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Suturovagina intermedia (Cheirolepidiaceae) from the Lower Cretaceous Dalazi Formation of Wangqing, Northeast China: cuticle ultrastructure and palaeoenvironmental insights

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Abstract The Cheirolepidiaceous conifer *Suturovagina intermedia* Chow et Tsao is reported from the Lower Cretaceous of Wangqing, Jilin Province in northeastern China, and its leaf cuticles were studied in detail using scanning and transmission electron microscopies and EDS. The data obtained were compared with those of the previous study on the same species from the Lower Cretaceous of the type locality Nanjing, Jiangsu Province in eastern China. Despite the general similarity in gross morphology and leaf cuticular structure, there are a number of differences in leaf cuticular ultrastructure and element composition (EDS data)

between specimens from the two localities. Among them, 14 differences are potential taxonomical characters. Of them, 7 might be of importance at the species/genus level, and 7 are possible characters at the family level. There are also 23 differences, i.e., 7 cuticle ultrastructural characters of ordinary epidermal cell cuticles and 5 subsidiary cell cuticles, plus 11 EDS ratios of cuticles, believed to be of ecological significances. This paper presents the first case to make comparative palaeoenvironmental analyses based on leaf cuticle ultrastructure and element composition of the same taxon from different areas. It shows that *Suturovagina intermedia* was an inhabitant of a lower xerothermic environment in Wangqing, instead of being a higher xerothermic environment, such as in the type locality Nanjing. The conclusion obtained is well-supported by the evidence of the element composition (EDX data) of the matrix and the associated biotic composition of the fossil-bearing beds from the two localities. It is the first attempt to search for the environmental footprint in fossil plant cuticles based on their element composition.

Key words: Cheirolepidiaceae, *Suturovagina*, cuticle ultrastructure, palaeoenvironment, EDS analyses

- **Introduction**

The extinct conifers Cheirolepidiaceae are known from the Late Triassic up to the Late Cretaceous and display quite a remarkable range of morphology, habits, and habitats (Watson, 1988). During the last few decades, researchers have revealed an extensive morphological variation, and a geographically and stratigraphically widespread fossil record of this group (Pole, 2000; Kunzmann et al., 2006; Tosolini et al., 2015; Hieger et al., 2015; Escapa et al., 2012; Escapa and Leslie, 2017). In China, since Chow and Tsao (1977) first recorded this important Mesozoic coniferous family, about fifty cheirolepidiaceous taxa have

been described (Zhou, 1995; Deng et al., 2005; Yang et al., 2006; Yang and Deng, 2007; Yang, 2008; Yang et al., 2009; Sun et al., 2010; Du et al., 2014).

The monotypic genus *Suturovagina* with the type species *S. intermedia* Chow et Tsao is endemic to China (Chow and Tsao, 1977; Zhou, 1983). It was instituted based on abundant material collected from the upper Lower Cretaceous Gecun Formation of Nanjing, Jiangsu Province, East China, consisting of heterophyllous leafy shoots, dispersed secondary xylem of *Protopodocarpoxylon*-type, and an associated male cone *Classostrobus cathayanus* Zhou (1983) yielding *Classopollis* pollen. *Suturovagina* was considered to be distinguished from *Pseudofrenelopsis* and other cheirolepidiaceous taxa mainly by having shoots characterized by predominant "open-sheathed" leaves and a characteristic stomatal apparatus (Zhou, 1983). Watson (1988), however, ascribed it to *Pseudofrenelopsis*, as individual shoots bearing "open-sheathed" leaves also exist in some species of *Pseudofrenelopsis*, such as *P. varians* (Fountaine) Watson and *P. parceramosa* (Fontaine) Watson (Alvin, 1977, 1982; Watson, 1977). Recently, the taxonomically controversial genus *Suturovagina* was restudied by ultra-thin sectioning and examination under scanning and transmission electron microscopes (Mairot et al., 2014). The new study shows that even though there exists a slight overlap in the gross morphology of leafy shoots between the two genera, *Suturovagina* is well distinguished from *Pseudofrenelopsis* and all other cheirolepidiaceous conifers in the micro- and ultrastructure of cuticles and stomata, especially in having very deep stomatal chambers composed of tiers of subsidiary cell circles (Mairot et al., 2014).

In addition to the type species from the upper part of the Lower Cretaceous Gecun Formation in Yanziji and Qixia districts, Nanjing City, Jiangsu Province (Zhou, 1983), *Suturovagina* has also been recorded from the upper Lower Cretaceous Dalazi Formation of Longjing, Jilin Province (Zhang et al., 1980) and the upper Lower Cretaceous Pulandian Formation of Pulandian, Liaoning Province (Li, 1992) (Fig. 1). The heterophyllous shoots of

Suturovagina intermedia, as recognized by Zhou (1983), bear three different types of leaves (Fig. 2): loosely arranged scale leaves (Type 1), close-sheathed leaves encircling the stem but interrupted by a suture (Type 2), or broad, scale-like to open-sheathed leaves (Type 3). The leaves borne on the shoot of *S. intermedia* from Longjing seems to belong to Type 2 (Zhang et al., 1980). The present author (X-J Yang) has recently procured new collections from the upper Lower Cretaceous Dalazi Formation in Wangqing County (not the type section of the Dalazi Formation in Longjing County), Jilin Province, which contain, besides well preserved *S. intermedia*, some other coniferous leafy shoots, pollen cones, and ovulate cones. Of them, another Cheirolepidiaceous conifer *Pseudofrenelopsis gansuensis* Deng, Yang et Lu (Deng et al., 2005) has already been studied in detail by using ultra-thin sectioning and examination under scanning and transmission electron microscopes (Yang and Deng, 2007; Guignard et al., 2017).

So far, there are very few studies about the palaeoenvironmental impacts on the cuticle ultrastructure of fossil plants (see Guignard et al., 2001 on sun and shade leaf cuticles of pteridosperm *Komlopteris nordenskioeldii*). The sensibility of cuticle ultrastructure to environmental factors has also been observed in the extant *Pinus* and *Erica* (Bartiromo et al., 2012, 2013). The purpose of the present paper is to study the leaf cuticle ultrastructure of *Suturovagina intermedia* from Wangqing County in detail. In addition, elemental analyses (Energy Dispersive Spectroscopy EDS and Energy Dispersive X-ray Spectroscopy EDX) are used respectively in studying the cuticles of this species, and the rock matrix of the plant-bearing beds. The results are further compared with those of the previous studies on the same species from a different palaeoenvironment in the type locality Nanjing, Jiangsu Province of eastern China to evaluate the potential environmental significance of cuticle ultrastructure and any differences in chemical composition between them.

2. Material and methods

The material studied in this paper was collected from a stratigraphical section in Luozigou Town, Wangqing County, Jilin Province, northeastern China (43°53′48″N, 130°03′26″E) (Fig. 1). The fossiliferous beds have been correlated with the Dalazi Formation in the Dalazi village, Zhixin Town, Longjing County, which is approximately 150 km southwest of Luozigou and is considered to be late Early Cretaceous in age (Zhou et al., 1980; Bureau of Geology and Mineral Resources of Jilin Province, 1997). Several cheirolepidiaceous conifers have been reported from the Dalazi Formation in Longjing County (Chow and Tsao, 1977; Zhang et al., 1980; Zhang, 1986; Zhou, 1995, Yang, 2008), but fossils of this family are rarely found in Luozigou, Wangqing County. Only one species *Pseudofrenelopsis gansuensis* was hitherto reported (Yang and Deng, 2007) and *Suturovagina intermedia* Chow and Tsao described in this paper is the second member of this family discovered in the latter locality.

Cuticle pieces of *S. intermedia* were treated with hydrofluoric acid (HF 70%) for 12-18 hours and subsequently macerated in Schulze's solution (concentrate nitric acid and potassium chlorate) for approximately 12 hours. When the cuticle pieces became yellow and translucent, they were rinsed with water, then treated with dilute ammonium hydroxide (5%) for a few seconds to half a minute, and thoroughly rinsed with water. Then, the samples for scanning electron microscopy (SEM) were mounted on stubs using double-sided adhesive tape, coated with gold, and observed and photographed under a LEO-1530 VP at 10 kV in the State Key Laboratory of Palaeobiology and Stratigraphy, Chinese Academy of Sciences, Nanjing. The samples for transmission electron microscopy (TEM) were prepared following Lugardon's technique (1971), which is also used for living plant cuticles (conifers and angiosperms; Bartiromo et al., 2012, 2013). In total, 22 pieces were embedded in Epon resin (8 blocks were made of macerated cuticles, and 14 of unmacerated cuticles). From these, 90 ultrathin 60-70 nm sections (30 from macerated and 60 from unmacerated cuticles)

were made and collected on uncoated 300 Mesh copper grids (70 being in transversal sections, i.e., perpendicular to the leaf length; 20 in longitudinal sections, i.e., parallel to the leaf length). Ultrathin sections were selected, observed and photographed with a Philips CM 120 at 80 kV, at the Centre de Technologie des Microstructures (CTμ) of Lyon-1 University, France.

To obtain more information about stomatal and cuticle structure, 1000 transversal sections and 500 paradermal sections of 1 μm thick were mounted on 150 glass slides (10 sections-per glass slide). These sections were stained following the methods used by Richardson et al. (1960) with methylene blue – Azur II solution (½-½), at the Centre de Technologie des Microstructures (CTμ) of Lyon-1 University France, before taking micrographs under a light microscope Olympas BX51 at the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, China.

EDS (Energy Dispersive Spectroscopic) analysis was performed on the transmission electron microscope using SIRIUS SD ENSOTECH and IDFIX software, an acceleration voltage of 120 kV, spot sizes of 1-3, a processing time of 60-120 seconds, and a time constant of 4 μseconds. Cuticle pieces of *S. intermedia* from Wangqing and the type locality Nanjing were prepared in this study for comparison. In total, 40 copper 300 Mesh uncoated grids were made (20 for each locality made, from 10 resin blocks respectively of macerated and unmacerated cuticles), and devoid of uranyl acetate and lead citrate staining. Among available elements, Cu and Al were eliminated in the results as belonging to the grid, Os as part of the embedding technique, Si as part of the oils used in the TEM, and C and O as well due to being major parts of the EPON embedding resin. Element analyses of the matrix containing fossil plants from the two localities were made using an Energy Dispersive X-ray Spectroscope (EDX) attached to LEO-1530VP in Nanjing.

Hand specimens and SEM stubs are housed in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, China. TEM material and negatives are stored in the Lyon-1 University, Villeurbanne, France.

3. Results

3. 1 Gross morphology and cuticle structure

The leafy shoots are unbranched segments (Figs. 3A-C). The longest shoot preserved is 10 cm long (Fig. 3A). The most slender shoot (Fig. 3B) is approximately 5-6 mm wide, while the widest (Fig. 3A) may reach 8-9 mm. The scale-like leaves are broad and encircle the axis laterally to form a complete sheath interrupted by a suture, slightly imbricate or overlapped, approximately 6 mm long, and with a broadly acute apex. The decurrent leaf base cushion is cylindrical with an oblique end. The adaxial surface of the leaf is crescent- to ring-shaped and approximately 1 mm high. The outer surface of the abaxial surface bears numerous more or less distinct, fine, parallel, longitudinal ridges and grooves that converge toward the leaf apex (Figs. 3A-C).

The outside view of the adaxial cuticle is smooth with longitudinal outlines of cell walls, and no papillae or hairs are visible on the surface (Fig. 5A). The adaxial cuticle is free of stomata. (Figs. 5A, B). It consists of polygonal, rectangular, and more or less isodiametric epidermal cells 20-38 μm long and 15-40 μm wide (Fig. 5B). The anticlinal walls are straight and approximately 2-3 μm thick.

The abaxial cuticles usually have defined, sometime more or less irregular, longitudinal stomatal and non-stomatal files (Fig. 3F, Fig. 5C, Fig. 6A), but at the base (Figs. 3D, E) of the leaf, they are usually free from stomata. On the outer surface, the outlines of epidermal cells are somewhat obscure, but sunken stomata in the grooves are clearly shown and arranged in roughly longitudinal uniseriate files, sometimes biseriate, and more or less

irregular (Fig. 5C). The cuticles are thick (approximately 20–30 μm) and sometimes reach up to 40 μm (Figs. 4A-I). The epidermal cells are clearly observed on the inner surface, approximately 20-50 μm long and 13–36 μm wide and are square, rounded or elongated in shape: most are approximately 22–42 μm × 20–30 μm in size (Fig. 6B-D). The anticlinal walls of the epidermal cells are approximately 2–7 μm in thickness. The epidermal cells adjacent to the stomatal apparatus usually differ slightly from the rest in shape and size, and sometimes form an incomplete encircling cell rings (Fig. 6C). Hypodermal cells have not been clearly seen below the epidermal cell cuticles. The stomatal apparatuses are dense, usually arranged in uniseriate rows, and are either separated from each other by 1–5 epidermal cells or contiguous; however, they never share subsidiary cells (Figs. 6A–C). Occasionally, there are two stomatal apparatuses arranged horizontally in one row (Fig. 5E; Fig. 6D). The stomatal density is approximately 43/mm^2 (31-52/mm^2). Stomatal apparatuses are actinocytic, rounded to elliptical when viewed from the surface (Figs. 5D-F; Figs. 6C, D), approximately 70–100 μm × 90–120 μm in size, and with the mouth of the stomatal chamber positioned slightly lower than the general upper surface of the ordinary epidermal cells. The guard cells are deeply sunken at the bottom of the stomatal chamber and are usually missing; the apertures are randomly orientated (Fig. 6B). The subsidiary cells, 7-9 (usually 8) in number, are more or less cylindrical and arranged in a rosette when viewed from the outer surface (Figs. 5D-F). The subsidiary cells are nearly uniform in shape and size, are round to elliptical in surface view, 20-30 μm in diameter, and do not have papilla or hair on the periclinal wall (Figs. 5C-F). The stomatal apparatuses are composed of several (usually three) tiers of subsidiary cell circles (Figs. 4A, D, H, I). This characteristic feature is clearly shown in the 1 μm thick ultrathin paradermal (Figs. 4J-T) and transverse sections (Figs. 4 A-I). While the shape and size of the stomatal apparatuses and subsidiary cells vary to a certain degree (usually subsidiary cells in the basal tier are quite long), the number of tiers seems to

be somewhat variable as well, mainly two to three tiers and with quite long cells in the basal tier (Figs. 4A-I). Each tier consists of around eight subsidiary cells in a circle (for instance, the two stomatal apparatuses in Figs. 4J-T), a total of 16 or 24 subsidiary cells in one stomatal apparatus.

3.2 Cuticle ultrastructure

The cuticle ultrastructure of the ordinary epidermal cells (Figs. 7-9), and the subsidiary and guard cells (Fig. 10) of the stomatal apparatus are shown in detail. In Table 1, the statistical values of different layers based on the data in Appendix A, include the means calculated using 30 measurements, along with the percentage of each layer of the cuticle.

Two kinds of cuticles (a thinner one with a mean of 23.8 µm, Fig. 7A, and a thicker one with a mean of 97.1 µm, Figs. 7B, C) were observed for the ordinary epidermal cell cuticle. As observed in TEM, the cuticular membrane (CM) of the ordinary epidermal cells is made of the outermost cuticle proper CP (= A) consisting of 17.2-18.4% of the total CM, composed of a wavy polylamellateA1 layer of different patterns as shown in Fig. 8A-G, with a mean of 0.1- 0.2 µm and a quite stable percentage (0.1-0.8%) of the total CM in thinner and thicker cuticles. In addition, the CP has a granular A2 layer, which has a mean thickness of 3.9-17.8 µm and is also quite stable at 16.4-18.3% of the total CM. Below is located the cuticular layer (CL) (= B), consisting of 81.6-82.8% of the total CM. The fibrilous B1 has a mean thickness of 19.2-76.5 µm and is quite stable in total CM percentage in either thinner or thicker cuticles, i.e., 78.8-80.7%. In addition, CL has a granular B2 layer with a mean thickness of 0.5-2.7 µm, which also very stable at 2.1-2.8% of the total CM (Figs. 8-9). In some cases (Fig. 8I), the A1 polylamellate layer is straight or absent (Fig. 8J). Some alveoli approximately 10 µm in diameter are also present in some places of the cuticle (Fig. 7D),

with a lumen and a ring of concentrated granules CG. Occasionally, remnants of cell walls may also be detected (Figs. 9K, M).

In the subsidiary and guard cell cuticles (Fig. 10), only the cuticle proper (CP) is present, with the cuticular layer (CL) always absent. The A1 polylamellate layer is thick, with a mean of 0.03-0.06 µm and 0.1-2.3% of the total thickness, and mainly wavy in subsidiary cell cuticle (Figs. 10D, E) including the outer chamber part of the stomatal apparatus (Fig. 10G), but can also be rather straight in rare cases (Fig. 10F). Conversely, the guard cell cuticle has always a straight A1 layer (Figs. 10L, M). The granular layer A2 is very developed in subsidiary and guard cell cuticles, attaining a thickness of 97.7-99.9% respectively for the total CM and a mean thickness of 1.3- and 17.9 µm.

3.3 EDS

When the cuticle elements are analyzed using the EDS system (Fig. 11; Tables 2, 3; Appendices C, D), the data obtained may include those from both the resin and meshes. As mentioned above in Material and Methods, a number of elements, such as Cu, Al, Os, Si, C and O are eliminated in order to provide only comparable values of cuticle elements. Among the 14 potential ratios, only 4 (N/F, N/Cl, F/Cl, K/Ca) with homogeneous values of resin (showing insignificant differences from the Mann-Whitney test for five measurements) are selected here for the four layers (A1, A2, B1, B2) of the ordinary epidermal cell cuticle, though providing similar eventual errors due to the elements in the resin. Added to the confidence interval values, all of these cuticle values were also determined to be significantly different or not by the Mann-Whitney test. Among the 16 possibilities (4 ratios for 4 layers), 11 are significantly different in ratio value (all ratios of the A1 layer, 3 ratios of the A2 and B1 layers, and only 1 ratio of the B2 layer). Five ratios show insignificant differences (K/Ca

for the A2 layer, N/Cl for the B1 layer, N/Cl and F/Cl and K/Ca for the B2 layer). In the EDS analyses, each layer has its own characteristics.

4. Discussions

4.1. General considerations

Suturovagina intermedia is highly heterophyllous and possesses some shoots with scale leaves of the *Brachyphyllum* (*Hirmeriella*) type, and occasionally shoots with close-sheathed leaves such as those of *Pseudofrenelopsis.* The predominant leafy shoots of *Suturovagina*, as noted by Zhou (1983) after examining thousands of leafy shoots, bear leaves of the open-sheathed type. In the Luozigou section of Wangqing, Jilin Province, only shoots with broad scale-like leaves encircling the axis and forming a lateral, complete sheath interrupted by a suture are present, and no branched shoots were found. In general morphology, the leaves of the present material are undoubtedly similar to the Type 2 of *Suturovagina intermedia* discovered from the Gecun Formation of Nanjing, Jiangsu Province (Zhou, 1983), although in the Gecun Formation of the type locality, shoots bearing Type 2 leaves are comparatively fewer.

The leaf cuticles from the two localities are essentially similar in structure, but hypodermal cells, which are occasionally present in some leaves of juvenile shoots from Nanjing, have not been clearly observed in the Wangqing specimens. A unique characteristic of *Suturovagina intermedia* is the stomatal apparatus, which differs markedly from that of all other genera so far known in the Cheirolepidiaceae. In the original description and illustrations, it was interpreted to consist of guard cells surrounded by a circle of vertically extending subsidiary cells (Chow and Tsao, 1977; Zhou, 1983). Recent light microscopic observations on the serial ultrathin sections and SEM examination on the material from the type locality (Mairot at al., 2014, Pl. II; Pl. III; Pl. IV, 5, 8, 13) and the present new locality

reveal that the stomatal apparatuses are composed of several tiers of subsidiary cell circles (Fig. 4). There are some insignificant differences in cuticular features between leaves of the same species from Wangqing and Nanjing: the leaf cuticle from the former locality is generally thinner, approximately 20–30 μm and sometimes up to 40 μm thick, while that from the latter locality is approximately 90–150 μm and sometimes up to 250 μm in thickness (Zhou, 1983); the stomatal apparatuses in the leaf cuticle of Wangqing specimens are slightly smaller (70–100 μm × 90–120) in size than in the leaf cuticle of Nanjing specimens (up to 100–150 μm in diameter). These differences are believed to be of no taxonomic value but are probably related to the regional ecology.

4.2. Cuticle ultrastructure of Cheirolepidiaceae

The previous study on the two species of *Pseudofrenelopsis* (Yang et al., 2009; Guignard et al., 2017), and also one species of *Hirmeriella* (Guignard et al. 1998) shows that 3 cuticle ultrastructural characters (i.e., the presence of the 4 layers of cuticles A1, A2, B1, B2, the wavy polylamellate, and possibly the thick transparent lamellae (TL) of A1) in the ordinary epidermal cell cuticles appear to be characteristic for the family Cheirolepidiaceae. Concerning the ultrastructural cuticles of the family Cheirolepidiaceae in China, this is the fourth detailed cuticle studies with statistical methods based on 30 measurements for each thicknesses of the four layers of cell cuticles respectively, including the study on *Suturovagina intermedia* from the type locality Nanjing, Jiangsu Province (Yang et al., 2009; Mairot et al., 2014; Guignard et al., 2017). Based on the present study and the results obtained from the previous works, the potential taxonomic and ecological significances of the cuticle details, measurements and statistic values are summarized below.

The studies on *Suturovagina intermedia* from Wangqing and Nanjing reveal the same 3 cuticle ultrastructural characters of the ordinary epidermal cell cuticles in the subsidiary cells.

Thus, 6 characters are believed to be of family importance. It is notable that no other fossil Families and Orders are identical to the Cheirolepidiaceae in these cuticle ultrustructural features, including: Czekanowskiales (Zhou & Guignard, 1998), Ginkgoales (Wang et al., 2005; Guignard and Zhou, 2005; Del Fueyo et al., 2006, 2013; Guignard et al., 2016), Pteridospermales (Guignard et al., 2001; Thévenard et al., 2005; Carrizo et al., 2014) including Corystospermaceae (Guignard et al., 2004), Bennettitales (Villar de Seoane, 1999, 2001, 2003); cycadalean cuticles (Archangelsky et al., 1995; Artabe et al., 1991; Passalia et al., 2010), Coniferales (fossil Miroviaceae, Nosova et al., 2016; living Pinaceae, Bartiromo et al., 2012),. For species/genus distinctions, only the thickness of B1 in the ordinary epidermal cells and subsidiary cells seems useful (Table 3; Guignard et al., 2017).

Although *Suturovagina intermedia* cuticles from the two localities are generally similar in the ultrastructure, there are significant differences in thickness between cuticle layers (Fig. 12; Table 3). Except for the measurements of the ordinary epidermal cell B1 layer, which are comparable between the cuticles from Nanjing and the thinner cuticles from Wangqing, all other measurements of the layers (7 of OEC and 5 of SC) are different. These are accordingly all related to cuticle thickness and ecological factors and will be addressed below.

4.3. EDS data

Although cuticle EDS analysis has been only scarcely use in the taxonomy of fossil plants, recent comparative studies of Cheirolepidiaceous conifers (Mairot et al., 2014; Guignard et al., 2017) reveal that elemental analyses might be of potential interest in this respect. Recent works also show that the biochemical properties of modern and fossil plants can yield taxonomically and phylogenetically useful information (Niklas and Gensel, 1977; D'Angelo et al. 2010, 2012; D'Angelo and Zodrow, 2015; Vajda et al., 2017).

For *Suturovagina intermedia* from both localities, 5 EDS ratios are homogeneous in their values: K/Ca (for the two layers A2 and B2) and N/Cl (for the two layers B1 and B2), followed by F/Cl (for the B2 layer) (Tables 2-3). In comparison with the EDS data of cuticles of the genus *Pseudofrenelopsis* (Guignard et al., 2017) where it shows different significant ratios of elements (N/S, N/K), except for one value (N/Cl ratio) being similar to that of the genus *Suturovagina*. It is possible that the N/Cl ratio of the B2 layer is of significance at the family level.

Despite the general similarity of the ratios mentioned above, *Suturovagina intermedia* specimens from the two localities differ from each other in 11 EDS data of the leaf cuticle (Table 2): A1 (N/F, N/Cl, F/Cl, K/Cl); A2 (N/F, N/Cl, F/Cl); B1 (N/F, F/Cl, K/ Ca); B2 (N/F), which are believed to be of ecological significance (see below).

4.4. Palaeoenvironments

Most Cheirolepidiaceae bear xeromorphic characters and are generally considered to be halophytic and thermophilous plants (Alvin, 1982; Watson, 1988). Recent studies on Cheirolepidiaceous conifers from different areas worldwide have shown an increase in ecological amplitude. The cheirolepidiaceous conifers of the English Wealden flora are believed to have lived in river margin swamps (Batten, 1974; Oldham, 1976), and *Pseudofrenelopsis* species inhabited soils with variable water availability (Alvin, 1982, 1983); *Pseudofrenelopsis parceramosa* from North America, Europe and China might have adapted to environments with different levels of salinity or even non-saline habitats (Upchurch and Doyle, 1981; Zhou, 1995). In addition, the cheirolepidiaceous conifer *Otwayia* from high-latitudes in southeastern Australia might have adapted to humid conditions (Tosolini et al., 2015).

Suturovagina intermedia has a xerophytic gross morphology and cuticle structure and is characterized by succulent shoots and greatly reduced adpressed leaves that are covered with thick cuticles. Its stomatal apparatus is composed of several tiers of subsidiary cell circles and a pair of guide cells at the bottom of the stomatal chamber. The stomatal apparatuses with a deep stomatal chamber that consists of tiers of subsidiary cells are a special adaptation to environmental stresses and almost unique in the Cheirolepidiaceae and conifers. Most cheirolepidiaceous conifers, such as *Pseudofrenelopsis*, have stomatal apparatuses with comparatively shallow stomatal pits usually surrounded by few (approximately 5-6) papillate and "none-tiered" subsidiary cells (Watson, 1977, 1988; Zhou, 1995; Deng et al., 2005; Axsmith, 2006; Yang et al., 2009). Only *Tarphyderma glabra* Archangelsky and Taylor (1986) from the Lower Cretaceous of Santa Cruz Province, Argentina, which probably belongs to Cheirolepidiaceae, has helically arranged rhomboidal leaves with stomatal apparatuses that are closely similar to those of *Suturovagina intermedia*. The guard cells are deeply sunken at the bottom of a stomatal chamber (or suprastomatal chamber), which is composed of tiers of vertically elongate subsidiary cells. Similarly, *Tarphyderma* possesses many other xeromorphic features, although Archangelsky and Taylor (1986) believed such features to be in response to different kinds of environmental stress, such as volcanic activity.

Despite the similarities in gross morphology and cuticle structure, *Suturovagina intermedia* described here from Wangqing of Jilin Province is different in cuticle ultrastructure and elemental composition from the same species of the type locality in Nanjing. As stated above, the leaves of the former locality bear much thinner cuticles (up to 40 μm) than those of the type locality (90–150 μm) and as a result, there are a number of differences (7 of OEC and 5 of SC) in cuticle ultrastructure measurements (Table 3). Among them, the difference in thickness in the A1 polylamellate layer is noticeable, as it is known to be correlated with the permeability of leaves in some living plants, such as *Agave americana*, *Plantago major* and

Ardisia crenata (Fischer and Bayer, 1972; Wattendorff and Holloway, 1980, 1982; Wattendorff, 1992; Jeffree, 2006). All of these differences indicate that in Wangqing, *Suturovagina intermedia* appears to have inhabited an environment that was not as hot or dry as that of the type locality.

A comparative elemental analysis (EDS) of the cuticles from both localities has come to the same conclusion (Appendices C-E; Tables 2-3). As mentioned above, there are 11 EDS data for the leaf cuticles: A1 (N/F, N/Cl, F/Cl, K/Cl); A2 (N/F, N/Cl, F/Cl); B1 (N/F, F/Cl, K/Ca); B2 (N/F), which are different between the same species from both localities and are believed to be of ecological significance. A higher xerothermy seems to be significantly related to lower N/F and K/Ca ratios (except for the A2 and B2 layers of K/Ca), and higher N/Cl (except the B1 and B2 layers) and F/Cl ratios (except the B2 layer) in the leaf cuticle of Nanjing. Conversely, a lower xerothermy seems to be related to higher N/F and K/Ca ratios, and lower N/Cl and F/Cl ratios in the cuticle of Wangqing. In leaves of a dicotyledon *Arabidopsis thaliana*, Benikhlef et al. (2013) reported a Ca peak on the leaf surface after "a soft mechanical stimulation" (= what they mean is artificial "stress"). Their study suggests that leaf cuticles are quite sensitive to environment, and Ca is a useful proxy for ecological evaluation. A higher Ca composition of the *Suturovagina* leaf cuticle in Nanjing, therefore, could be correlated with a higher stress for water (higher xerothermy), while a lower Ca composition in Wangqing could be correlated with a lower stress (lower xerothermy). In *Pinus ponderosa* and *Pseudotsuga menziesii* the cuticular transpiration is revealed to be higher during exposure to chlorine gas (Schreuder and Brewer, 2001). Although the Cl content of the cuticles was not analyzed, an increase of transpiration may indicate a lower permeability, which is congruent with the thinner leaf cuticle of Wangqing having a higher Cl content, as compared with a lower Cl content in the thicker leaf cuticle of Nanjing due to a higher xerothermic palaeoenvironment (= less permeable cuticle).

In the Lower Cretaceous Gecun Formation of Nanjing, Jiangsu Province, China, fossil plants are very rare. The only species so far known to be associated with *Suturovagina intermedia* is a unique conifer named *Glenrosa nanjingensis* Zhou, Thévenard, Barale et Guignard, which also exhibits xeromorphic gross morphology and cuticle micro- and ultrastructure (Thévenard et al., 2000; Zhou et al., 2000). The scale leaves of *Glenrosa nanjingensis* have a thick cuticle with scattered stomatal chambers on the abaxial surface. More than one stoma has been detected in the stomatal chamber (or nest) filled with papillae. As noted by Watson and Fisher (1984), the genus *Glenrosa* resembles the living angiosperms *Nerium oleander* L. and *N. odorum* Aiton of the Apocynaceae (Metcalfe and Chalk, 1950; Coste and Flahault, 1990), as well as *Blossfeldia* Wedermann (Cactaceae) (Gorelick, 2004; Butterworth, 2006). They are all typical members of xerothermic plant communities. The stomata are confined to deeply sunken chambers (or nests) with hair-like papillae filling the pit opening to trap atmospheric moisture and restrict evapotranspiration.

In the Dalazi Formation of Wangqing, however, *Suturovagina intermedia*, is associated with a number of fossil plants as listed and reported early in the last century, including *Cladophlebis exiliformis* (Geyler) Ôishi, *Gleichenites nipponensis* Ôishi, *Onychiopsis elongata* (Geyler) Yok., *Coniopteris saportana* (Heer) Vachr., *Otozamites* sp., *Elatocladus curvifolius* (Dunker), *Sphenolepis sternbergiana* Dunker, *Pityocladus iwaiana* (Ôishi) Chow, *Pityolepis* sp., *Pagiophyllum* sp., *Brachyphyllum crassum* Lesq., *Brachyphyllum ningshiaense* Chow et Tsao, *Frenelopsis*? sp., *Sassafras*? sp., *Paliurus*? sp. (Ôishi, 1941; Zhou et al., 1980). In recent years, during field work in this area, further specimens of megafossil plants were collected from the same formation. Besides *Suturovagina intermedia*, there are the following: *Selaginellites* cf. *fausta* (Wu) Sun et Zheng (Fig. 13A), *Ruffordia goeppertii* (Dunker) Seward (Fig. 13B), *Gleichenites nipponensis* Ôishi (Fig. 13C), *Onychiopsis elongata* (Geyler) Yokoyama (Fig. 13D), *Otozamites* sp. (Fig. 13E), *Pseudofrenelopsis*

gansuensis Deng, Yang et Lu (Fig. 13F; Yang and Deng, 2007; Guignard et al., 2017, *Elatocladus* sp. (Fig. 13G), *Cupressinocladus elegans* (Chow) Chow (Fig. 13H), *Dicotylophyllum* sp. (Fig. 13I)**.** It is noteworthy that *Suturovagina intermedia* can be found here amid a quite diverse plant assemblage compared with the rather monotonous assemblage of the type locality Nanjing. Although the floral composition suggests a hot and dry environment, as indicated by the presence of the xeromorphic conifer *Pseudofrenelopsis gansuensis* (Yang and Deng, 2007; Guignard et al., 2017), the occurrence of lycopodialeans, ferns, bennettitaleans and dicotyledons in the assemblage suggests an environment with less water stress than that one in Nanjing. In addition to fossil plants, there are conchostracans, insects and fishes, which are hitherto unknown in the Gecun Formation of Nanjing (Zhou et al., 1980).

All of the differences mentioned above coincide with the lithological character and elemental composition of the matrix. The Gecun Formation of Nanjing is composed mainly of variegated clastic sediments with rich calcareous compounds and gypsum. EDX analyses of the *Suturovagina* bed show a high Ca (CaO up to 33.64%) and very low, or even the absence of carbon (C) (Mairot et al., 2014). The Dalazi Formation in Wangqing is a riparian/lacustrine deposit containing oil-shale (Zhou et al., 1980) and the matrix of the plant-bearing beds contain a higher degree of carbon (up to 49.63%), but relatively lower Ca (or even absent) (Guignard et al., 2017) as compared with the data from the Gecun Formation of Nanjing.

As far as we are aware, some other widespread species of the Cheirolepidiaceae, such as *Pseudofrenelopsis parceramosa*, have occupied a range of habitats (Oldham, 1976; Alvin et al., 1981; Upchurch and Doyle, 1981; Alvin, 1982; Srinivasan, 1995 ; Axsmith, 2006). A restudy of the *Suturovagina* specimens of probably of the same species from the upper Lower Cretaceous Dalazi Formation of Longjing, Jilin Province and the upper Lower

Cretaceous Pulandian Formation of Pulandian, Liaoning Province reported by Zhang et al. (1980) and Li (1992) may shed further light on the infraspecific palaeoenvironmental diversity of this plant.

5. Conclusions

This is the fourth work to study cheirolepidiaceous conifers by using scanning and transmission electron microscopies to assess the potential taxonomic significance of cuticle micro- and ultrastructures. So far, we have found 3 cuticle ultrastructural features (i.e., the presence of 4 layers of cuticles A1, A2, B1, B2, the wavy polylamellate, and possibly the thick transparent lamellae (TL) of A1 in the ordinary epidermal cell cuticles) to be characteristic of the family Cheirolepidiaceae. The present study reveals the same 3 cuticle ultrastructural characters in the subsidiary cells, which are also of importance at family level. For species/genus distinctions, only the thickness of B1 in the ordinary epidermal cells and subsidiary cells seems useful.

The present study is the first one to make comparisons between the cuticles of the same taxon, *Suturovagina intermedia*, from two different localities. In addition to further verifying the taxonomic value of the cuticle micro- and ultrastructures, attention is also paid to their ecological significance. It reveals that almost all cuticular layers (Table 3) are thinner in the specimens from Wangqing than in those from the type locality Nanjing due to a less xerothermic environment.

Comparisons of elemental compositions are also made between the cuticles of the same taxon from both localities. In the Cheirolepidiaceae, ratios of K/Ca (for the two layers A2 and B2) and N/Cl (for the two layers B1 and B2), followed by F/Cl (for the B2 layer) are believed to be of importance at species/generic level. EDS analyses of *Suturovagina intermedia* leaf

cuticles from both localities show that they bear a general similarity in element ratios which are of taxomonical importance. They differ from each other, however, in 11 EDS data (Table 3), including a higher ratio of K/Ca and a lower ratio of N/Cl and F/Cl in the different cuticle layers of the Wangqing specimens compared to data from leaf cuticles of Nanjing. This suggests that *Suturovagina intermedia* grew in a less xerothermic environment in Wangqing compared to that in Nanjing. This work is the first attempt to search for the environmental footprint in fossil plant cuticles based on their cuticle elemental composition. The differences in environmental factors between the *Suturovagina intermedia* trees from the two localities are fully in accordance with the evidence concerning the chemical composition of the matrix and associated biotic assemblages.

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Caption of figures, tables, appendices

Fig. 1. Distribution of *Suturovagina intermedia* in China.

Fig. 2. Schematic figures of heterophyllous shoots of *Suturovagina intermedia*, showing the both sides of compressed shoots. A. Loosely arranged scale leaves. B. Broad scale-like leaves. C. Close-sheathed leaves (upper part) and open-sheathed leaves (lower part) on one shoot. D. Close-sheathed leaves encircling the stem but interrupted by a suture (redrawn from Zhou 1983).

Fig. 3. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. A-C. Segments of shoot, showing a long shoot without lateral branches, note fine parallel, longitudinal ridges and grooves on the outer surface (B). Specimens PB22714-22716. D-G. Light micrographys, cuticle from specimen PB22714. D, E. Cuticle of base part of leave, no stomata in the margin area of leaf. F, G. Cuticle from the middle part of leaves, note the arrangement of stomatal complexes in longitudinal rows.

Fig. 4. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Light micrographs of glass slide 1μm sections, cuticle from specimen PB22714. Abbreviations: OP = outer part, IP = inner part, EC = epidermal cell cuticle (with arrows), ST = stomatal apparatus. A-D. Four successive transverse sections from one side (A) to the opposite side (D) of one stomatal apparatus. Gradual changes in the shape and size of the subsidiary cell cuticles are visible, showing a maximum of three tiers of subsidiary cells (numbered 1 to 3). E-G. Cuticle of stomatal apparatuses and epidermal cells. H, I. Two connected stomatal apparatuses (ST1 and ST2) in two longitudinal sections, with 1 to 3 tiers of subsidiary cells (clearly observable in ST2). J. Paradermal section with two connected

stomatal apparatuses (ST3 and ST4). K-O, P-T. Successive paradermal cuticle sections from the outermost (K and P) to the basal (O and T) parts of two stomatal apparatuses. K-O, the number of subsidiary cells changes from 7 in the outmost part, then to 8, 9, 9, finally 8 in the lower most part (arrows show new cells appearing). P-T, the number of subsidiary cells changes from 8 in the outmost part, then raises to 9, 9, then goes back to 8, finally 6 in the lower most part (arrow shows new cell appearing).

Fig. 5. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Scanning electron micrographs, cuticle from specimen PB22714. A. Outer view of adaxial cuticle, showing thick epidermal cells cuticle with smooth surface, and without stoma in the adxial cuticle. B. Inner view of adaxial cuticle, note the rectangle outline of epidermal cells along margin and square or polygonal ordinary epidermal cells. C. Outer view of abaxial cuticle, showing stomata arranged in irregularly longitudinal files, out layer of cuticle was partly preserved. D-F. Outer view of abaxial cuticle, showing stomata arranged continuously in two or three or isolate.

Fig. 6. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Scanning electron micrographs, cuticle from specimen PB22714. A. Inner view of abaxial cuticle, showing stomata arranged in longitudinal files and stoma distributed densely. B. Inner view of abaxial cuticle, showing two stomata arranged tilted in one files. C. Inner view of abaxial cuticle, showing a stoma with eight subsidiary cells and incomplete surrounding cell rings, epidermal cells of non-stomata files nearly square, rectangular or isodiametric. D. Inner view of abaxial cuticle, showing two stomata arranged tilted in horizontal files. E, F. Tilted vertical sections of abaxial cuticle, showing thickly cutinized ordinary epidermal cells, the stomatal pits and hollow subsidiary cells.

Fig. 7. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Ultrastructural transmission electron micrographs, epidermal and hypodermal cell cuticles, cuticle from specimen PB22714. Abbreviations: OP = outer part, ECR = epidermal cell remnants, HCR = hypodermal cell remnants, AW = anticlinal wall, CP = cuticle proper, CL = cuticular layer, B2 = B2 granular layer of the cuticular layer, G = granules, Fb = fibrils, CG = concentrated granules, lumen = lumen of one alveolus. All micrographs are from untreated material, transversal sections. A-C. General views, A is a thin cuticle while B and C are thick cuticle. In the case of B, epidermal cell remnants (ECR) can be observed as a lighter zone. The cuticular layer makes various coloured zones, from light grey to almost black colours. D. Alveolus in the cuticle, with the lumen surrounded by one zone of concentrated granules. E. Detail of one alveolus, with the lumen and the zone of concentrated granules, among B1 layer showing in this part very various concenttrations of fibrilous (Fb) and granular (G) zones.

Fig. 8. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Ultrastructural transmission electron micrographs of details of A1 polylamellate layer of the ordinary epidermal cell cuticle, cuticle from specimen B22714. Abbreviations: OP = outer part, EXCM = extracuticular material, OEC = ordinary epidermal cell cuticle, A1 and A2 = polylamellate and granular layer of the cuticle proper. All micrographs are from untreated material, transversal sections. A, B. Connection between two adjacent ordinary epidermal cell cuticles (OEC 1 and 2), B being a detail of A. Very wavy A1 layer making many curves is observed. C-H. Polylamellate A1 layer making various wavy schemes, above the A2 granular layer of the cuticle proper. I. Rarely observed A1

polylamellate layer, straight in this case. J. Rarely observed cuticle, devoid of A1 layer, where A2 granular layer is directly at the outermost part of the cuticle.

Fig. 9. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Ultrastructural transmission electron micrographs of details of the ordinary epidermal cell and hypodermal cell cuticles, cuticle from specimen PB22714. Abbreviations: ECR = epidermal cell remnants, HCR = hypodermal cell remnants, CW = cell wall. Except micrograph D which is from treated material and longitudinal section, all other micrographs are from untreated material, transversal sections. A-D. A2 granular layer of the cuticle proper, located below A1 layer (not polylamellate in the rare case of C), above B1 fibrilous layer of the cuticular layer mixed with various concentrations of granules. E-G. In some cases, epidermal cell remnants ECR are visible in the upper part of the cuticle, with membrane-like lines which are probably residues of cell contents, very different is aspect from the B1 fibrilous layer. H-J. B1 fibrilous layer, mixed with various concentrations of granules, making various colours. K-M. Lower part of the cuticle, with B2 granular layer (absent in M), the cell wall CW of the hypodermal cell cuticle showing in some regions some very parallel and concentrated lines. In the case of M, some lines are even going towards the cuticular layer just above.

Fig. 10. *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China. Ultrastructural transmission electron micrographs of stomatal apparatus cuticle, details of subsidiary and guard cell cuticles, cuticle from specimen PB22714. Abbreviations: OP = outer part, IP = inner part, SC = subsidiary cell cuticle, GC = guard cell cuticle, EXCM = extra-cuticular material. All micrographs are from untreated material, transversal sections. A-J. Subsidiary cell cuticles. A-C. General views of stomatal

apparatuses, B being a detail of A. D-G. Details of outermost part of subsidiary cell cuticle, with A1 polylamellate layer, mostly wavy but in some cases straight (photo F), G being the outer chamber zone with a connection between two cell cuticles. H-J. Details of middle (H-I) and lower (J) parts of subsidiary cell cuticles, with only A2 granular layer, cuticular layer being absent. K-M. Guard cell cuticles. K. General view. L, M. Details, with a straight polylamellate A1 layer over the A2 granular layer, cuticular layer is absent.

Fig. 11. Examples of EDS analysis for *Suturovagina intermedia* Chow et Tsao. Cuticle spectra are on the left side, resin spectra on the right side

Fig. 12. Three-dimensional reconstruction of the cuticle of *Suturovagina intermedia* Chow et Tsao from the two localities Nanging and Wangqing, after figure 3 from Mairot et al. (2014). Statistical percentages of A1, A2, B1 and B2 layers are indicated for each layer.

Fig. 13. Plant fossils associated with *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing, Jilin Province, China, scale bars: 1 cm. All specimens housed in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences. A. *Selaginellites* cf. *fausta* (Wu) Sun et Zheng; specimen PB22605A. B. *Ruffordia goeppertii* (Dunker) Seward; specimen PB22717. C. *Gleichenites nipponensis* Ôishi; specimen PB227178. D. *Onychiopsis elongata* (Geyler) Yokoyama; specimen PB22719. E. *Otozamites* sp.; specimen PB22720. F. *Pseudofrenelopsis gansuensis* Deng, Yang et Lu; specimen PB22721. G. *Elatocladus* sp.; specimen PB22722. H. *Cupressinocladus elegans* (Chow) Chow; specimen PB22723. I. *Dicotylophyllum* sp.; specimen PB22724.

Table 1. Statistical values, based on 30 measurements of the thicknesses for the four kinds of cell cuticles of *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing.

Note. Min-max = minimum and maximum values of thickness observed; % = percentage of each detailed part of the cuticle; st-d = standard deviation; Nb = number of lamellae. The cuticular membrane CM is made of cuticle proper CP (= A = A1 + A2 layers) and cuticular layer CL (= B = B1 + B2 layers). A1 layer cuticle is composed of alternate opaque OL and translucent TL lamellae. Except for the very thin OL and TL measured in nm, all other measurements are in µm.

Table 2. Statistical values of the EDS ratios for the four layers of ordinary epidermal cells of *Suturovagina intermedia* from the two localities Wangqing and Nanjing.

Note: Mann Whitney test was made based on 5 EDS data (Appendix C) of each ratio. *Suturovagina intermedia* data of Nanjing are from Mairot et al. (2014). Only the thicker cuticle of the Wangqing material was studied using EDS.

Table 3. Comparisons of the cuticle EDS and ultrastructural characters between *Suturovagina intermedia* from the two localities Wangqing and Nanjing.

Note. Figures in blue colour, indicating the mean, are significantly different between the cuticles of the same species from two different localities using the confidence interval CI (= x 1.96. giving 95 % α risk). These are believed to be palaeoenvironmental signature. The figures in grey colour, indicating the minimum and maximum mean, are not significantly different between specimens from the two localities; they might have a potential specific-generic value. *Suturovagina intermedia* data from Nanjing are from Mairot et al. (2014). For ordinary epidermal cell features, as this cuticle may be either thinner or thicker, when the two values are very different, they are both listed in the table to show there are two possibilities

for this type of cuticle. For the guard cell cuticle, it is absent from Nanjing material previously studied and left without any colour. OL = opaque lamellae, TL = translucent lamellae of the A1 polylamellate layer.

Appendix A. Measurements and statistical data of ultrastructural cuticle *Suturovagina intermedia* Chow et Tsao from Wangqing

Appendix B. Comparisons among the layers of the cuticle of *Suturovagina intermedia* Chow et Tsao from Wangqing, using the confidence interval.

Appendix C. EDS data for 5 measurements from the two localities Wangqing and Nanjing.

Appendix D. EDS comparisons between the cuticles of *Suturovagina intermedia* Chow et Tsao from the two localities Wangqing and Nanjing, test Mann-Whitney with 5 measurements

Appendix E. EDS ratio means and confidence intervals of the layers of *Suturovagina intermedia* Chow et Tsao from the two localities Wangqing and Nanjing.

Table 1. Statistical values, based on 30 measurements of the thicknesses for the four layers of cell cuticles of *Suturovagina intermedia* Chow et Tsao from the Lower Cretaceous of Wangqing.

Note. Min-max = minimum and maximum values of thickness observed; % = percentage of each detailed part of the cuticle; st-d = standard deviation; Nb = number of lamellae. The cuticular membrane CM is made of cuticle proper CP (= A = A1 + A2 layers) and cuticular layer CL (= B = B1 + B2 layers). A1 layer cuticle is composed of alternate opaque OL and translucent TL lamellae. Except for the very thin OL and TL measured in nm, all other measurements are in μm.

	thick cuticle of ordinary epidermal cell				thin cuticle of ordinary epidermal cell			
	mean	min-max	%	st-d	mean	min-max	%	st-d
CM	97.1	80.7-116.9	100	11.8	23.8	10.3-46.5	100	13.2
CP (A)	17.9	10.7-26.6	18.4	4.8	4.1	0.5-8.4	17.2	2.7
A1	0.1	0.02-0.6	0.1	0.2	0.2	0.03-1.1	0.8	0.2
OL/nm	2.1	1.4-3.1		0.4	3.1	1.2-6.1		1.1
TL/nm	6.2	3.9-8.4		1.4	6.7	3.7-10.2		2
Nb	3.9	2-9		1.8	6.5	2-10		2.2
A2	17.8	10.6-26.5	18.3	4.8	3.9	0.4-8.3	16.4	2.7
CL(B)	79.2	54.2-101.8	81.6	12.3	19.7	4.9-41.2	82.8	12.8
B1	76.5	51.6-99.7	78.8	12.2	19.2	4.6-40.4	80.7	12.6
B2	2.7	1.5-6.3	2.8	1	0.5	0.1-1.7	2.1	0.4
	stomatal apparatus							
	subsidiary cell cuticle				guard cell cuticle			
	mean	min-max	%	st-d	mean	min-max	%	st-d
CM	17.1	10.9-21.9	100	3.4	1.3	0.7-2.1	100	0.3
CP (A)	17.1	10.9-21.9	100	3.4	1.3	0.7-2.1	100	0.3
A1	0.06	0.01-0.1	0.1	0.03	0.03	0.01-0.06	2.3	0.01
OL/nm	12.3	4.3-22.3		4.5	6.8	4.3-11.5		1.9
TL/nm	6.4	4-7.9		1.1	17.3	6.9-26.5		5.8
Nb	4.8	2-8		1.7	2.8	1-5		1.5
A2	17.09	10.8-21.8	99.9	3.4	1.3	0.7-2	97.7	0.3

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Table 2. Statistical values of the EDS ratios for the four layers of ordinary epidermal cells of *Suturovagina intermedia* from the two localities Wangqing and Nanjing.

		mean	minimum-maximum values	standard deviation	confidence interval	differences, Mann-Whitney test
Nanjing	**A1, ratio N/F**	0.09	0.08-0.10	0.01	0.01	significant, ecological character
Wangqing		0.42	0.37-0.46	0.04	0.03	
Nanjing	**A1, ratio N/Cl**	0.57	0.30-0.80	0.22	0.19	significant, ecological character
Wangqing		0.09	0.08-0.10	0.01	0.01	
Nanjing	**A1, ratio F/Cl**	6.39	3.31-8.67	2.34	2.05	significant, ecological character
Wangqing		0.21	0.19	0.22	0.01	
Nanjing	**A1, ratio K/Ca**	0.13	0.10-0.19	0.04	0.03	significant, ecological character
Wangqing		0.73	0.60-0.86	0.13	0.11	
Nanjing	**A2, ratio N/F**	0.08	0.07-0.09	0.01	0.00	significant, ecological character
Wangqing		0.44	0.38-0.53	0.06	0.05	
Nanjing	**A2, ratio N/Cl**	1.04	0.87-1.39	0.20	0.17	significant, ecological character
Wangqing		0.11	0.08-0.14	0.02	0.02	
Nanjing	**A2, ratio F/Cl**	13.25	11.89-16.33	1.90	1.66	significant, ecological character
Wangqing		0.25	0.21-0.29	0.03	0.03	
Nanjing	**A2, ratio K/Ca**	0.11	0.06-0.22	0.06	0.06	insignificant, specific-generic character
Wangqing		0.51	0.18-0.70	0.22	0.19	
Nanjing	**B1, ratio N/F**	0.04	0.03-0.04	0.00	0.00	significant, ecological character
Wangqing		0.40	0.37-0.42	0.02	0.02	
Nanjing	**B1, ratio N/Cl**	0.94	0.76-1.13	0.14	0.13	insignificant, specific-generic character
Wangqing		0.68	0.43-0.94	0.19	0.17	
Nanjing	**B1, ratio F/Cl**	25.92	22.39-30.91	3.57	3.13	significant, ecological character
Wangqing		1.71	1.05-2.23	0.43	0.38	
Nanjing	**B1, ratio K/Ca**	0.03	0.03-0.04	0.00	0.00	significant, ecological character
Wangqing		0.33	0.06-0.56	0.18	0.16	
Nanjing	**B2, ratio N/F**	0.18	0.08-0.27	0.08	0.07	significant, ecological character
Wangqing		0.42	0.39-0.45	0.02	0.02	
Nanjing	**B2, ratio N/Cl**	0.29	0.07-0.72	0.30	0.26	insignificant, specific-generic character
Wangqing		0.16	0.14-0.19	0.02	0.01	
Nanjing	**B2, ratio F/Cl**	2.82	0.27-6.71	3.37	2.95	insignificant, specific-generic character
Wangqing		0.39	0.33-0.44	0.05	0.04	
Nanjing	**B2,**	0.44	0.16-1.08	0.38	0.33	insignificant,

Wangqing	**ratio K/Ca**	0.31	0.23-0.40	0.08	0.07	specific-generic character

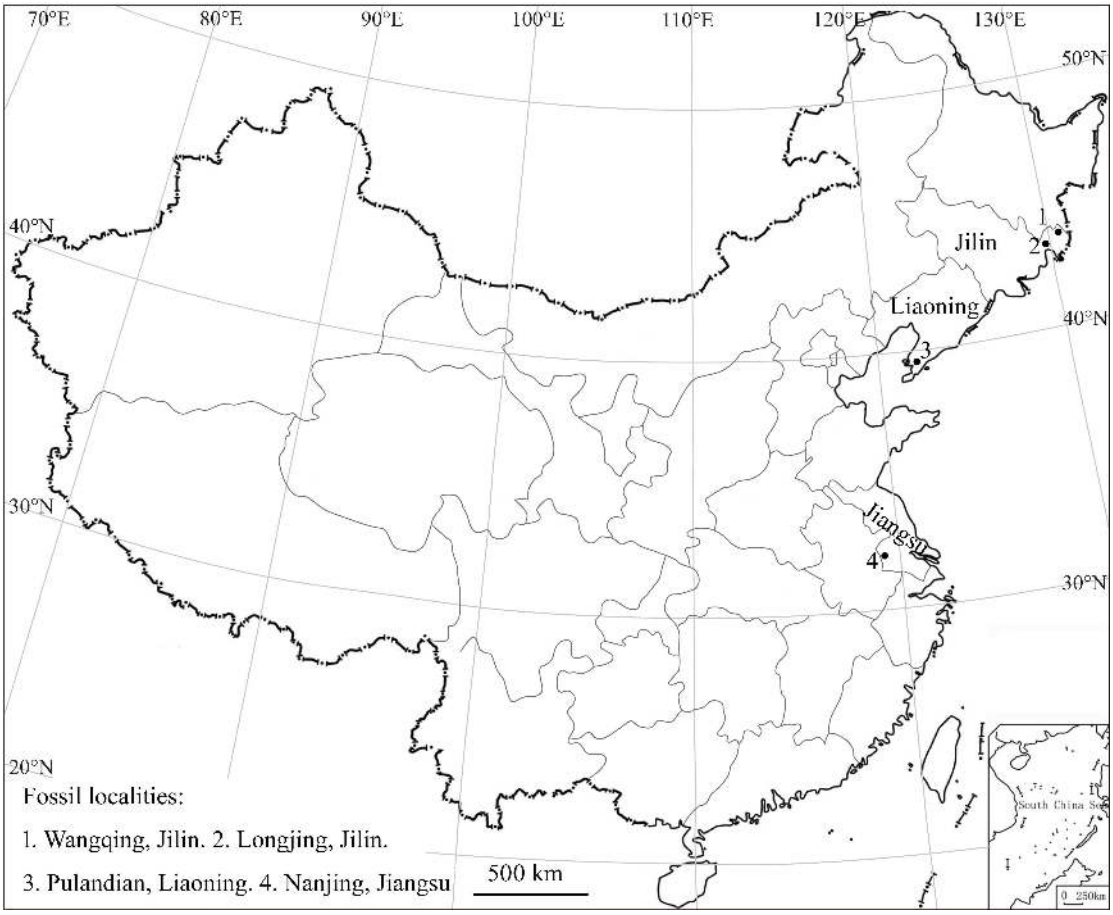
Note: Mann Whitney test was made based on 5 EDS data (Appendix C) of each ratio. *Suturovagina intermedia* data of Nanjing are from Mairot et al. (2014). Only the thicker cuticle of the Wangqing material was studied using EDS.

Table 3. Comparisons of the cuticle EDS and ultrastructural characters between *Suturovagina intermedia* from the two localities Wangqing and Nanjing

		ordinary epidermal cell cuticle			stomatal apparatus cuticle			
					subsidiary cell cuticle		guard cell cuticle	
		Nanjing	Wangqing		Nanjing	Wangqing	Nanjing	Wangqing
			Thicker or thinner cuticle					
CM cuticle membrane	**A + B**	114.59 µm	97.10 µm or 23.81 µm		117.39 µm	17.15 µm	unmeasured	1.34 µm
CP cuticle proper	**A = A1 + A2**	73.91 µm	17.92 µm or 4.07 µm		117.39 µm	17.15 µm	unmeasured	1.34 µm
CP cuticle proper	**A1**	0.26 µm	0.15-0.16 µm		0.25 µm	0.06 µm	unmeasured	0.031µm
	N/F ratio	0.09	0.42		unmeasured		unmeasured	
	N/Cl ratio	0.57	0.09					
	F/Cl ratio	6.39	0.21					
	K/Ca ratio	0.13	0.73					
CP cuticle proper	**A2**	73.64 µm	17.77 µm or 3.91 µm		117.14 µm	17.09 µm	unmeasured	1.31 µm
	N/F ratio	0.08	0.44		unmeasured		unmeasured	
	N/Cl ratio	1.04	0.11					
	F/Cl ratio	13.25	0.25					
	K/Ca ratio	0.11-0.51						
CL cuticular layer	**B = B1 + B2**	40.68 µm	79.18 µm or 19.74 µm		absent		unmeasured	absent
CL cuticular layer	**B1**	20.86 µm	76.46 µm	19.22 µm	absent		unmeasured	absent
	N/F ratio	0.04	0.40		unmeasured		unmeasured	
	N/Cl ratio	0.68-0.94						
	F/Cl ratio	25.92	1.71					
	K/Ca ratio	0.03	0.33					
CL		19.82	2.72 µm		absent			

cuticular layer	**B2**	µm	or 0.52 µm			unmeasured	absent
	N/F ratio	0.18	0.42	unmeasured		unmeasured	
	N/Cl ratio	0.16-0.29					
	F/Cl ratio	0.39-2.82					
	K/Ca ratio	0.31-0.44					
CP cuticle proper	**OL/nm**	6.54 nm	2.15 nm or 3.06 nm	8.27 nm	12.33 nm	unmeasured	6.78 nm
	TL/nm	5.73-6.72 nm		6.32-6.44 nm		unmeasured	17.32 nm

Note. Figures in blue colour, indicating the means, are significantly different between the cuticles of the same species from two different localities using the confidence interval CI (= $\bar{x} \pm \sqrt{\frac{\text{var}}{n}} \times 1.96$. giving 95 % α risk). These are believed to be palaeoenvironmental signatures. The figures in grey colour, indicating the minimum and maximum means, are not significantly different between specimens from the two localities; they might have a potential specific-generic value. *Suturovagina intermedia* data from Nanjing are from Mairot et al. (2014). For ordinary epidermal cell features, as this cuticle may be either thinner or thicker, when the two values are very different, they are both listed in the table to show there are two possibilities for this type of cuticle. For the guard cell cuticle, it is absent from Nanjing material previously studied and left without any colour. OL = opaque lamellae, TL = translucent lamellae of the A1 polylamellate layer.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Jilin
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1
2
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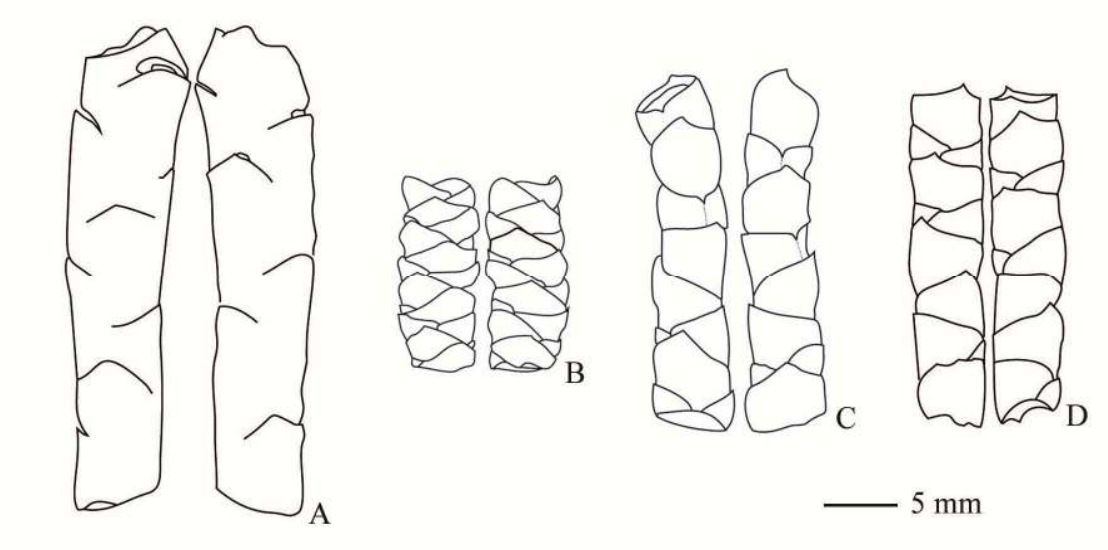
Fossil localities:
1. Wangqing, Jilin. 2. Longjing, Jilin.
3. Pulandian, Liaoning. 4. Nanjing, Jiangsu
500 km
South China Sea
0 250km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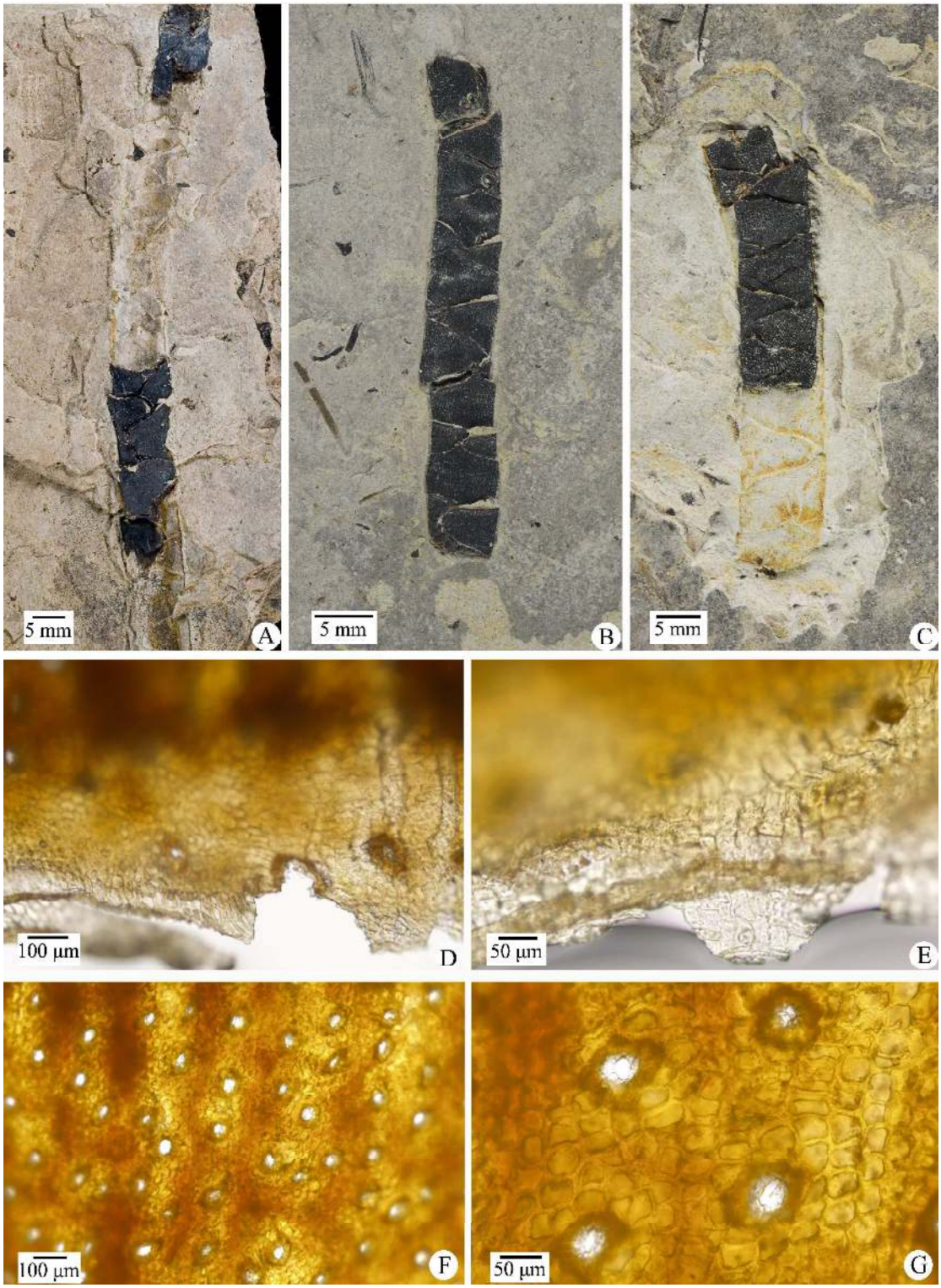
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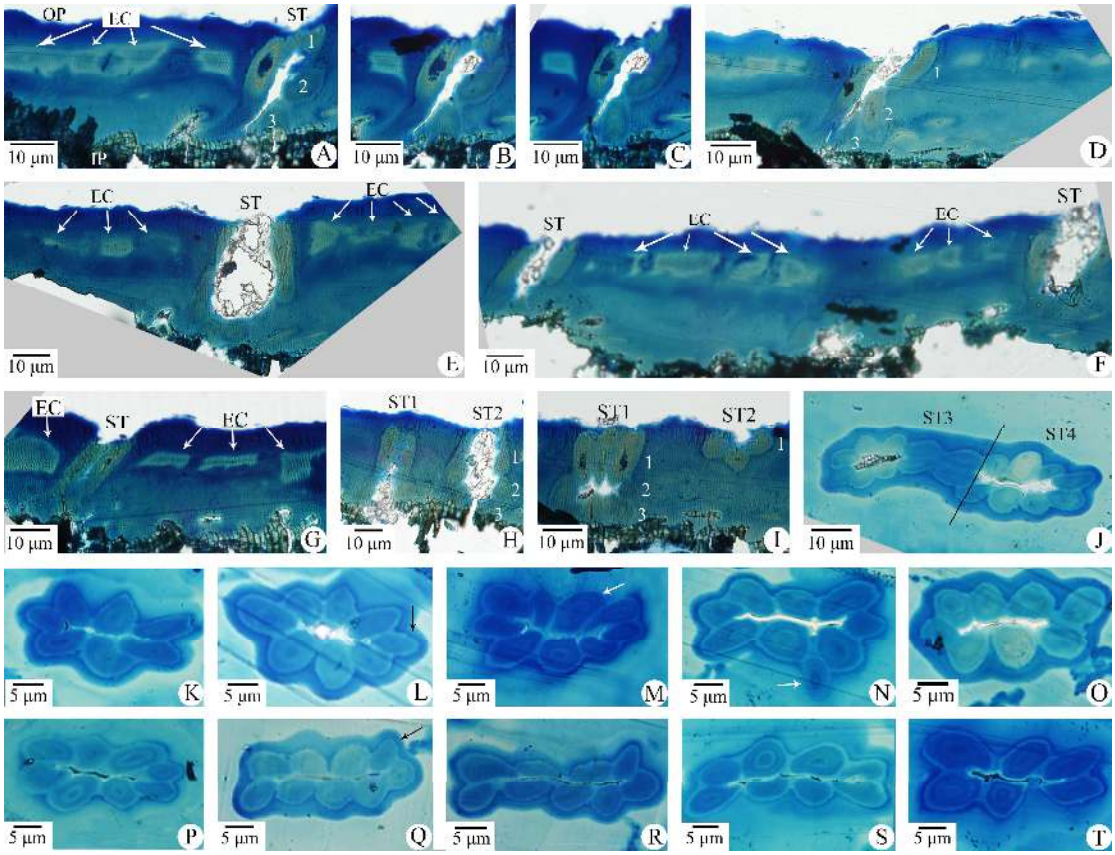
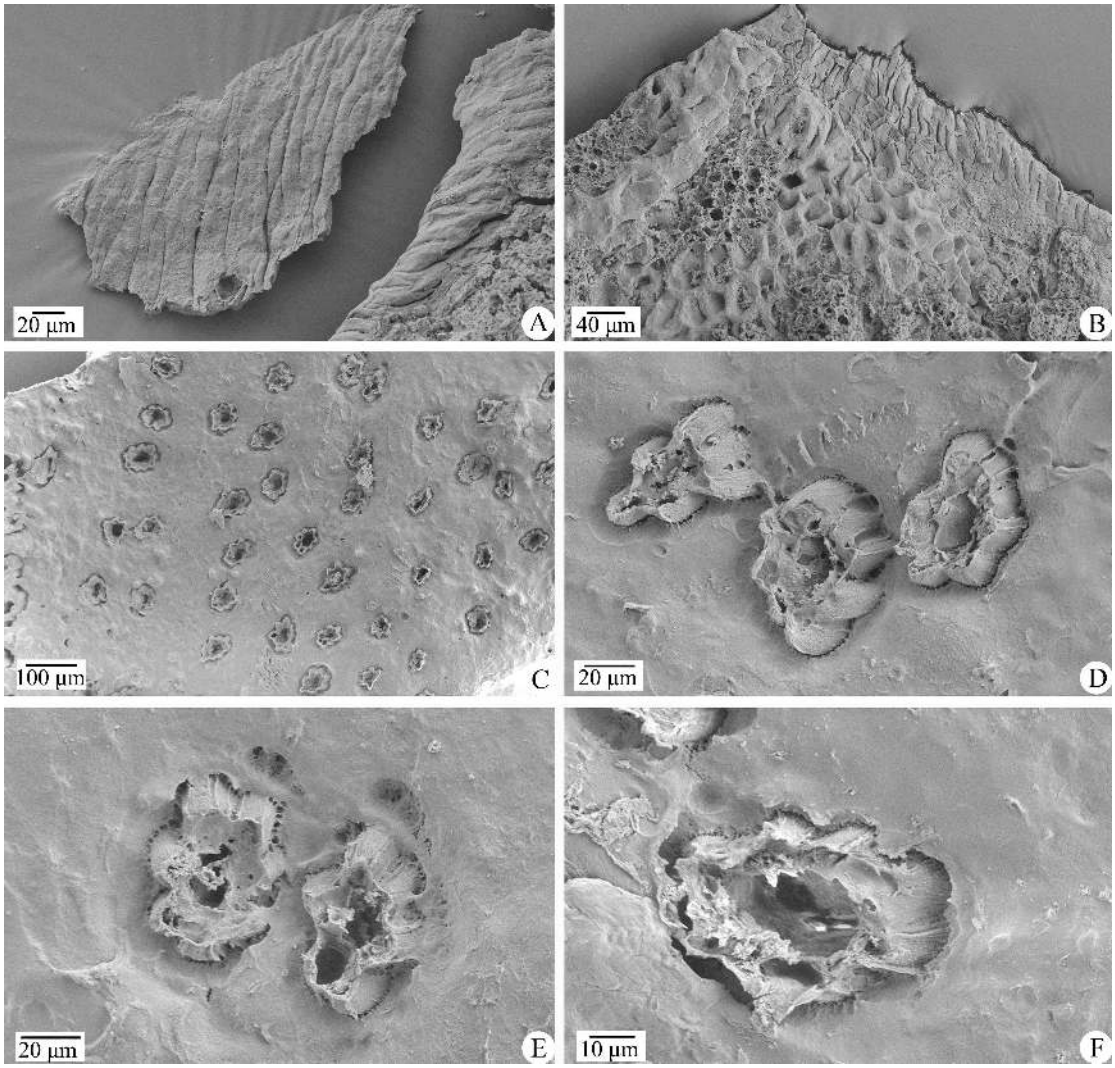
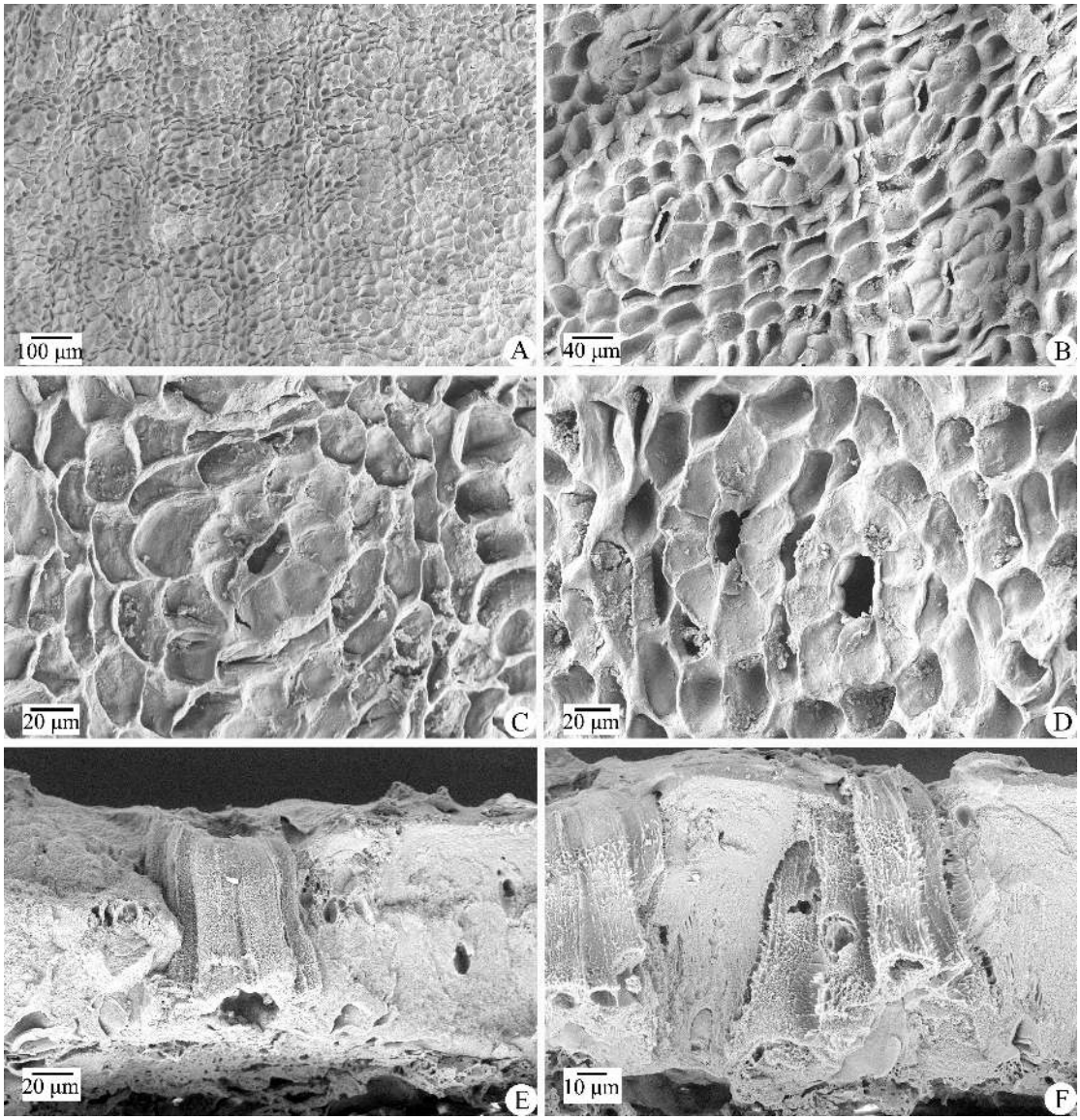
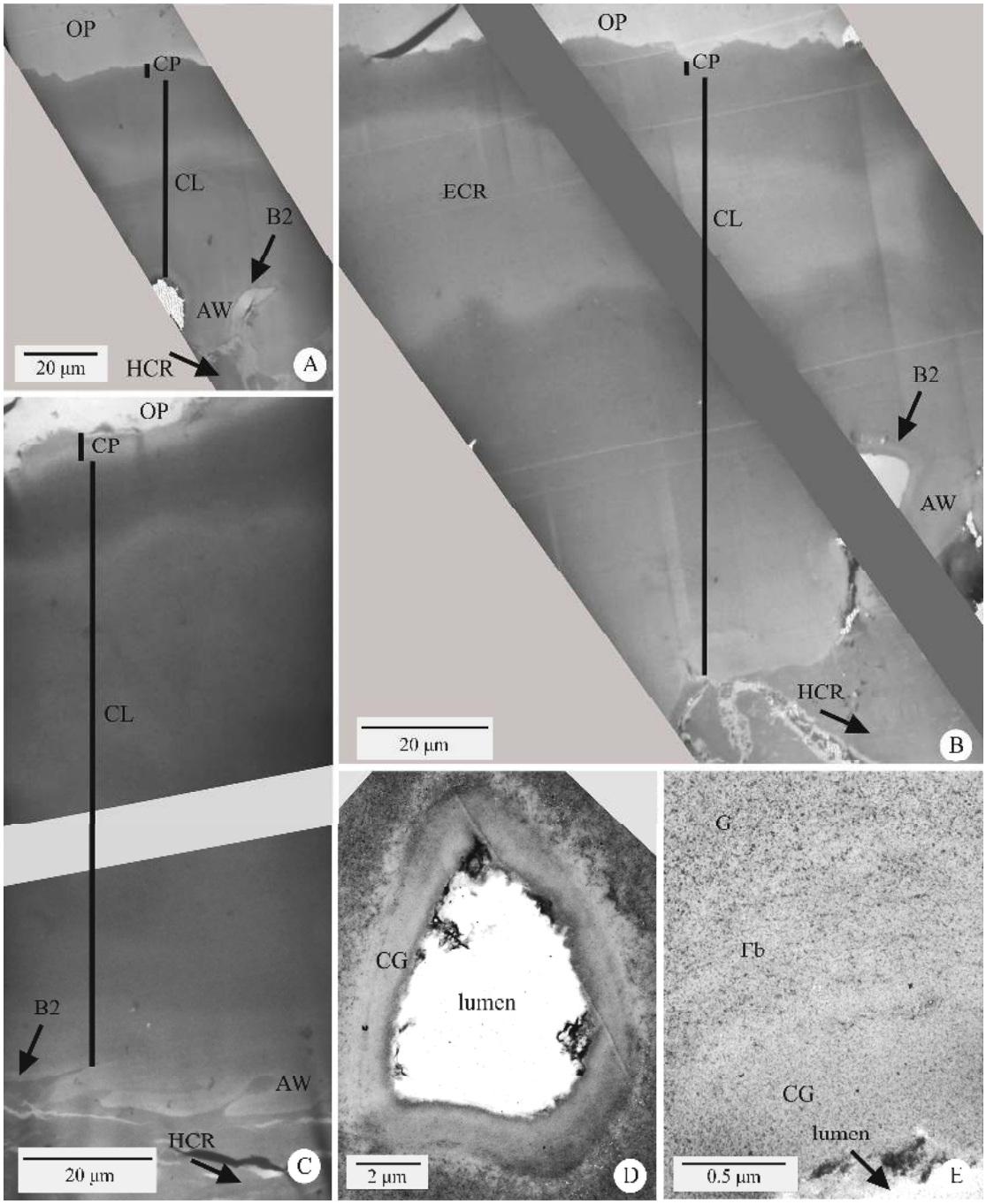
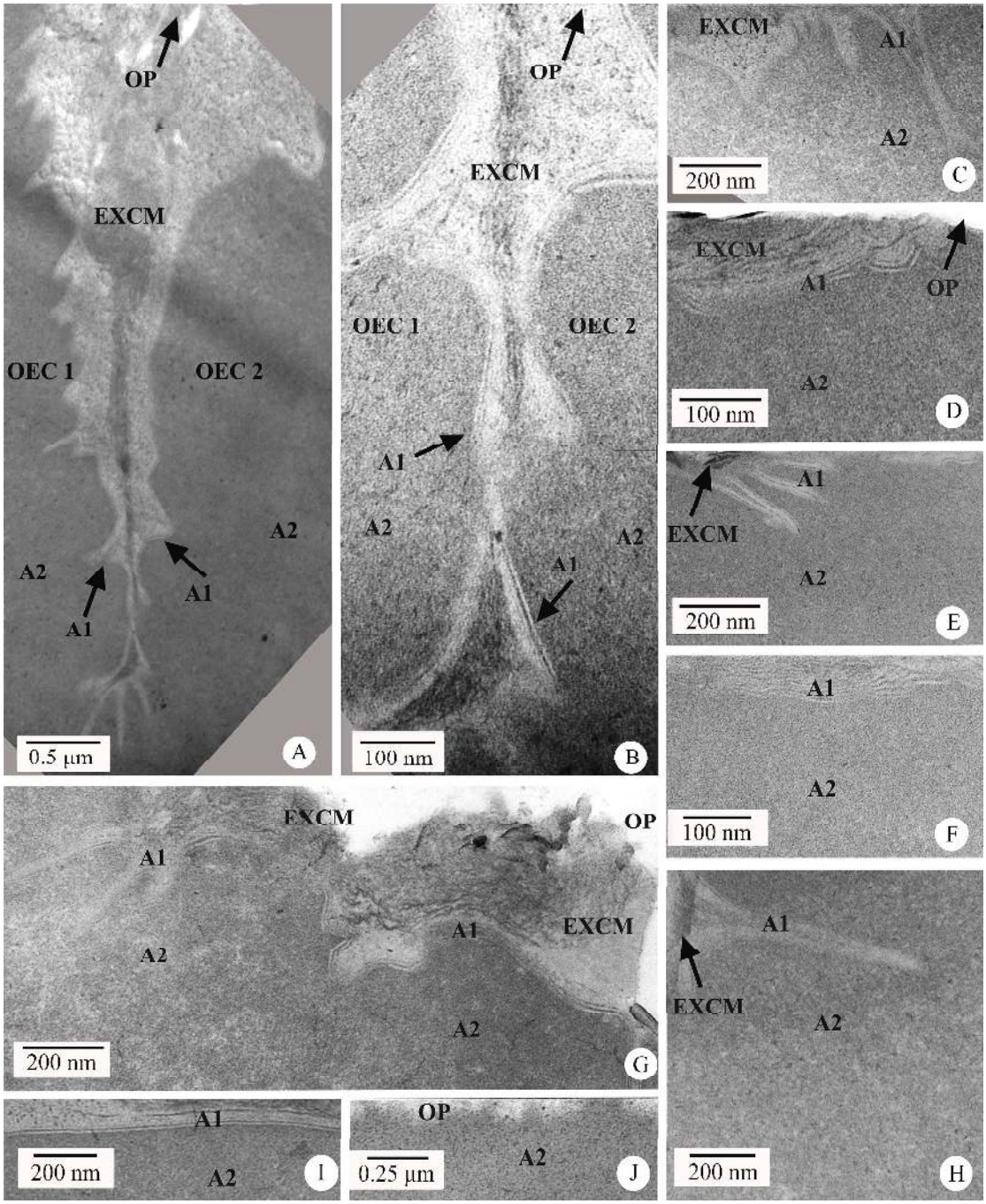
50 µm
G

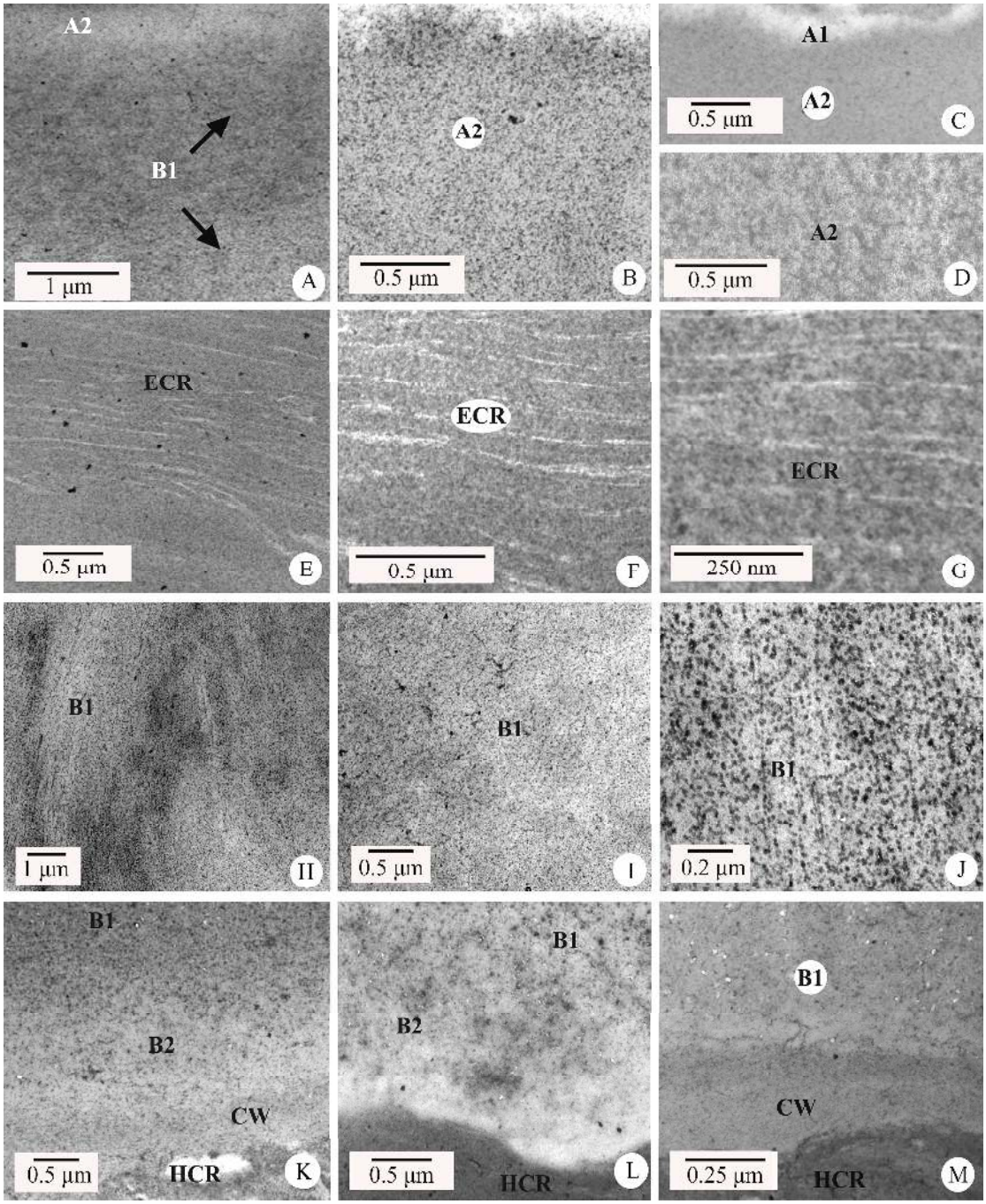

OP
EC
ST
1
2
3
IP
10 µm
A
B
C
D
E
F
G
ST1
ST2
H
I
S13
S14
J
5 µm
K
L
M
N
O
P
Q
R
S
T

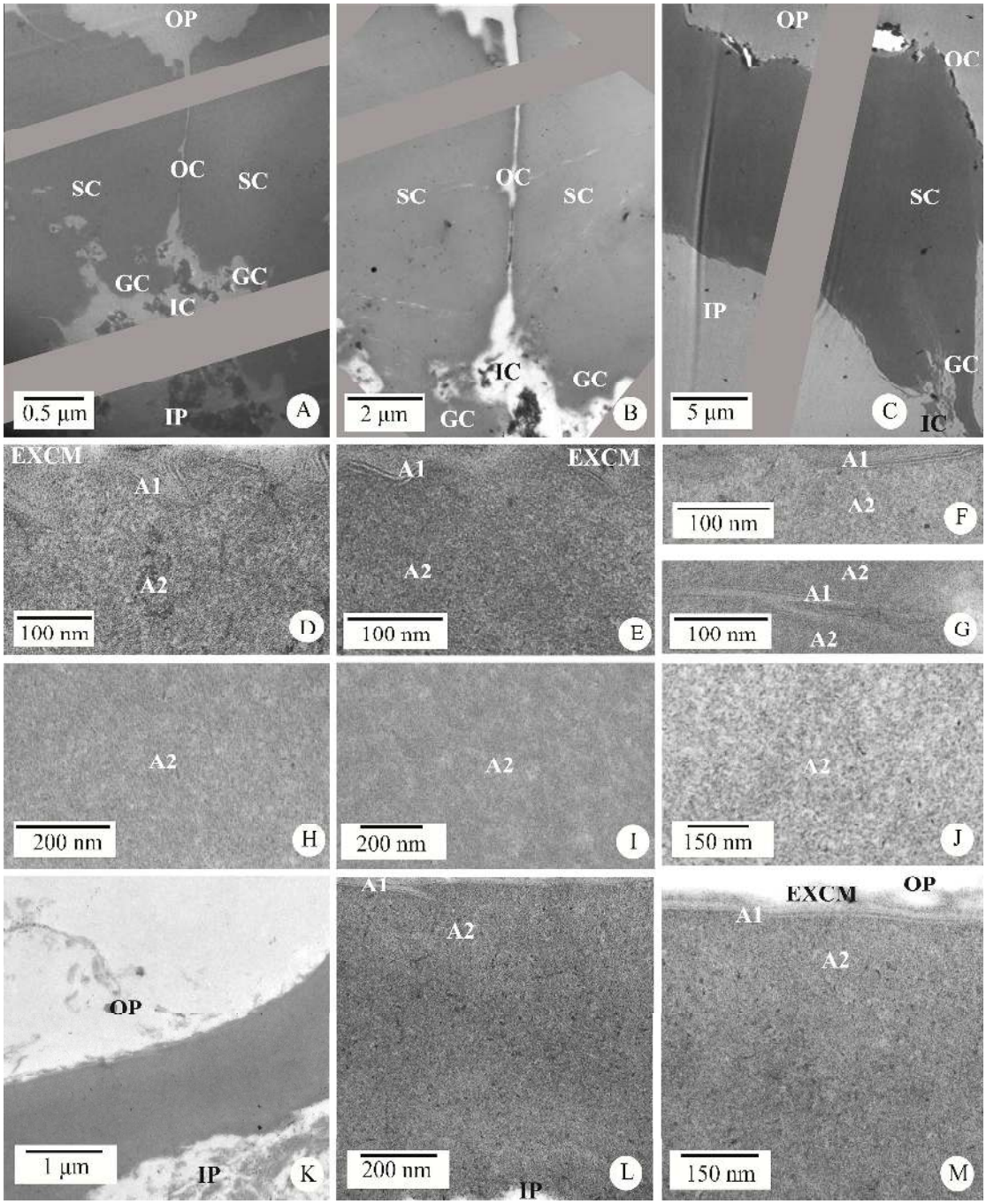

20 µm
A
40 µm
B
100 µm
C
20 µm
D
20 µm
E
10 µm
F


100 µm
A
40 µm
B
20 µm
C
20 µm
D
20 µm
E
10 µm
F

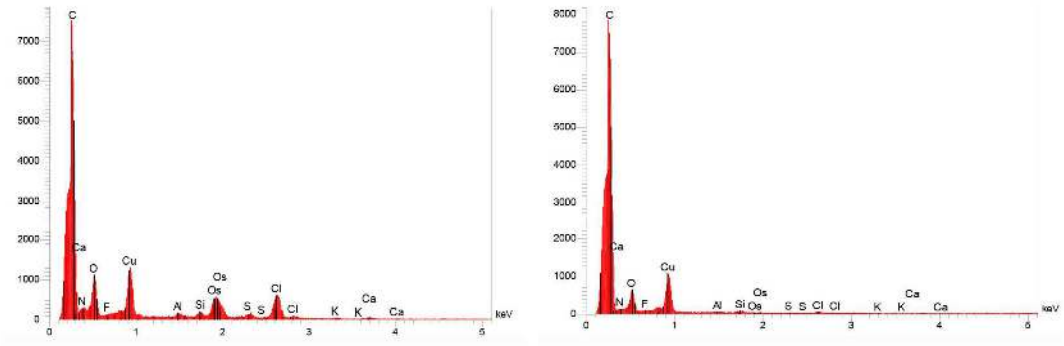

OP
CP
CL
B2
AW
HCR
20 µm
A
OP
CP
ECR
CL
B2
AW
HCR
20 µm
B
OP
CP
CL
B2
AW
HCR
20 µm
C
CG
lumen
2 µm
D
G
Fb
CG
lumen
0.5 µm
E


OP
EXCM
OEC 1
OEC 2
A1
A2
0.5 µm
A
OP
EXCM
OEC 1
OEC 2
A1
A2
A2
A1
100 nm
B
EXCM
A1
A2
200 nm
C
EXCM
A1
OP
A2
100 nm
D
A1
EXCM
A2
200 nm
E
A1
A2
100 nm
F
EXCM
OP
A1
A2
A1
EXCM
A2
200 nm
G
A1
EXCM
A2
200 nm
H
A1
200 nm
A2
I
OP
0.25 µm
A2
J

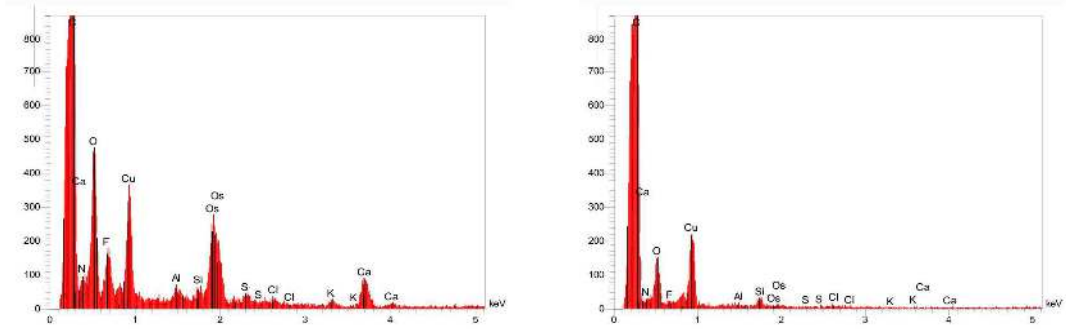

A2
B1
1 μm
A
A2
0.5 μm
B
A1
A2
0.5 μm
C
A2
0.5 μm
D
ECR
0.5 μm
E
ECR
0.5 μm
F
ECR
250 nm
G
B1
1 μm
H
B1
0.5 μm
I
B1
0.2 μm
J
B1
B2
CW
0.5 μm
HCR
K
B1
B2
0.5 μm
HCR
L
B1
CW
0.25 μm
HCR
M

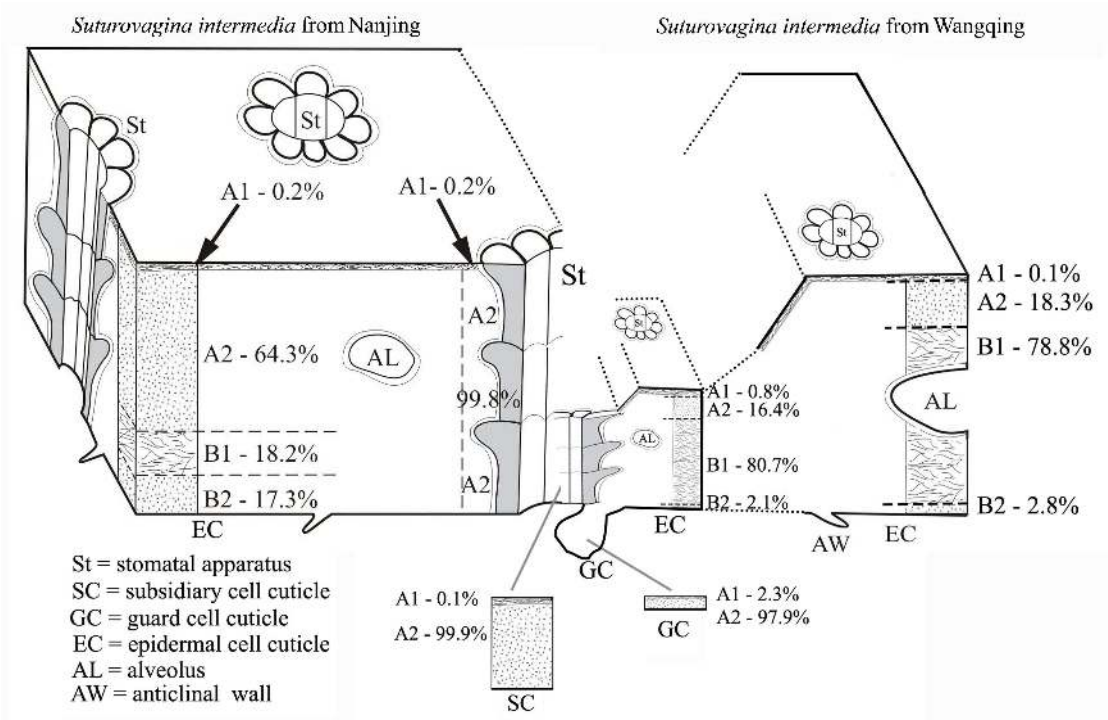

OP
OC
SC
SC
GC
GC
IC
IP
0.5 µm
A
SC
OC
SC
IC
GC
GC
2 µm
B
OP
OC
SC
IP
GC
IC
5 µm
C
EXCM
A1
A2
100 nm
D
A1
EXCM
A2
100 nm
E
A1
A2
100 nm
F
A2
A1
A2
100 nm
G
A2
200 nm
H
A2
200 nm
I
A2
150 nm
J
OP
1 µm
IP
K
A1
A2
200 nm
IP
L
EXCM
OP
A1
A2
150 nm
M

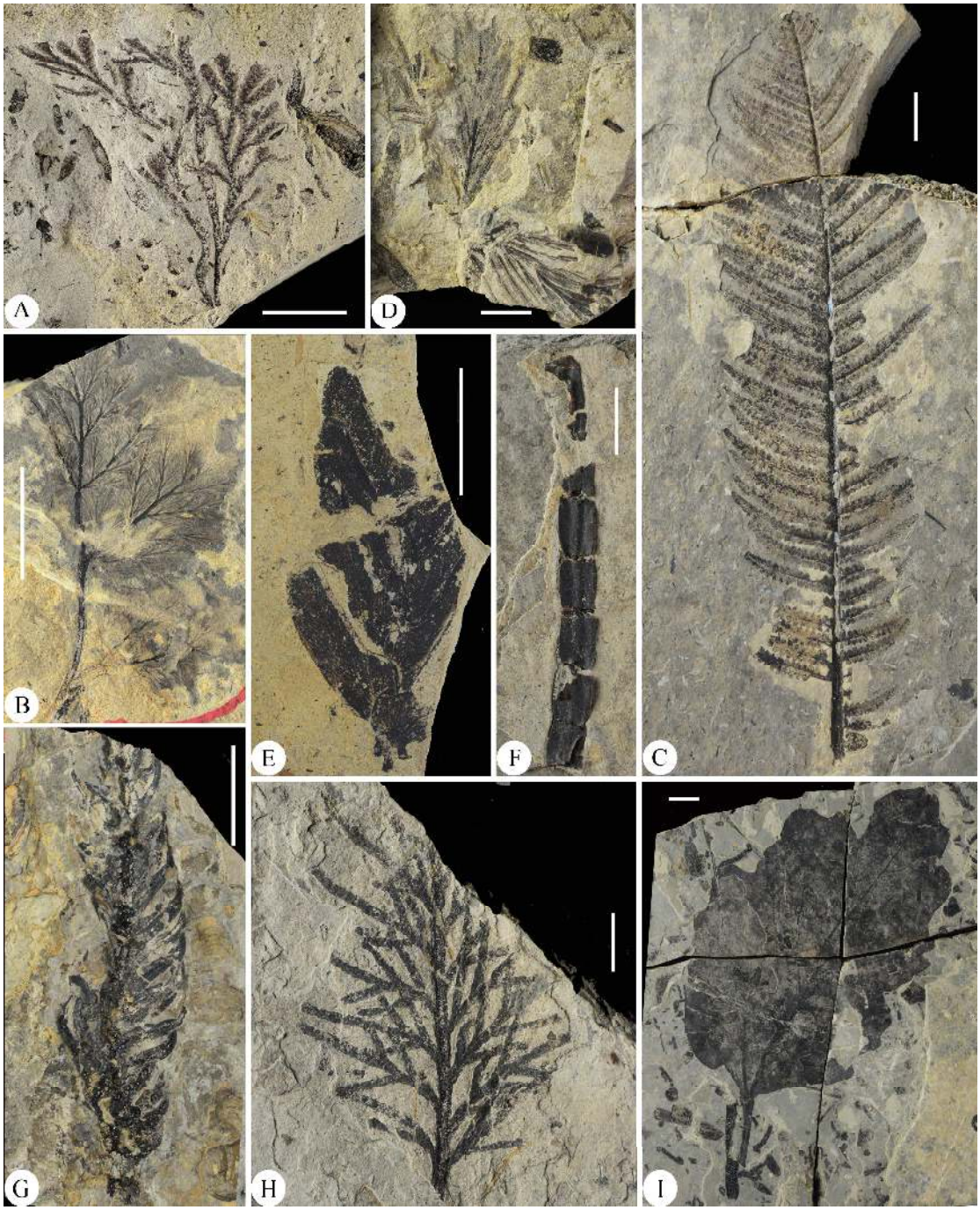
Spectra of A1 layer of cuticle (left) and resin (right) of *Suturovagina intermedia* from Wangqing, 120 seconds, spot 3



Spectra of A1 layer of cuticle (left) and resin (right) of *Suturovagina intermedia* from Nanjing, 120 seconds, spot 3




Suturovagina intermedia from Nanjing
Suturovagina intermedia from Wangqing
St
St
A1 - 0.2%
A1- 0.2%
St
A2
A2 - 64.3%
AL
99.8%
B1 - 18.2%
B2 - 17.3%
A2
EC
St
A1 - 0.8%
A2 - 16.4%
AL
B1 - 80.7%
B2 - 2.1%
EC
GC
A1 - 0.1%
A2 - 18.3%
B1 - 78.8%
AL
B2 - 2.8%
AW
EC
St = stomatal apparatus
SC = subsidiary cell cuticle
GC = guard cell cuticle
EC = epidermal cell cuticle
AL = alveolus
AW = anticlinal wall
A1 - 0.1%
A2 - 99.9%
SC
A1 - 2.3%
A2 - 97.9%
GC


A
D
B
E
F
C
G
H
I

Highlights

Suturovagina intermedia (Cheirolepidiaceae) is reported from Wangqing, Jilin, China.

There are differences (7 cuticle ultrastructural characters and 11 EDS ratios of cuticle element composition) of ecological significances, between the specimens from Wangqing and the type locality Nanjing, Jiangsu.

S. intermedia in Wangqing inhabited an environment which was not as hot or dry as that of the type locality.

The conclusion is supported by the evidence of matrix element composition (EDX data) and the associated biotic composition of the fossil-bearing beds.