



## The systematic and palaeoecological significance of *Neuropteris ovata* (Medullosales) cuticles from the Stephanian Stage of the Puertollano Basin, Spain

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### ABSTRACT

Cuticles from *Neuropteris ovata* foliage (Medullosales) from Stephanian strata in the Puertollano Basin show epidermal structures similar to those seen in Kasimovian coal balls from North America. They are assigned to a new taxonomic variety, *Neuropteris ovata* var. *puertollanensis*.

**Keywords:** Palaeobotany, Medullosales, cuticles, Puertollano, Stephanian.

### RESUMEN

Cutículas de *Neuropteris ovata* (Medullosales) obtenidas de materiales del Estefaniense de la cuenca de Puertollano muestran estructuras epidérmicas similares a las que se encuentran en *coal balls* del Kasimoviense de Norte América. Se han asignado a una nueva variedad, *Neuropteris ovata* var. *puertollanensis*.

**Palabras clave:** Paleobotánica, Medullosales, cutículas, Puertollano, Estefaniense.

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## 1. INTRODUCTION

*Neuropteris ovata* Hoffmann is one of the most widespread fossil-species of medullosalean foliage found in the Pennsylvanian (late Carboniferous) floras of the palaeotropical belt, having been reported from North America, Europe, North Africa and China (Jongmans & Dijkstra, 1961, 1968; Dijkstra & van Amerom, 1983). Its sudden appearance in the upper Westphalian Stage (middle Moscovian Stage) is used as an index to the base of the Westphalian D (“Asturian”) Substage (Laveine, 1977; Cleal, 1984). In Euramerica it then ranges up through towards the end of the Carboniferous System (e.g., Wagner & Álvarez-Vázquez, 2010), and in China into the Cisuralian Series (lower Permian – as summarised by Shen *in* Li, 1995).

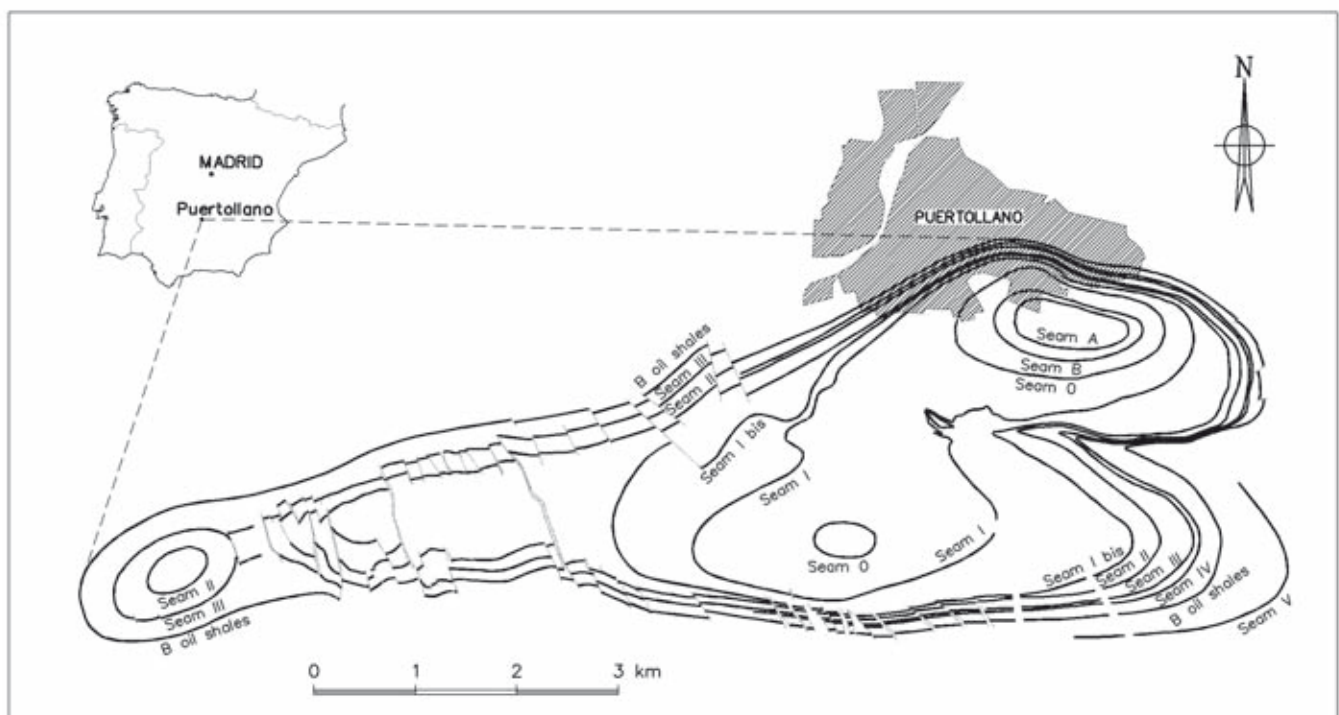
However, there is evidence of variation within the taxonomic concept of *Neuropteris ovata* that has resulted in the recognition of fossil-varieties, and even for the species itself to be subdivided. This was first suggested by Bertrand (1930), who distinguished a number of species within the traditionally-recognised *N. ovata* concept based on morphological criteria. Wagner (1963) later also used morphological criteria to argue that many of the Stephanian (Kasimovian – Gzhelian) *N. ovata* foliage was different from those found in the Westphalian Stage (including the types), naming the former *Neuropteris ovata* var. *grandeuryi* Wagner, 1963.

Cuticles and coal-ball studies have added support to the idea that *N. ovata* was a heterogeneous taxonomic concept (Gothan, 1915; Florin, 1925; Barthel, 1962; Beeler, 1983; Cleal & Zodrow, 1989; Cleal *et al.*, 1999). Most of these cuticles have been of Westphalian (Moscovian age), and so as to expand our understanding of the heterogeneity of epidermal structure in this species, we describe here newly prepared cuticles from Stephanian *N. ovata* from the Puertollano Basin in southern Spain.

## 2. GEOLOGICAL BACKGROUND

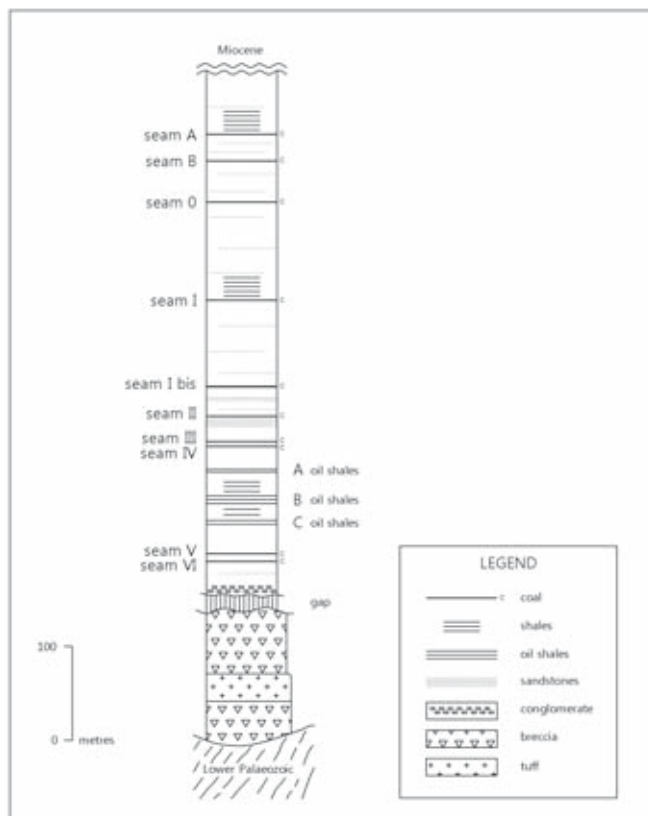
The fossils described in this paper were obtained from Stephanian strata of the Puertollano Basin, Ciudad Real province (southern Spain) (Fig. 1). This is a relatively small (ca. 50 km<sup>2</sup>) outlier of predominantly lacustrine strata including tuffaceous layers (Wagner, 1985, 1989; Wagner *et al.*, 2003; Wagner & Álvarez-Vázquez, 2015). Wagner (1985) and Wagner & Álvarez-Vázquez (2015) have suggested a Stephanian C (≡ Gzhelian) age for the deposits, but recent unpublished radiometric dating has indicated a somewhat older, at about “Saberian”/Stephanian B boundary (≡ late Kasimovian) age (S. Opluštil, pers. comm., 2015).

The fill of the basin is more than 450 m (Wagner, 1985). Economically important coal seams are named in



**Figure 1.** Map of the Iberian Peninsula with situation of the Puertollano Basin. Modified from Wagner & Álvarez-Vázquez (2015, text-fig.1).

ascending stratigraphical order III, II and I. The distance between coal seams III and II is about 30 m, and coal seams II and I is about 130 m. Coal seam III is 1.5 to 2 m thick and is recognised by a fossiliferous tuff parting. Coal seam II is about 3 m thick and contains thin clayey partings. Coal seam I is about 5 m thick and, in addition to a few very thin partings, contains two several decimetre thick partings (Fig. 2).



**Figure 2.** General stratigraphic column of the Puertollano Basin showing the position of coal seams. Modified from Wagner & Álvarez-Vázquez (2015, text-fig.2).

### 3. MATERIAL AND METHODS

Cuticles described in this paper were obtained from two sources. *In situ* cuticles were taken from adpressions identifiable as *Neuropteris ovata* collected from the roof shales of Coal seam II (Czech Geological Survey Collection, ZŠ 585 – ZŠ 595). They were prepared using the standard method (Cleal & Zodrow, 1989; Kerp, 1990; Cleal & Shute, 1991; Krings & Kerp, 1997a; Kerp & Krings, 1999). Small fragments of coalified phytoliteim were separated from the rock by hydrofluoric acid (HF) and then macerated for about 1 day in Schulze's reagent (20% HNO<sub>3</sub> with few crystals of KClO<sub>3</sub>). After washing, a few drops of 2% KOH were added to the macerated fragments in water in a Petri dish, but this tended to cause the cuticles to further fragment.

Dispersed cuticles were obtained from coal samples taken from two coal seams: from the upper part of Seam III (Sample 20; Slides 621/1-34) and approximately ¼ of the way up Seam I (Sample 12; Slides 638/1-25). These dispersed cuticles are very well preserved and can be compared directly with the fragments obtained from the adpressions. The coals were macerated using a modification of the method described by Šimůnek & Bureš (2015). A sample of about 3 g of coal was macerated in 40 ml Schulze's reagent (based on either concentrated or 55% diluted nitric acid) for ca. 2 days and 18 hours. The black residue was fully washed under running water in a sieve and then treated with 10% potassium hydroxide (KOH) for up to one hour. The resulting residue consisted mainly cuticles and vascular tissue.

The cuticles were stained in Safranin, Bismarck brown, Malachite green or Neutral red, and mounted in Glycerine Jelly, or were attached to an SEM stub for observation under a scanning electron microscope.

### 4. DESCRIPTIONS

**Gross morphology.** Fragments of ultimate and penultimate pinnae, and isolated pinnules. Penultimate rachis 2 mm wide, bears three fragments of ultimate pinna, < 80 mm long. Ultimate rachis 0.5-1 mm wide. Twenty-five isolated and attached pinnules (Figs 3a-3d), 6-18 mm long (mean 11.66 mm) and 3.5-8 mm wide (mean 5.84 mm); length/breadth ratio 1.6-2.33 (mean 1.99). Pinnules ovoid, lingulate, with broadly rounded apices and contracted at the base with typically an auricle on basiscopic side of the pinnule. Midvein not prominent, formed by several veins that individually continue in the upper half of the pinnule. Lateral veins extend obliquely from midvein, then bend and reach pinnule margin at 60-75° (Figs 3c-3d); vein density 40-50 per cm on pinnule margin.

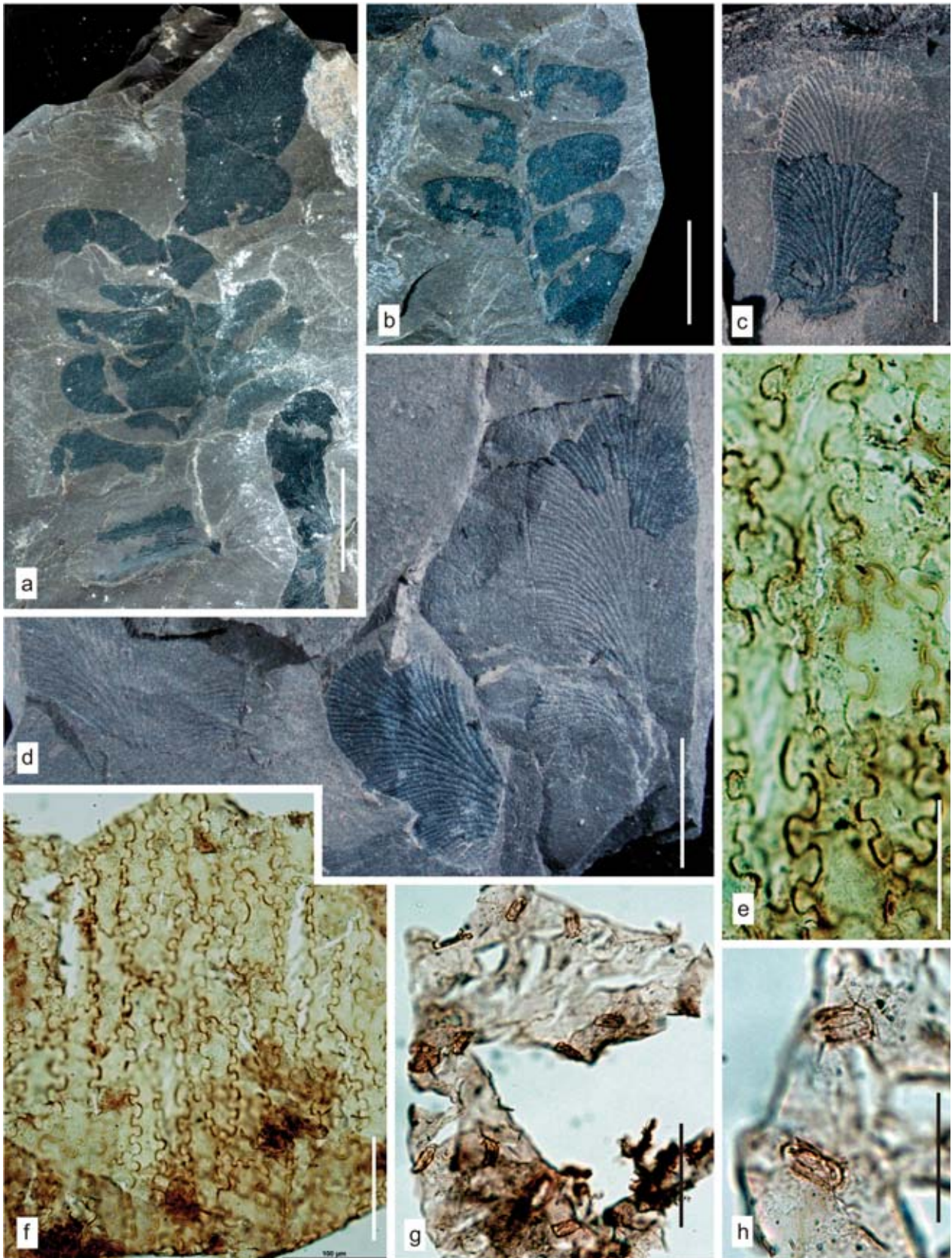
***In situ* cuticles.** Only small fragments of adaxial cuticle were obtained (Figs 3e-3f), showing elongate cells with strongly sinuous ( $\Omega$ ) anticlinal walls, oriented parallel to veins. Cells 100-150  $\mu$ m long, 30-50  $\mu$ m wide (due to sinuous margins).

One fragment of abaxial cuticle (Figs 3g-3h) has stomata with elliptical or oval guard cells, 25-30  $\mu$ m long, 12-15  $\mu$ m wide. The anticlinal cell walls are indiscernible.

**Dispersed cuticles.** In many cases, adaxial and abaxial cuticles were attached to a pinnule margin (Figs 4a-4c), confirming they belonged to *Neuropteris ovata* (Barthel, 1962; Cleal & Zodrow, 1989).

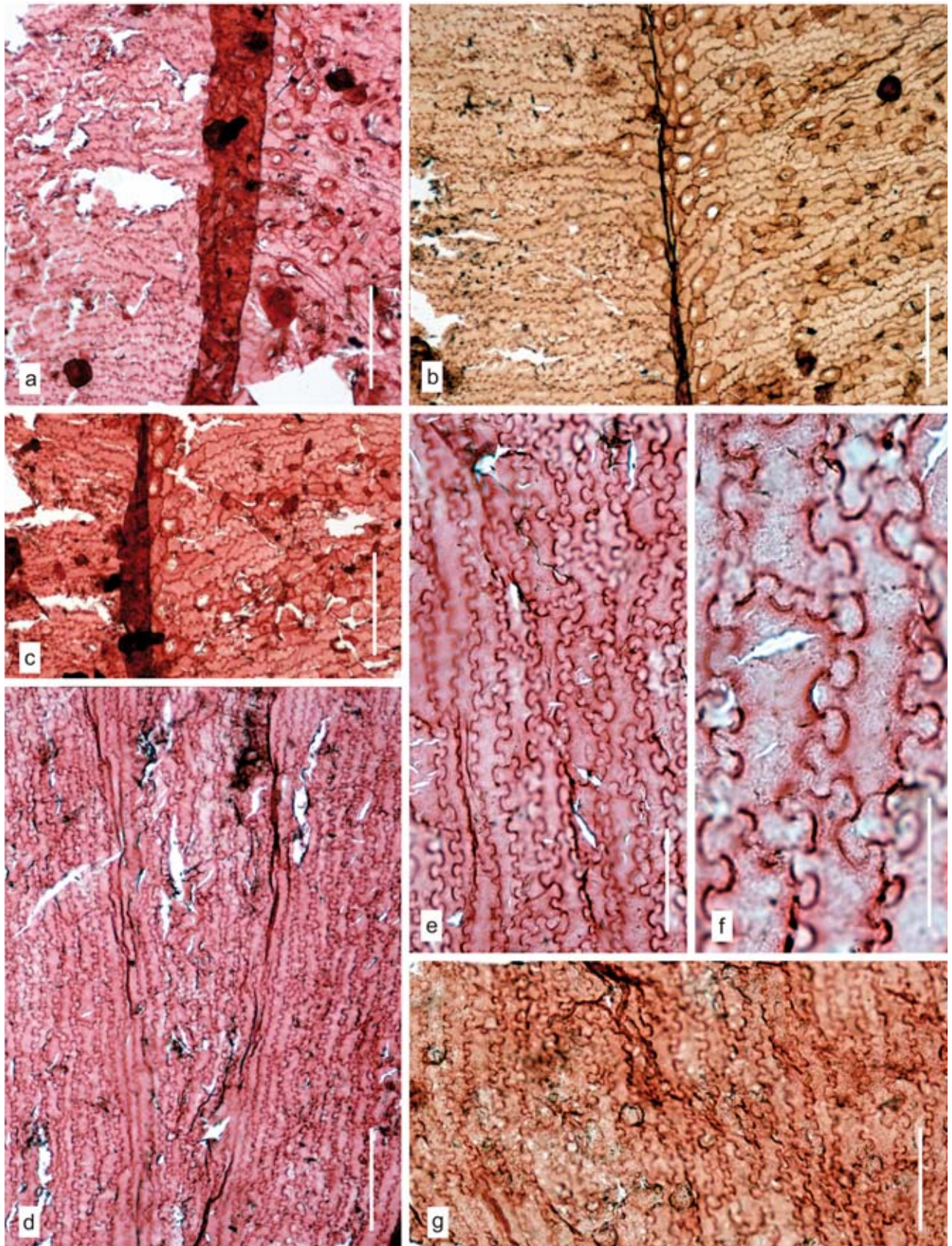
Epidermis differentiated between costal and intercostal fields on both adaxial (Fig. 4d) and abaxial (Figs 6b-6c, 6h) surfaces. Adaxial epidermis consisted of mostly





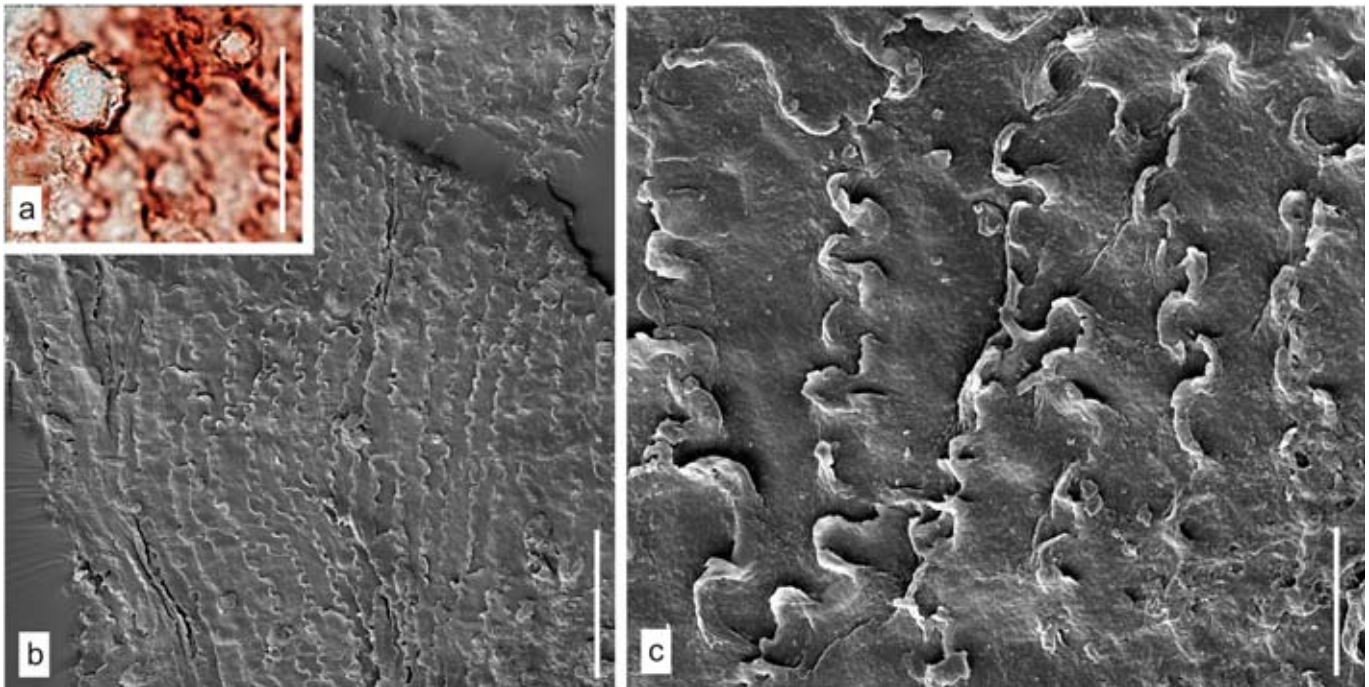
**Figure 3.** *Neuropteris ovata* var. *puertollanensis*, Puertollano, Emma quarry, Coal seam II, Stephanian. **a)** Holotype: fragment of an ultimate order pinna with terminal pinnule. Collection: Czech Geological Survey, Prague, No. ZŠ 585. Scale bar = 1 cm. **b)** Ultimate pinna fragment. Collection: Czech Geological Survey, Prague, No. ZŠ 586. Scale bar = 1 cm. **c, d)** Pinnule fragments used for maceration. Scale bar = 5 mm. **e, f)** Adaxial cuticle, slide 646/1. Scale bar: **(e)** = 100 μm, **(f)** = 50 μm. **g, h)** Abaxial cuticle with stomata, slide 646/4. Scale bar: **(g)** = 100 μm, **(h)** = 50 μm.





**Figure 4.** Cuticles of *Neuropteris ovata* var. *puertollanensis*, Puertollano, Emma quarry, Stephanian. **a-c)** Still attached adaxial (left) and abaxial (right) cuticles. Note the hydathodes near the margin of abaxial side. Coal seam III. **(a)** Slide 621/10; **(b)** Slide 621/18; **(c)** Slide 621/34. Scale bar = 200  $\mu\text{m}$ . **d-f)** Adaxial cuticle. Coal seam III, slide 621/10. Scale bar: **(d)** = 200  $\mu\text{m}$ , **(e)** = 100  $\mu\text{m}$  and **(f)** = 50  $\mu\text{m}$ . **g)** Adaxial cuticle with circular objects. Coal seam I, slide 638/14. Scale bar = 100  $\mu\text{m}$ .





**Figure 5.** Adaxial cuticles of *Neuropteris ovata* var. *puertollanensis*, Puertollano, Emma quarry, Coal seam III, Stephanian. **a)** Close up of a circular object from Fig. 4a. Scale bar = 50  $\mu\text{m}$ . **b, c)** SEM images of adaxial cuticles, stub 100. Scale bar: **(a)** = 100  $\mu\text{m}$ , **(b)** = 20  $\mu\text{m}$ .

subrectangular intercostal cells with long axes parallel to the veins, 60–100  $\mu\text{m}$  long, 20–50  $\mu\text{m}$  wide. Cell walls markedly sinuous, with “U” or “ $\Omega$ ” shaped walls (Figs 4f, 5a–5c). Costal cells narrower and more elongate, 100–200  $\mu\text{m}$  long, usually 15–20  $\mu\text{m}$  wide. No trichomes observed.

Abaxial intercostal cells mostly polygonal with gently to strongly curved anticlinal walls; cells generally 30–40  $\mu\text{m}$  in diameter, but some are elongated, up to 80  $\mu\text{m}$  long and only 15–20  $\mu\text{m}$  wide. Costal cells more subrectangular and elongate, 80–250  $\mu\text{m}$  long, 15–30  $\mu\text{m}$  wide. Midvein represented by a few hundred  $\mu\text{m}$  wide costal field (Fig. 7g) with parallel oriented, tetragonal cells, mostly with straight anticlinal walls; cells 60–140  $\mu\text{m}$  long, 10–30  $\mu\text{m}$  wide.

Trichomes and trichome bases occur on both, costal and intercostal fields of abaxial surface (Figs 6a–6i, 7e–7h, 8g–8k). Trichome bases are nearly circular, ca. 32–45  $\mu\text{m}$  in diameter (Figs 6a, 6d, 6g, 6i, 8g, 8i), or elongated-oval, up to 70 (100)  $\mu\text{m}$  long. Trichomes are multicellular files, 100–300  $\mu\text{m}$  long, 40  $\mu\text{m}$  wide, formed of up to six cells and narrowing towards the apex (Figs 6e–6f, 6h, 8h–8i). Along midvein, trichome bases are on darker cells up to 75  $\mu\text{m}$  long, each with a hole 30  $\mu\text{m}$  long, 10  $\mu\text{m}$  wide.

Randomly oriented, sunken stomata occur throughout intercostal fields; guard cells 25–32  $\mu\text{m}$  long, 14–20  $\mu\text{m}$  wide. Stomata mainly brachyparacytic (Cleal & Zodrow, 1989). Polar areas of guard cells not cutinised (Figs 6d, 6f, 6h–6i, 7i, 8a–8f).

Cells with rounded to oval holes 20–27  $\mu\text{m}$  in diameter occur in abaxial cuticle near pinnule margin (Figs 4a–4c, 7a–7d); these are interpreted as hydathodes.

Stomatal densities (SD) and stomatal indices (SI) were counted from four cuticular fragments from each specimen:

Sample 621: SD 288–352 (mean = 321) stomata per  $\text{mm}^2$ ; SI ca. 24.

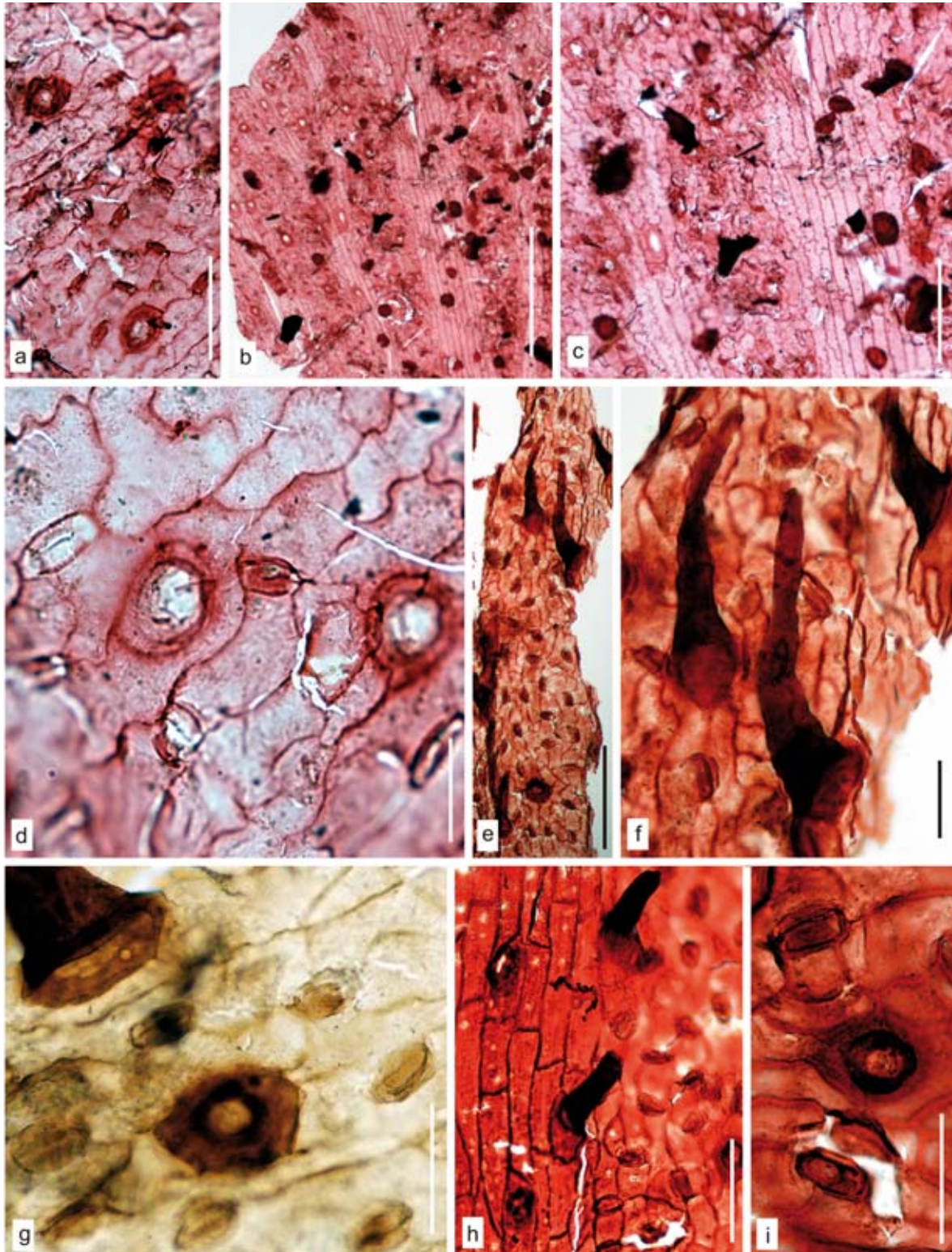
Sample 638: SD 257–308 (mean = 280) stomata per  $\text{mm}^2$ ; SI ca. 23.

One apparently anomalous specimen with many small cells around trichome bases, has an SI of 19.

## 5. COMPARISONS

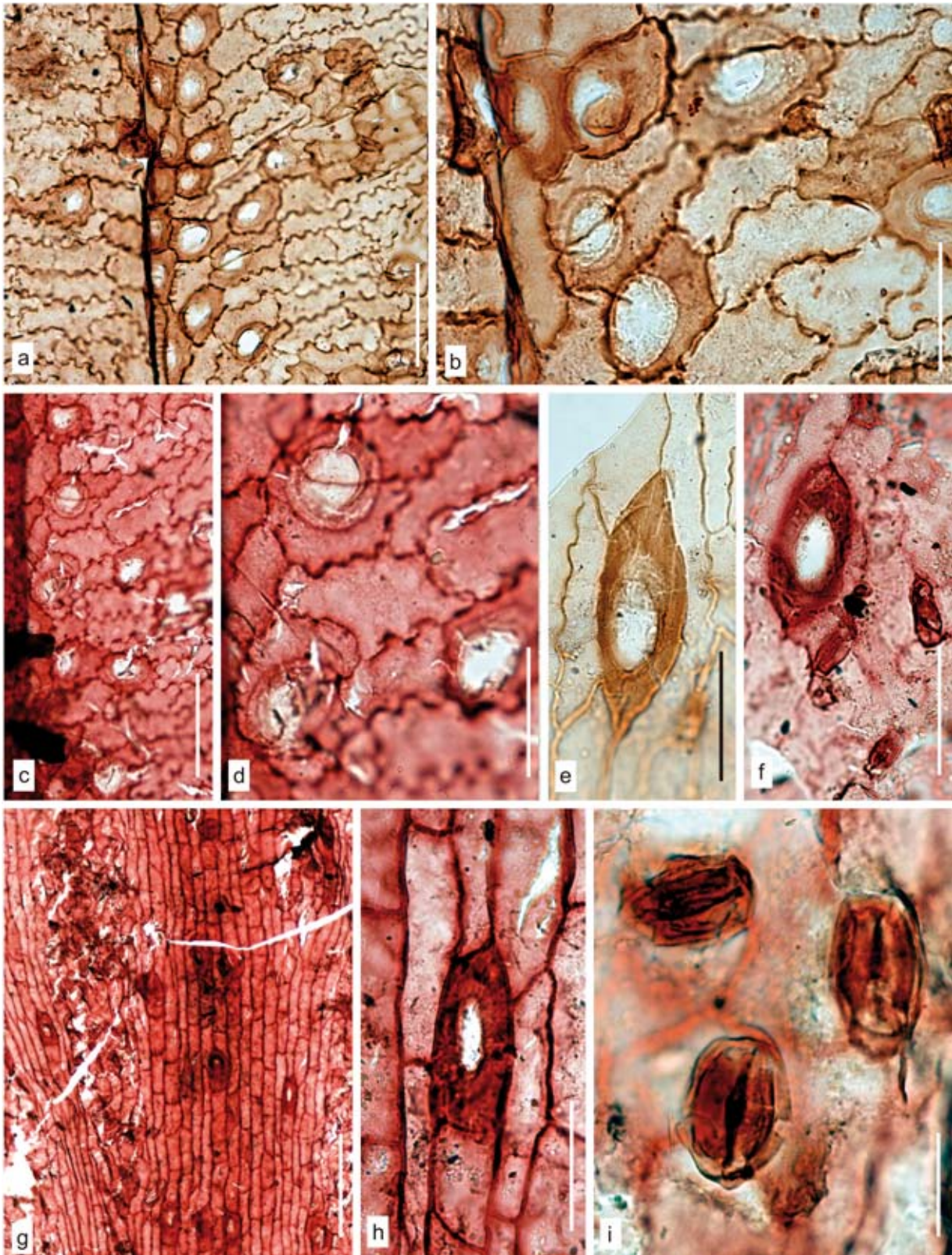
The characteristic features of the five main varieties of *Neuropteris ovata* currently recognised, together with these of the Puertollano specimens, are summarised in Table 1. The cuticles from the Puertollano specimens have many features in common with most of the Moscovian cuticles assigned to *N. ovata* from Saarland and Nova Scotia (Gothan, 1915; Barthel, 1962; Cleal & Zodrow, 1989; Cleal *et al.*, 1999). Most notable are the sinuous anticlinal walls on the adaxial surface, and the anomocytic to brachyparacytic stomata, hydathodes and prominent trichomes on the abaxial surface. Also very similar





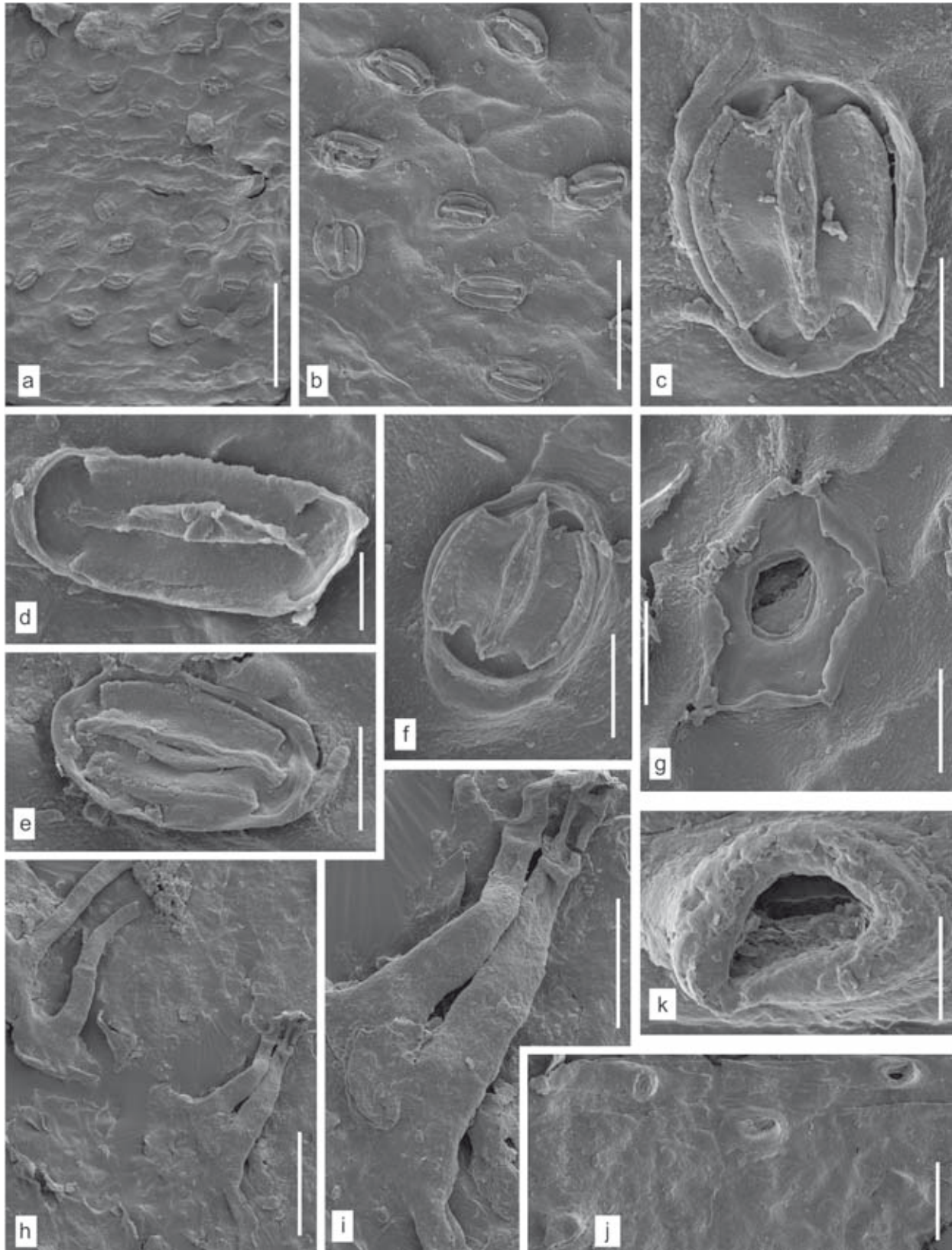
**Figure 6.** Abaxial cuticles of *Neuropteris ovata* var. *puertollanensis*, Puertollano, Emma quarry, Stephanian. **a)** Abaxial cuticle with stomata and two hair bases from the cuticle on Fig. 4a. Scale bar = 100  $\mu\text{m}$ . **b)** Abaxial cuticle with costal and intercostals fields distinguished. Coal seam III. Slide 621/11. Scale bar = 500  $\mu\text{m}$ . **c)** Close up of Fig. 6b with trichome bases in the costal fields and stomata in the intercostal fields. Scale bar = 200  $\mu\text{m}$ . **d)** Close up of Fig. 6c with two trichome bases and stomata in the intercostal field. Scale bar = 50  $\mu\text{m}$ . **e)** Abaxial cuticle with trichomes and stomata. Coal seam I. Slide 638/13. Scale bar = 500  $\mu\text{m}$ . **f)** Close up of Fig. 6e with three trichomes and stomata. Scale bar = 50  $\mu\text{m}$ . **g)** Trichome bases and stomata. Coal seam I. Slide 638/5. Scale bar = 50  $\mu\text{m}$ . **h)** Costal field with trichomes and trichome bases and intercostals field with stomata. Coal seam I. Slide 638/12. Scale bar = 100  $\mu\text{m}$ . **i)** Close up of Fig. 6h with a trichome bases and two stomata. Scale bar = 50  $\mu\text{m}$ .





**Figure 7.** Abaxial cuticles of *Neuropteris ovata* var. *puertollanensis*, Puertollano, Emma quarry, Stephanian. **a)** Hydathodes on the margin of abaxial cuticle (a hydathode is on the adaxial cuticle). Detail from Fig. 4b. Scale bar = 100  $\mu\text{m}$ . **b)** Close up of several hydathodes from Fig. 7a. Scale bar = 50  $\mu\text{m}$ . **c)** Margin of abaxial cuticle with hydathodes from Fig. 4c. Scale bar = 100  $\mu\text{m}$ . **d)** Close up of Fig. 7c with three hydathodes. Scale bar = 50  $\mu\text{m}$ . **e, f)** Details of trichome bases. Coal seam III. Slides: **(e)** 621/7, **(f)** 621/31. Scale bars = 50  $\mu\text{m}$ . **g)** Costal field (midvein) with trichome bases. Coal seam III. Slide 621/33. Scale bar = 200  $\mu\text{m}$ . **h)** Close up of Fig. 7g with a trichome base. Scale bar = 50  $\mu\text{m}$ . **i)** Detail of abaxial cuticle with 3 stomata. Slide 638/13. Scale bar = 20  $\mu\text{m}$ .





**Figure 8.** Abaxial cuticles of *Neuropteris ovata* var. *puertollanensis* in SEM, Puertollano, Emma quarry, Coal seam III. Stephanian. SEM stub 100. **a)** Inner view to the cuticle with stomata. Scale bar = 100  $\mu\text{m}$ . **b)** Close up of stomata from Fig. 8a. Scale bar = 50  $\mu\text{m}$ . **c)** Close up of a stoma from Fig. 8b. Scale bar = 10  $\mu\text{m}$ . **d-f)** Close up of stomata. Scale bar: **(d)** = 5  $\mu\text{m}$ , **(e)** and **(f)** = 10  $\mu\text{m}$ . **g)** Inner view to a trichome basis from Fig. 8a. Scale bar = 20  $\mu\text{m}$ . **h)** Outer view to the abaxial cuticle with trichomes. Scale bar = 100  $\mu\text{m}$ . **i)** Close up of two stomata from Fig. 8h. Scale bar = 50  $\mu\text{m}$ . **j)** Outer view to the cuticle with four trichome bases. Scale bar = 50  $\mu\text{m}$ . **k)** Close up of a trichome base from Fig. 8j. Scale bar = 10  $\mu\text{m}$ .



**Table 1.** Key characteristics for differentiating the main varieties of *Neuropteris ovata*. Sources of data: <sup>1</sup>Saltzwedel (1969); <sup>2</sup>Cleal & Zodrow (1989); <sup>3</sup>Wagner (1963); <sup>4</sup>present paper.

	var. <i>ovata</i> <sup>1</sup>	var. <i>simonii</i> <sup>2</sup>	var. <i>sarana</i> <sup>2</sup>	var. <i>aconiensis</i> <sup>2</sup>	var. <i>grandeuryi</i> <sup>3</sup>	var. <i>puertollanensis</i> <sup>4</sup>
Typical pinnule form	Subrectangular to ovoid	Subrectangular to ovoid	Subrectangular to ovoid	Subrectangular to ovoid	Squat subrectangular	Subrectangular to ovoid
Vein density (per cm)	45–55	35–45	40	40	65–75	40–50
Orientation of veins to pinnule margin	Obtuse	Oblique	Oblique	Oblique	Obtuse	Oblique
Apical pinnule	Large, subrhomboidal	Small, ovoid	Large, subrhomboidal	Small, ovoid	Large, subrhomboidal	Large, subrhomboidal
Adaxial epidermal cell walls	—	Sinuuous	Sinuuous	Straight	—	Sinuuous
Stomatal orientation	—	Parallel to veins	Parallel to veins	Parallel to veins	—	Random
Stomatal bands	—	Broad	Narrow	Broad	—	Broad

are the stomatal density and index. However, there are differences, most notably in the more random orientation of the stomata in the Puertollano cuticles. The latter is in fact quite different from other neuropterids, in which the stomata tend to be oriented parallel to the veins (Cleal & Shute, 2012) and is more comparable to what is seen in *Odontopteris* cuticles (Barthel, 1961, 1962; Krings, 1997; Krings & Kerp, 1997b; Kerp & Krings, 2003; Šimůnek & Cleal, 2004; Cleal *et al.*, 2007).

Given the late stratigraphical occurrence of the Puertollano specimens, it might be expected that they would belong to *N. ovata* var. *grandeuryi* Wagner, as this is the form that normally occurs in strata of this age in Spain (Wagner & Álvarez-Vázquez, 2010). As pointed out by Wagner (1985), however, the Puertollano specimens differ from the typical var. *grandeuryi* in having veins that are less dense and a little more oblique to the pinnule margin; in this they compare more closely morphologically with some of the varieties more typically found in late Moscovian floras, such as var. *simonii* and var. *sarana* (see Cleal & Zodrow, 1989). Wagner (1985) mentioned that he had also seen *N. ovata*-like foliage similar to that at Puertollano in a Stephanian C (Gzhelian) flora at El Bierzo in NW León. Wagner (1985) suggested that this may be reflecting a reversion in these very late examples of *N. ovata* (at least for Euramerica) to morphologies seen in the earlier, Moscovian forms. Alternatively, the differences may be reflecting differences in substrates on which the plants were growing: the Puertollano fossils probably represent plants growing in more organic-rich, if not peaty substrates (Wagner, 1985) whereas *N. ovata*

var. *grandeuryi* adpressions occur in the type of leafbeds that are widespread in Pennsylvanian coal-bearing sequences and probably represent plants growing in somewhat-better drained, clastic substrates (Gastaldo *et al.*, 1995). This distinction is also supported by the *N. ovata* pinnules preserved in Kasimovian (Stephanian B) coal balls from Ohio (USA) (Beeler, 1983), which must also have been from plants growing on peaty substrates: they have pinnules of similar shape and nervation density, and seem to have randomly orientated stomata.

We therefore propose that these distinctive *N. ovata* foliage, probably from plants growing on peaty or organic rich substrates in the Euramerican coal swamps, should be assigned to a separate, new variety.

## 6. SYSTEMATIC PALAEOLOGY

Order MEDULLOSALES Corsin, 1960

Family **Neurodontopteridaceae** Laveine *ex* Cleal & Shute, 2003

Fossil-genus *Neuropteris* (Brongniart, 1822) Sternberg, 1825 (= *Filicites* (*Nevropteris*) Brongniart, 1822)

Type species *Neuropteris heterophylla* (Brongniart, 1822) Sternberg, 1825 emend. Cleal & Shute, 1991 (= *Filicites* (*Nevropteris*) *heterophyllus* Brongniart, 1822)

**Remarks.** We have followed the generic diagnosis as emended by Cleal & Shute (1991). A slightly modified



diagnosis was given by Laveine (2008), which omitted any mention of epidermal characters. However, a recent review of medullosalean foliage anatomy (Cleal & Shute, 2012) has shown that such characters are of taxonomic value and so we have retained the Cleal & Shute (1991) diagnosis.

*Neuropteris ovata* Hoffmann, 1826

*Neuropteris ovata* Hoffmann var. *puertollanensis*

Šimůnek & Cleal var. nov.

**Type.** Specimen No. ZŠ 585, Czech Geological Survey, Prague (Fig. 3a).

**Derivatio nominis.** Named after the Puertollano Basin where the type originated.

**Diagnosis.** Auriculate, subrectangular to ovoid pinnules, 6–20 mm long, 3–10 mm wide, rounded apex, constricted base. Weak midvein; fine lateral veins meet pinnule margin at 60–80°; marginal vein density typically 40–50 per cm. Adaxial epidermal cells differentiated between coastal and intercostal fields, with more or less sinuous anticlinal walls. Stomata brachyparacytic, restricted to intercostal fields of abaxial surface, randomly orientated.

## 7. CONCLUSIONS

The specimens of the medullosalean foliage *Neuropteris ovata* found in the Stephanian mudstones of the Puertollano Basin (southern Spain) differ from those found in Stephanian floras of Europe, and are morphologically more similar to those found in the older, Westphalian floras, such as in Saar-Lorraine. However, the cuticles of the Puertollano specimens are different from those found on the Westphalian fronds and provide further evidence of the taxonomic (? varietal) heterogeneity of *N. ovata*. To understand the significance of this heterogeneity – whether it is of phylogenetic or ecological (perhaps climatic or substrate) significance – requires yet more data. Particularly informative would be evidence of the epidermal structure of some of the other Stephanian age forms of *Neuropteris ovata*, especially those referred to as var. *grandeuryi*.

The similar stomatal density and index values for these specimens and those reported from Moscovian *N. ovata* might also be regarded as unexpected, given that post-Moscovian times are widely regarded as representing “greenhouse” conditions with higher levels of atmospheric CO<sub>2</sub> (Gastaldo *et al.*, 1996; Cleal & Thomas, 1999, 2005; Cleal *et al.*, 1999). However, various other factors in addition to atmospheric CO<sub>2</sub> can affect stomatal development (e.g., McElwain & Chaloner, 1996; Poole

*et al.*, 1996) and the more peaty substrates on which the Puertollano *N. ovata*-bearing plants may have resulted in a reduced stomatal density. Alternatively, Gzhelian times may have seen the re-introduction of ice-house conditions and a concomitant reduction in atmospheric CO<sub>2</sub> (e.g., Montañez & Poulsen, 2013), which would also potentially produce an increase in stomatal densities nearer to those seen during the Moscovian glacial pulse (Cleal *et al.*, 1999).

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