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Noteworthy range extensions of two *Aneura* (Jungermanniopsida, Metzgeriales) species new for the Iberian Peninsula: *Aneura maxima* (Schiffn.) Steph. and *A. pseudopinguis* (Herzog) Pócs

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Abstract – The recognition of *Aneura maxima* (Schiffn.) Steph. in Iberian Peninsula and the discovery of *Aneura pseudopinguis* (Herzog) Pócs in Portuguese bryoflora are given. The identity of two misunderstood and unknown *Aneura* from Iberian Peninsula is clarified based in herbarium material. The morpho-anatomical observations carried out in herbarium samples are summarized in a table and illustrated. The geographic distribution of the two species is offered for the Iberian Peninsula. *A. pseudopinguis* is also reported new to the Western Africa.

***Aneura* / Hepaticae / Iberian Peninsula / Portugal / Spain / Western Africa**

INTRODUCTION

During the identification of *Aneura* material stored in LISU collections, two interesting discoveries were made: the recognition of *Aneura maxima* (Schiffn.) Steph. in the Iberian Peninsula and the discovery of *Aneura pseudopinguis* (Herzog) Pócs in the Portuguese bryoflora. *Aneura maxima* was reported for the first time in Europe during the 1990's and its presence was to be expected in the Iberian flora. *Aneura pseudopinguis* was restricted to Central Africa and Tropical America and its presence in the Iberian flora was not so predictable.

In the Iberian Peninsula, distribution data of *Aneura* are scarce not including in floristic works as in Basque region (Infante, 2000) and so far there is no taxonomic revision. So, this paper presents the first taxonomic revision based in selected herbarium material, with the aim to detect the presence of *Aneura maxima* in the Iberian territory.

Finally, we intend to give an accurate characterization of the tree species, although this data will be completed with new field work in the near future.

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MATERIAL AND METHODS

All herbarium material stored in LISU, COI and a selection of *Aneura pinguis* (L.) Dumort. specimens from Spain in BCB and MUB collections was studied using recent taxonomic criteria for this genus. To confirm the discovery of *A. pseudopinguis*, certain collections at LISU and COI from São Tomé e Principe and Angola were considered and studied. So, the morpho-anatomical studies were carried out only based in herbarium samples. A total of more than 50 dried herbarium specimens were studied (list of specimens annexed). Each specimen was examined, measured and some sections photographed. From each sample, normally three thalli and at least ten sections were observed. Thallus sections were made in the middle part of the thalli supported by the largest and/or fertile plants of the specimen. As oil bodies in dried plants disappear in a short period of time they were not considered in our observations. We considered the specimen sexual condition, however the plants are frequently without sporophytes, so we have observed only vegetative characters.

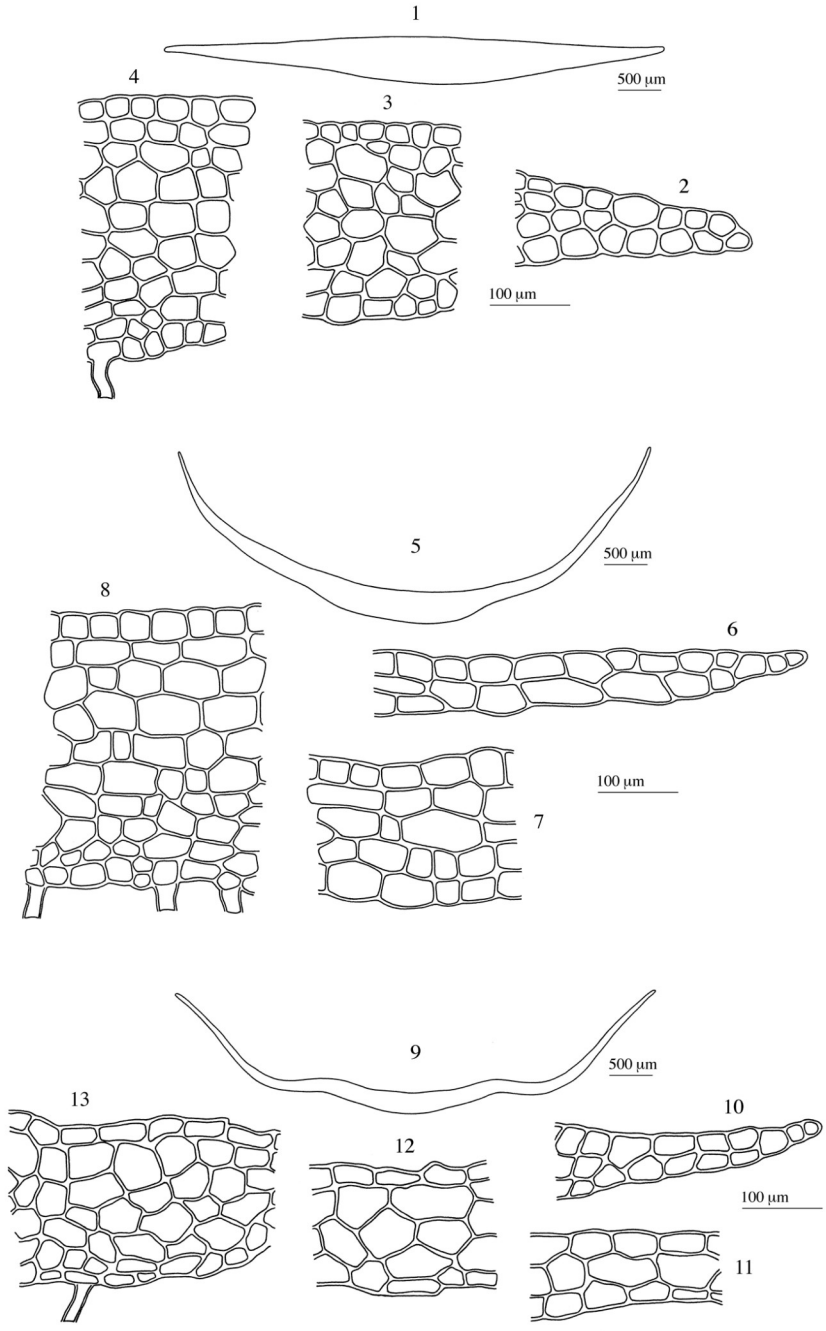
RESULTS

***Aneura maxima* (Schiffn.) Steph.** **(Figs 1-4)**

Aneura maxima is a species with an Asian-Eastern North American distribution, and it was discovered in Europe Belgium only in the 1990's by Andriessen *et al.* (1995). Its known range has been extended since then into different European countries: France (Sotiaux & Sotiaux, 1996), Finland (Frahm, 1997), Denmark (Thingsgaard, 2002), Luxemburg (Werner, 2003), Czech Republic (Kučera, 2004) and Poland (Buczkowska & Baczkiewicz, 2006). This expansion reflects the increase in the taxonomic knowledge since the first report in Belgium. So, *Aneura maxima* present a scattered, but quite wide distribution: was found outside North America (Darigo, 2004) from East Asia to Japan (Furuki, 1991). The European records are predominantly from Poland (Wachowiak *et al.,* 2007) nevertheless it was until recently only recorded from Finland to France (Vanderpoorten *et al.,* 2006). Therefore, one obvious reason for the few known localities of *Aneura maxima* is the rarity of fertile plants, as it is a dioicous species.

Bearing in mind that most morphological characters are variable in *Aneura* taxa, this renders the identification between the two European species sometimes difficult. The explanation for the high variability of *Aneura pinguis* was presented with the prediction of cryptic speciation in five distant regions in Poland (Wachowiak *et al.,* 2007). The plasticity of the species as an aquatic plant also contributes to this variability, although this study indicates that the *A. pinguis* complex is highly differentiated at molecular level. Recently new results of DNA analysis confirm the division of four cryptic species of *Aneura pinguis* in Europe, and also separate it from *Aneura maxima* (Baczkiewicz *et al.,* 2008).

In recent works, *A. maxima* is considered "conservation dependent" to "near threatened" within the category "lower risk" bryophyte (Vanderpoorten *et al.,* 2006) but it seems not very infrequent in Atlantic areas of Europe.



Figs 1-13. **1-4**. *Aneura pinguis* (L.) Dumort.; **5-8**. *Aneura maxima* (Schiffn.) Steph.; **9-13**. *Aneura pseudopinguis* (Herzog) Pócs. 1, 5 and 9, cross sections of thallus; 2, 6 and 10, cross sections of wing margin; 4, 8 and 13, cross sections of median part of thallus; 3, 7, 11 and 12, cross sections of wing (± 1/3).

To this moment *Aneura maxima* seems much more extended in Portugal than in Spain as in a total of 14 Spanish specimens studied only 5 correspond to this species. All others are *Aneura pinguis*. Data suggest a scattered distribution in Spain in relation to Portugal as have found 13 specimens of *A. maxima* to 14 *A. pinguis*. This may be due to the absence of spore dispersal, as the sporophytes are absent, makes infrequent *A. maxima*.

In general *Aneura maxima* has an Atlantic distribution and a recent modeling distribution in Belgium is noteworthy (Vanderpoorten *et al.,* 2006). However Thingsgaard (2002) has included this liverwort in a boreal-montane species assemblage.

***Aneura pseudopinguis* (Herzog) Pócs** **(Figs 5-8)**

The identification using Jones's criteria for African flora (e.g. Wigginton, 2004a) and with our own knowledge of African material (São Tomé Island) led to the recognition of these specimens as *A. pseudopinguis,* a liverwort new to Portugal and the European bryoflora. The thallus sections of Portuguese plants appear to have very few median cells thick in the thalli, features that are very distinct from both species of *Aneura* in Europe. Although gatherings of this species are somewhat insufficient and some only with few plants to permit observation of sexual features (only female plants), the thallus sections are totally similar to *A. pseudopinguis* from Africa.

According to Meenks & Pócs (1985), *Aneura pseudopinguis* is clearly related to *A. pinguis* but the differences between the two species are accepted as significant as very few median cells thick in the thalli. However, some characters correspond to fertile plants, male lobes and antheridium number and spore size. More recently Perold (2001) has considered that studied plants from South Africa are *Aneura pinguis,* but for this author is not meant to imply that *A. pseudopinguis* not occurs in this area. Gradstein & da Costa (2003) consider also the two species as independent taxa (*A. pseudopinguis* and *A. pinguis*). According to these authors, the first diagnostic differences are related to the structure of the sterile thallus.

In reality the distribution map of this species presented by Gradstein *et al.* (1983) is compared with other tropical Afro-American elements, but this distribution map does not include Western Africa. However, at present we can confirm the presence of *A. pseudopinguis* in Angola and in São Tomé Island (see list of studied material), as well as extend the occurrence of this liverwort to southwestern part of Europe (Fig. 15). According Wigginton (2004b) the African countries of occurrence of *A. pseudopinguis* are: Ivory Coast, Lesotho, Malawi, Rio Muni, South Africa, Seychelles, Tanzania, Uganda, Democratic Republic of the Congo and Zimbabwe.

Despite the apparent difficult in examining dry *Aneura* specimens, we have observed that the tree species have morphological differences that support the maintenance of their specific status. An updated synthesis of the main diagnostic characters of the tree species is now given, including illustrations and data on their possible ecology and distribution.

Aneura pinguis, *A. maxima* and *A. pseudopinguis* present simplicity on thallus organization with little anatomic differentiation, usually with some distinction between wings and midrib or nerve (the epithet *Aneura* indicates the absence of a nerve) but the thallus sections are quite different (Table 1).

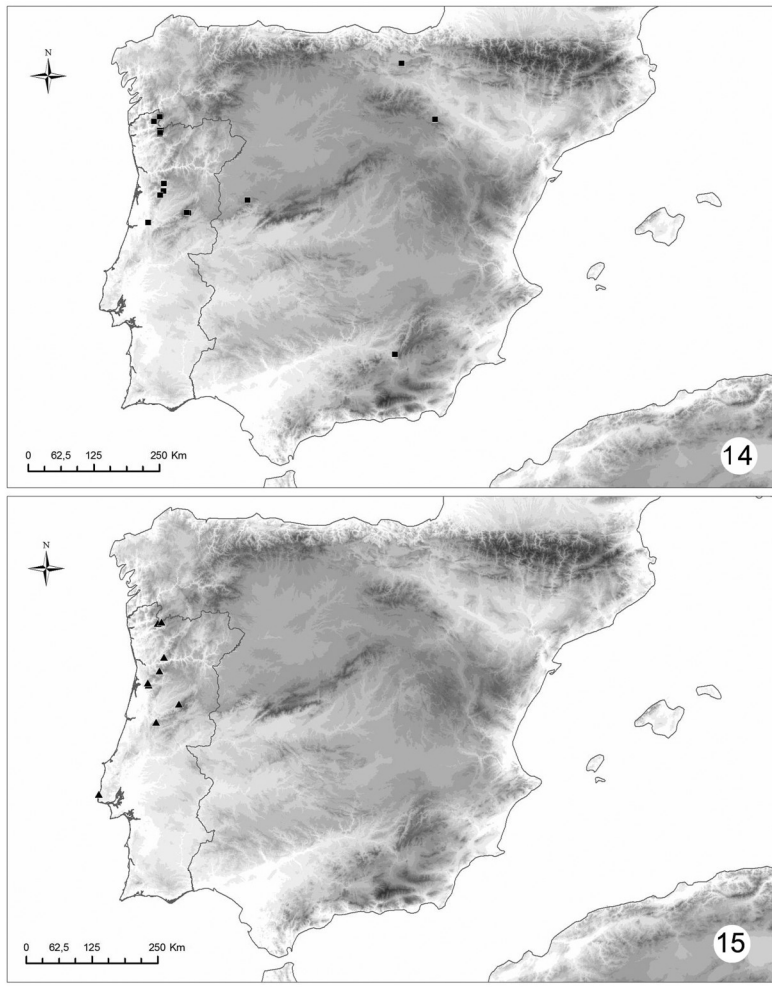
We have found a strong correlation between the features of sterile thalli and the dorsal surface cells of the thallus in *Aneura pseudopinguis*. In this species

Table 1. Differential morphological characters between *Aneura pinguis*, *A. maxima* and *A. pseudopinguis* (based in Iberian material), fertility and distribution pattern based in herbarium specimens (Figs 1-13 and 14-15).

Thallus characters	*Aneura pinguis*	*Aneura maxima*	*Aneura pseudopinguis*
Thallus cross section (clearness, form)	Thallus not translucent, without an apparent central midrib, margin plano-convex to biconvex with wings weakly recurved or plane.	Thallus margins translucent, with clear central midrib, plano-convex to concavo-convex with wings weakly incurved.	Thallus regularly translucent, very thin, with an incipient midrib, concavo-convex or plane with wings weakly undulate to flattened.
Number of middle cells thick	(9) 10-13 (15)	(8) 10-12 (14)	(5) 6-9 (10)
Wings (form and number of border cells)	Becoming gradually thinner to margin; margin obtuse in section to acute, bordered by 1-2 single uni-stratose row, and wing with 2, 3 to 4 stratus close to the margin; wing less than 10 cells wide.	Markedly thinner to margin; margin acute skinny bordered by 2-4 single uni-stratose row and wing totally 2, 3 to 4 stratose from the margin to near the central part; wing large more than 20 to 25 or more cells wide.	Becoming gradually thinner to margin; margin acute skinny bordered by 2-4 single uni-stratose row and wing totally 2, 3 to 4 stratose from the margin to near the central part; wing imperceptible as whole thallus is thin.
Epidermal cells in section	Quadrangular, isodiametric with firm walls, not collapsed.	Quadrangular, iso-diametric with firm not collapsed walls.	Rectangular, not iso-diametric, with flexible collapsed walls.
Fertility in Portuguese populations	Sometimes fertile developing capsula and spores (near 20% of observed specimens). Female plants apparently more frequent.	Never fertile and spores not observed. Female plants apparently more frequent.	Never fertile and spores not observed. Female plants apparently more frequent. (In African specimens male plants are more frequent).
Distribution in the Iberian Peninsula	It seems to be widespread in mountain zones in Iberian territory ascending to 3300 m, but down to 20 m in Atlantic areas.	It appears to be spread in mountain areas in the Northern half part of the Peninsula ranging from 200 to 2300 m.	Mainly in lowland, ascending to at least 800 in central Western part of Portugal. Not in Mediterranean enclaves.
(number of specimens studied)	(Portugal 14, Spain 9)	(Portugal 13, Spain 5)	(Portugal 12, Spain 0)

they are, in section, rectangular and not isodiametric with flexible walls, whereas in *A. maxima* and *A. pinguis* are quadrangular, isodiametric and with firm walls, not collapsed (Figs 1-13).

The morpho-anatomical observations carried out in herbarium Iberian samples are summarized and illustrated in table 1 and figures 1-13.



Figs 14-15. Iberian distribution of: **14**. *Aneura maxima* (Schiffn.) Steph.; **15**. *Aneura pseudopinguis* (Herzog) Pócs.

DISCUSSION

As explained above, the morphological differences among the three species are relatively easy to observe and it is not crucial to have fertile plants to distinguish each one. However this scheme for morpho-anatomical characters based only in herbarium material needs to be supported by fresh and if possible by fertile plants. The relationships with other taxa included within the synonymes of *A. maxima* need to be recognized. It is the example of *Aneura pellioides* (Horik.) Inoue from Japan, included by Schuster (1992) in the same complex of *A. maxima.*

We consider that the taxa arrangement of *Aneura pinguis s. lat*. requires a world-wide study, not only with molecular studies using European populations (Baczkiewicz *et al.,* 2008), but together with African and American material, to identify genetic similarity and its phylogeography.

The Iberian populations of *Aneura pseudopinguis* are considerably disjunct from those in Africa and South America. Thus, they are apparently geographically isolated, but this may merely reflect our poor knowledge of the hepatic flora of Northern Africa and/or also the Mediterranean region. On the other hand, a possible explanation for the current distribution of *A. pseudopinguis* in Portugal suggests that the populations survived in the area as refugia during the glacial episodes. In effect, numerous disjunct termophytic species occur in habitats that would likely have been available in such periglacial refugia.

In fact, the localities where *Aneura pseudopinguis* was found correspond to areas with a strong Atlantic influence, with moderate temperatures and a large amount of humidity.

We can point out some bryophytes with similar distribution in Portugal such as *Dumortiera hirsuta* (Sw.) Nees, *Lejeunea eckloniana* Lindenb., *Asterella africana* (Mont.) A.Evans. and *Racomitrium lamprocarpum* (Müll.Hal.) A.Jaeger. So *Aneura pseudopinguis*, recognized as a European taxon, needs to be included in analyses to confirm the similarity between different taxa of the complex *A. pinguis* and to support the possible Iberian vegetation histories.

Selection of studied specimens (Iberian Peninsula and Western Africa)

***Aneura maxima* (Schiffn.) Steph.**

Portugal

Beira Alta: S. Pedro do Sul, Rio Sul, 2 km a jusante da Vila, 08.1949, *E. Mendes 1209*, LISU221079; Serra da Estrela, estrada para o Poço do Inferno, 1020 m, 29TPE2572, 26.07.1995, *C. Sérgio & J. Jansen 10134*, LISU176458; Serra da Estrela, Fontes., 1430, 29TPE1470, 29.06.1996, *J. Jansen 96-074*, LISU177915; Serra da Estrela, Manteigas, entre a Senhora dos Verdes e os Covais, Queda de água junto da ponte do Aceiro, 1050 m, 29TPE2371, 02.07.2000, *C. Garcia*, LISU180996. – **Beira Litoral**: Coimbra, Corrente, 01.03.1968, *J. Ormonde* 431, COI10000005; Entre Águeda e Caramulo, pr. rio do Águeda, 22.02.1967, *J. Ormonde 438*, COI10000004. – **Minho**: Arcos de Valdevez, Rio Vez, 29TNG4541, 16.07.2004, *A. Albuquerque & Rodriguez-González*, LISU147533; Serra de Castro Laboreiro, 07.1949, *E. Mendes 1134*, LISU221063; Serra do Gerês, Albergaria, 700 m, NG72, 1982, *C. Sérgio 4046*, LISU153693; Serra do Gerês, Parque Tude de Sousa, 350 m, 1948, *I. Tavares*, LISU147533; Serra do Gerês, próximo da Bouça da Mó, 600 m, 1949, *C. Tavares*, LISU147535; Serra do Gerês, Ribeira da Lage, estrada para Leonte, 750 m, 29TNG72, 1984, *C. Sérgio & R. Schumacker 5330*, LISU153695.

Spain

Jaén: Sierra de Cazorla, Barranco de la Canal (Quesada), 1600 m, WG0382, 04.1996, *R.M. Ros & R. Monreal*, MUB14378. – **Salamanca**: Entre el Maíllo y Monsagro, 29TQE38-39, 1984, *Elías*, BCB19635. – **Zaragoza**: Sierra del Moncayo, Parque de Agramonte, 1100 m, 30TWM92, 02.06.1982, *R.M. Ros*, MUB6257. – **Álava**: Eguileta, puerto de Azáceta, 800 m, 30TWN3937, 14.01.1986, *P. Hera*s, MUB1686.

***Aneura pinguis* (L.) Dumort.**

Portugal

Beira Alta: Serra da Estrela, Casal do Rei, 400-450m, 29TPE0663, 25.01.1995, *C. Sérgio, M. Brugués & R.M. Cros,* LISU180107; Serra da Estrela, descida do Vale do Zêzere para Manteigas, 1300m, 29TPE2373, 25.07.1995, *C. Sérgio & J. Jansen 10113*, LISU176435; Serra da Estrela, Fonte da Maria Samarra, spring with *Chrysosplenium*, 860 m, 29TPE1273,

27.07.1995, *J. Jansen* 95-032, LISU177366; Serra da Estrela, Rapa, 600-650 m, 29TPE4093, 01.03.2000, *C. Garcia & C. Sérgio*, LISU181621; Serra da Estrela, from S. Romão, just after Ponte de Jugais, spring with *Chrysosplenium*, 550 m, 29TPE0971, 27.07.1995, *J. Jansen 95-033*, LISU177373. – **Beira Litoral**: Mata de Foja, 06.1880, *Moller 129,* COI10000010; Ribeira Fria, entre Albergaria-a-Velha e S. João de Loure, 23.04.1965, *A. Fernandes, J. Paiva & Cardoso 55*, COI10000008. – **Minho**: Serra do Gerês, a 1 km de Albergaria, estrada para a Geira Romana, 650 m, 29TNG72, 1984, *C. Sérgio & R. Schumacker 5392*, LISU153694; Serra do Gerês, entre Albergaria e Bouça da Mó, 650.m, 04.1949, *E. Mendes 401*, LISU221070; Serra do Gerês, Fonte do Rio do Forno, 04.1949, *E. Mendes 540 and 532*, LISU221065 and LISU2210766. – **Trás-os-Montes e Alto Douro**: Serra de Montesinho, Bragança, Vale da Coroa, 04.1953, *E. Mendes & C. Romariz 1414*, LISU2210769.

Spain

Alicante: Benifallim, Serra dels Plans, Barranc de Clots, 750 m, YH2482, 25.02.1993, *M.J. Cano & R.M. Ros*, MUB16982 & MUB5900; Callosa d'en Sarrià, Fonts del riu Algar, Font del Moro, 350 m, YH58, 08.02.1983, *Brugués, Cros, Sérgio & Sim-Sim 21.1*, LISU149834. – **Asturias**: Ballota, 29TQJ12, 1982*, C. Casas*, BCB34268. – **Cantabria**: Lamasón, Venta de Fresnedo, 30TUN79, 200 m, 1988, *Aedo* BCB45139; Camaleño, Vega de Tarna, 30TUN56, 1600 m, 1994, *Casas, Cros & Brugués*, BCB40977. – **Granada**: Sierra Nevada, nacimiento Río Veleta, 30SVG60, *Cros, Brugués & Sérgio*, BCB52501. – **León**: Ancares de León, Valle del Cuiña, PH84, 1100 m, 1984, *R.M. Cros & Lloret*, BCB19800. – **Lérida**: Vall d'Aran, Salardú, Vall de Ruda, 1850 m, 31TCH32, 05.09.1988, *C. Sérgio et al. 6491*, LISU155032. – **Tarragona**: Serra de Prades, Alforja, 31TCF36, 1952, *C. Casas* BCB18325; Els Reguers, Barranc de la Caramella, 290-480 m, BF61, 05.06.1984*, Casas*, *Sérgio, Brugués & Cros* 21.1, LISU150404.

***Aneura pseudopinguis* (Schiffn.) Steph.**

Portugal

Beira Alta: Serra da Estrela, Senhora do Desterro, junto ao Alva, 750 m, 1952, *C. Tavares*, LISU147534; Serra da Estrela. Senhora do Desterro, próximo da Ponte da Gaia, no vale do Alva, ± 800 m, 29TPE1072, 08.1954, *C. Tavares*, LISU183386. – **Beira Litoral**: Entre Pessegueiro do Vouga e Sever do Vouga, 02.12.1967, *C. Sérgio 418*, COI10000006; Sever do Vouga, Silva Escura, 29.12.1969, *C. Sérgio 879*, COI10000003; Serra da Lousã, Candal, 01.1950, *E. Mendes 1107*, LISU221064; Zombaria, pr. Coimbra, 1.01.1879, *Henriques 37*, COI10000009 (aff. *Aneura pseudopinguis* (Schiffn.) Steph.). – **Douro Litoral**: Próximo de Cinfães, Ribeira das Bichas, próximo de Baião, 09.1945, *F. Resende 1495*, LISU221072; Serra de Montemuro, próximo de Alvarenga, 400 m, 07.1949, *E. Mendes 1200*, LISU221071. – **Estremadura**: Azenhas do Mar, arribas junto à praia, 24.01.1999, *C. Garcia*, LISU225896. – **Minho**: Serra do Gerês, 10.1879, *M. Ferreira*, COI10000011; Serra do Gerês, entre Albergaria e Bouça da Mó, 04.1949, *E. Mendes 515*, LISU221068; Serra do Gerês, Ponte de Bacelo, 04.1949, *E. Mendes 372*, LISU221067.

São Tomé e Príncipe

Novo Destino, an altitude about 2500 ft., Welwitsch 267, LISU; Nova Moka, 900 m, 1885, *A. Moller* 57, COI; Picada para a Lagoa Amélia, 1400 m, 1975, *A. Sérgio* 94, LISU; estrada para Bombaim, junto à estrada para a picada de Zampalma, 700 m, 1975, *A. Sérgio* 36, LISU; Descida do Pico de São Tomé, caminho para Ponta Figo, 1109 m. 5.07.2007, *C. Garcia* Lev. 79, LISU; Descida do Pico de São Tomé, caminho para Ponta Figo, 802 m, 5.07.2007, *C. Garcia* Lev. 83, LISU.

Angola

Huila, 1887, *F. Newton*, COI.

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