·1–)2·5–3·2(–3·5) times as long as wide (n = 32).

Specimen examined. **Portugal:** Serra da Sintra, Estrada da Pena, *Quercus* forest, 381 m, 38°47'22"N, 9°23'40"W, 2015, *Zaca* 7303.

Graphis leptospora Vain.

Ann. Bot. Soc. Zool.-Bot. fenn. Vanamo **1**(3): 53 (1921); type: Thailand, Doi Suthep, 1904, *C. C. Hosseus* (TUR-V 27807—holotype)

(Figs 1D, 2A & B)

Thallus whitish, KOH+ yellow to red (TLC: norstictic acid). *Lirellae acharii*-morph, rather thick, striate, hymenium not inspersed.

Ascospores up to 15-septate and very variable in size, (27–)40–75(–100) × (7·2–)8·0–9·5(–11·5) µm, (3·4–)5–8(–9·7) times as long as wide (n = 197).

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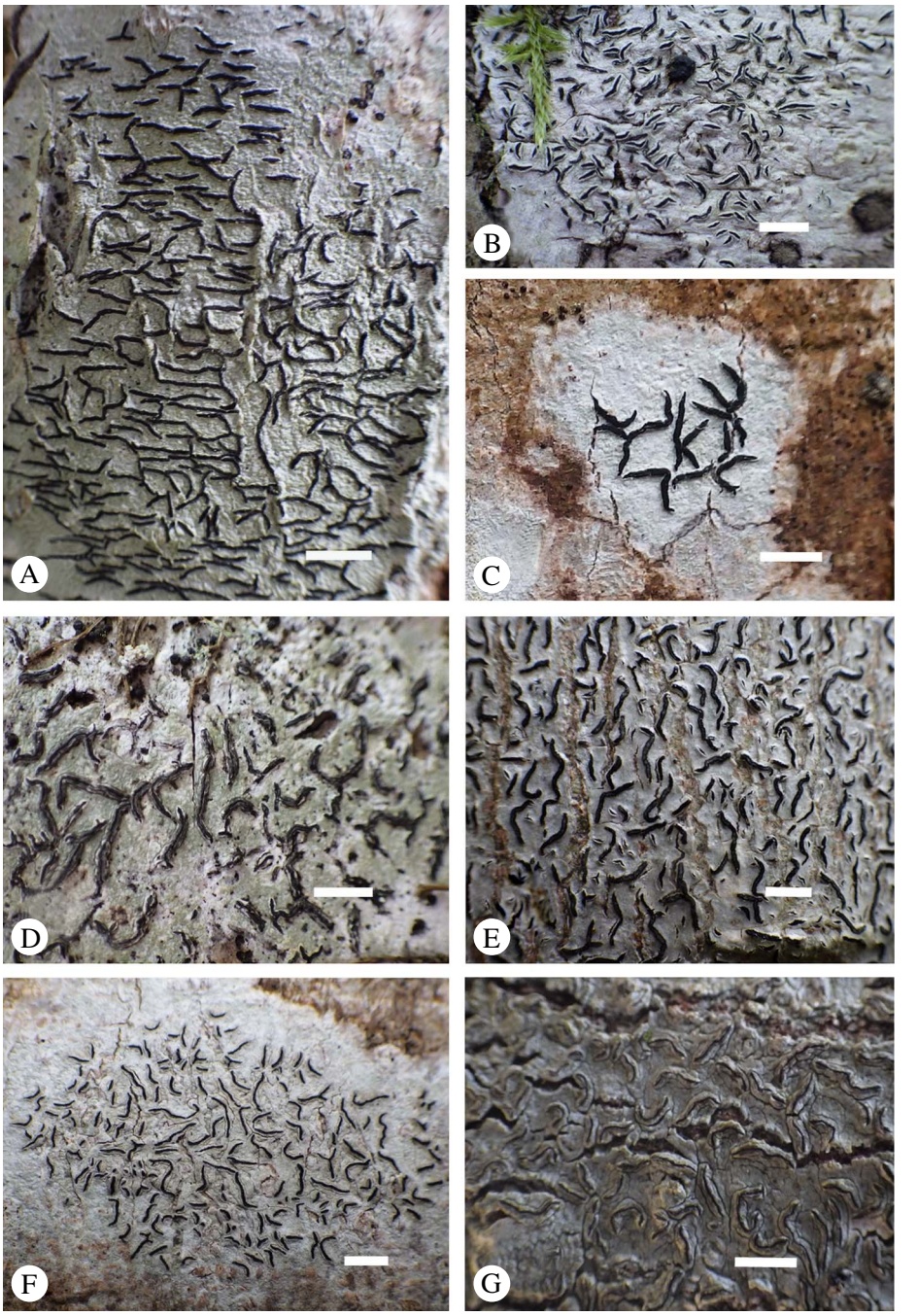

FIG. 1. *Graphis* species in Portugal, habitus. A, *Graphis cincta*, *Zaca* 6595; B, *G. crebra*, *Zaca* 6647; C, *G. handelii*, *Zaca* 7303; D, *G. leptospora*, *Zaca* 6611; E, *G. lineola*, *Zaca* 7293; F, *G. plumierae*, *Zaca* 6573; G, *G. verminosa*, *Zaca* 8622. Scale = 2 mm. In colour online.

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Specimens examined. **Portugal:** Serra da Sintra, Sítio de Santa Eufémia, *Quercus* forest, 430–440 m, 38°47'15"N, 9°23'05"W, 2014, *Zaca* 6602 & 6611; 38°47'15"N, 9°23'08"W, 2014, *Zaca* 6557; Planalto das Cezaredas, Lourinhã, Casal da Curtinha, *Quercus coccifera* habitat, 150 m, 39°17'21"N, 9°13'30"W, 2014, *Zaca* 3768; 39°18'10"N, 9°13'19"W, 2014, *Zaca* 9151; 39°17'54"N, 9°15'21"W, 2014, *Zaca* 1763.

Graphis lineola Ach.

Lich. Univ.: 264 (1810); type: Dominican Republic, on *Clusia, O. P. Swartz* (UPS-ACH—holotype).

(Figs 1E, 2C)

Thallus KOH−. *Lirellae lineola*-morph, with lateral thalline margin, no striation, disc exposed, white pruinose, excipulum laterally carbonized, hymenium inspersed.

Ascospores 5–9-septate, 25–35 × 6–8 µm, (3·0–)3·6–4·7(–5·6) times as long as wide (n = 37).

Specimen examined. **Portugal:** Serra da Sintra, Estrada da Pena, *Quercus* forest, 381 m, 38°47'22"N, 9°23'40"W, 2015, *Zaca* 7293.

Graphis plumierae Vain.

Ann. Acad. Sci. Fenn., Ser. A **6**(7): 161 (1915); type: Guadeloupe, Gourbeyre, P. Duss 1189 (TUR-V 27732—holotype).

(Fig. 1F)

Thallus KOH+ yellow to red. *Lirellae lineola*-morph, no striation, excipulum laterally carbonized, hymenium inspersed.

Ascospores 7–10-septate, (26·5–)32·5–39·9 (–43·3) × (6·4–)6·9–7·8(–7·9) µm, (4·1–)4·3–5·6(–6·1) times as long as wide (n = 24).

Specimen examined. **Portugal:** Serra da Sintra, Sítio de Santa Eufémia, *Quercus* forest, 441 m, 38°47'16"N, 9°23'09"W, 2014, *Zaca* 6573.

Graphis verminosa Müll. Arg.

J. Linn. Soc. London, Bot. **29**: 224 (1892); type: India, Manipur, 1882, *G. Watt* (G—holotype).

(Figs 1G, 2D)

Thallus KOH+ yellow to red. *Lirellae acharii*-morph, with striation, excipulum completely carbonized, hymenium not inspersed.

Ascospores 10–15-septate, (41·8–)50–71 (–73·8) × (7·2–)7·4–10·4(–12·9) µm, (4·9–)5·9–7·5(–8·0) times as long as wide (n = 41).

Specimen examined. **Portugal:** Planalto das Cezaredas, Peniche, Bolhos, *Quercus coccifera* habitat, 133 m, 39°18'54"N, 9°16'29"W, 2014, *Zaca* 8622.

Key to the species of *Graphis* known from Europe

1 Ascospores only transversely septate 2
Ascospores muriform 10

2(1) Lirellae becoming striate; hymenium never inspersed 3
Lirellae never striate (try both options for young material); hymenium often inspersed with oil droplets 5

3(2) Ascospores (small to) medium-sized (35–60 µm long), 7–13-septate, conspicuously thick-walled (wall usually over 2 µm thick); subcosmopolitan, in Europe mostly Atlantic, but east to the Carpathians and north to southern Scandinavia **G. elegans**
Ascospores medium-sized to large (27–100 × 7·5–11·5 µm), 10–15-septate, not conspicuously thick-walled (wall under 2 µm thick) 4

4(3) Excipulum completely carbonized, also below the hymenium; palaeotropical . . . . . **G. leptospora**
Excipulum only laterally carbonized, open below; palaeotropical . . . . **G. verminosa**

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5(2) Hymenium not inspersed; thallus KOH−; disc often exposed and pruinose; northern temperate . . . . . **G. scripta** complex
Hymenium inspersed with oil droplets 6

6(5) Thallus KOH−; disc concealed; labia not pruinose; lirellae erumpent, with lateral thalline margins; pantropical. . . . . **G. lineola**
Thallus KOH+ yellow to red; other characters variable (see below) 7

7(6) Disc exposed; lirellae erumpent, with lateral thalline margins 8
Disc concealed; lirellae variable 9

8(7) Disc white-pruinose; pantropical . . . . . **G. crebra**
Disc not pruinose; pantropical . . . . . **G. handelii**

9(7) Labia white-pruinose; lirellae immersed; pantropical . . . . . **G. plumierae**
Labia not pruinose; lirellae erumpent; pantropical . . . . . **G. cincta**

10(1) Apothecia emergent; disc becoming exposed; temperate western Europe . . . . . **G. inustuloides**
Apothecia superficial; disc closed; cosmopolitan, in Europe mainly in the south-west Atlantic, north to Great Britain and east to Germany . . . . . **G. ruiziana**

Discussion

The Serra da Sintra was already known as the richest region in Europe for graphidoid *Graphidaceae* (Jones 2002; Carballal *et al.* 2004), as Portugal is the only country in Europe where almost all the European species of *Graphis* and *Phaeographis* occur (Poelt & Vězda 1981). Apparently, the combination of the Atlantic climate and the presence of extended forests at low elevations is favourable for members of this predominantly tropical family. This is further exemplified by *Glyphis cicatricosa*, a widespread tropical lichen which, in Europe, is known only from Portugal, from the Serra da Sintra and from the Azores (Poelt & Vězda 1981).

The discovery in the region of seven *Graphis* species that were previously known only from tropical areas raises several questions. First, have these species been present for a long time or could they have arrived rather recently? Second, if the latter, could this be an effect of global warming? Third, could some have been inadvertently introduced with tropical trees? Another question is whether these species might have been ignored or overlooked in other European countries.

The possibility that the tropical graphidoid *Graphidaceae* have been present in the study area for a long time cannot be completely ruled out, but it is rather unlikely. For some time, hamathecium inspersion has not been recognized as a relevant character and there might be some previously studied specimens of the species reported here in European herbaria. Additionally, a distinction should be made between the two collecting regions where the species were found. The Planalto das Cezaredas had not been investigated for epiphytic lichens previously, as far as we know, so the species may or may not have been present there for some time before this study. The Sintra region has been known as a rich area for epiphytic lichens and especially for *Graphidaceae*. For example, it was known as the only mainland European locality of *Glyphis cicatricosa* (Poelt & Vězda 1981). In view of the ample attention that has been paid to this group in this region recently (López de Silanes & Álvarez 2003; Carballal *et al.* 2004), it is quite likely that at least some of the species have been there for only a short time.

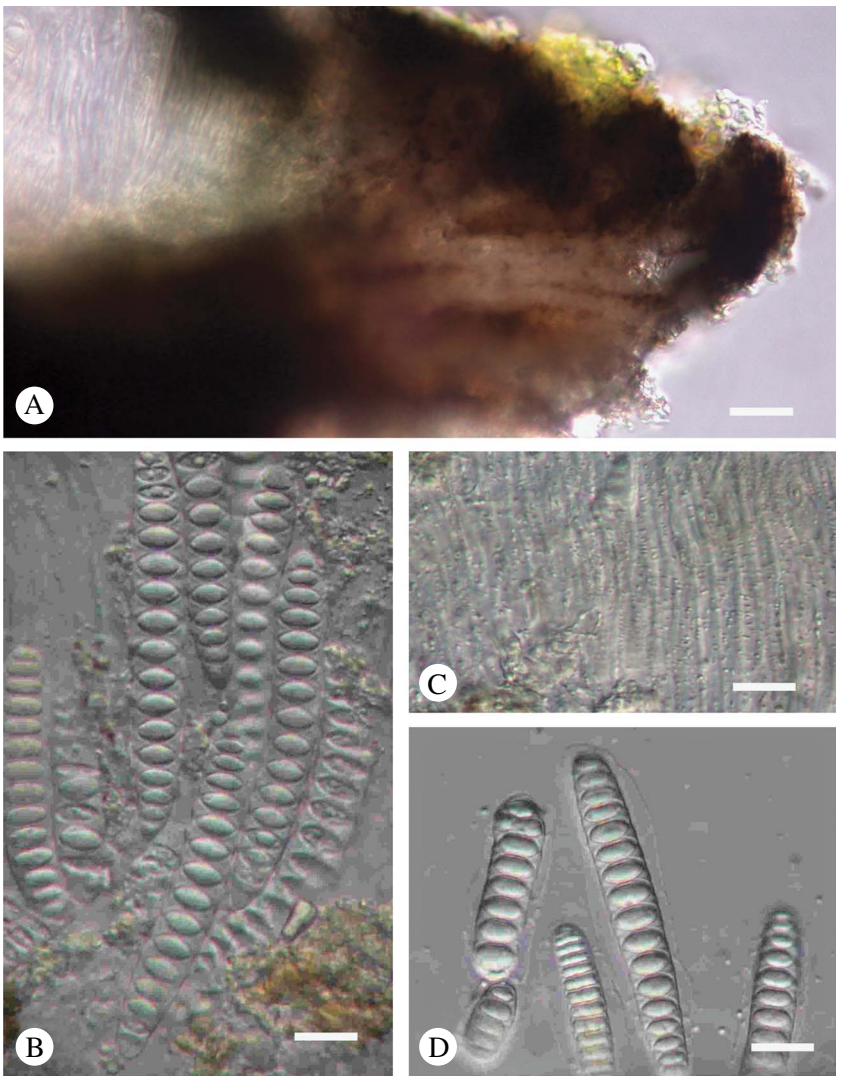

FIG. 2. A & B, *Graphis leptospora*, *Zaca* 3768; A, section through striate margin of lirella; B, ascospores. C, *G. lineola*, *Zaca* 7293, inspersed hymenium. D, *G. verminosa*, *Zaca* 8622, ascospores. Scales: A = 50 µm; B–D = 10 µm. In colour online.

The theory that the tropical graphidoid *Graphidaceae* arrived rather recently in Portugal in response to a warmer and wetter climate might be plausible. The climate in Portugal has become wetter and warmer in recent decades (Abiodun & Adedoyin 2015). Global warming has been reported to be the cause of major and rapid changes in the epiphytic lichen flora of other Atlantic regions in Europe (e.g. van Herk *et al.* 2002; Aptroot *et al.* 2015). The species reported here invariably have the bulk of their distribution in areas closer to the equator. In the case of Portugal, this means northern Africa or Macaronesia, or even further south. The principally tropical *Graphis* species that we are now reporting from Portugal are pantropical and generally occur in open areas

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such as coastal forests and dry tropical forests (Lücking *et al.* 2008), or on branches and twigs in rainforests.

Furthermore, all lichens with trentepohlioid phycobionts (the photobiont of all *Graphidaceae*) have been reported to have increased their range extensively as a result of climate change (Aptroot & van Herk 2007). That this colonization can occur very rapidly is shown in the Netherlands, where as many as three graphidoid *Graphidaceae* (viz. *Graphis inustuloides, Phaeographis dendritica* and *P. smithii*) were found new to the country one a year for the past three years. (van Dort & van der Pluijm 2013; van der Pluijm 2014, 2015), and all are establishing themselves in several widely separated places. It must be noted, however, that one species occurred before 1900 and the other was present until the 1970s. There is no doubt that they vanished completely in the intervening period, and are now recolonizing rapidly. Their former disappearance can probably be mostly attributed to air pollution, but the rapid recolonization in the past few years cannot be explained by this; changes in air pollution occur over a much longer timescale and, more importantly, there has been little change in the last decade.

Another possibility that cannot be ruled out is that some of these tropical species have inadvertently been introduced with plant material. Introduction of lichens on living trees has been demonstrated worldwide (e.g. Alstrup & Alstrup 1989; Galloway 1998; Aptroot 2011; Moncada *et al.* 2014) and may occur more often than generally thought. There even exists a monitoring programme (Sparrius *et al.* 2014) to follow the fate of these introductions. Such introductions may be increasing in these times of a global economy, now that it is cheaper and more profitable to grow trees in, for example, France or Poland and transport them to the Netherlands when mature, rather than to grow them *in situ*. However, trees have been introduced from all over the world in the past when a forest or park was established. Part of Sintra is a park and contains planted exotic trees, notably *Eucalyptus*. In fact, there are observations of tropical *Graphis* species being introduced into Europe. Aptroot (1993) reported the presence of *Graphis lineola* and *Glyphis cicatricosa* on a living *Dracaena* and similar observations have been documented in the literature.

It is unlikely that these tropical *Graphis* species have been ignored or overlooked in other European countries. Graphidoid *Graphidaceae* have been studied rather intensely in Europe (e.g. López de Silanes & Álvarez 2003; Carballal *et al.* 2004; Benfield *et al.* 2009; van Dort & van der Pluijm 2013; van der Pluijm 2014, 2015), and even more so in recent years after the publication by Neuwirth & Aptroot (2011) prompted the re-examination of *Graphis* material in many countries (e.g. Neuwirth 2013).

It cannot be ruled out that several mechanisms are at work to a greater or lesser extent, at the same time, to cause the relatively high diversity of *Graphis* in the study area. Whatever the cause, the forests of Portugal are, as far as is known, the biodiversity hot spot for graphidoid *Graphidaceae* in Europe.

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