Cretaceous Research 71 (2017) 24–39

Contents lists available at ScienceDirect

Cretaceous Research

journal homepage: www.elsevier.com/locate/CretRes

Cuticle ultrastructure of *Pseudofrenelopsis gansuensis*: Further taxonomical implications for Cheirolepidiaceae

Gaëtan Guignard [a, b], Xiao-Ju Yang [c, *], Yong-Dong Wang [c]

[a] Université Lyon 1, F-69622, Lyon, France
[b] CNRS, UMR 5023 LEHNA, 7-9 rue raphaël Dubois, 69622, Villeurbanne cedex, France
[c] Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, 39 East Beijing Road, Nanjing, 210008, China

ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form
10 November 2016
Accepted in revised form 14 November 2016
Available online 19 November 2016

Keywords:
Pseudofrenelopsis
Cheirolepidiceae
Cuticle ultrastructure
Taxonomy
Palaeoecology
EDS analyses

ABSTRACT

A study of the cheirolepidiaceous conifer *Pseudofrenelopsis gansuensis* from the Lower Cretaceous of Wangqing Jilin Province in China was conducted in detail using scanning and transmission electron microscopy techniques. In total, nine ultrastructural features were recognized for the cuticle of this fossil plant, which are helpful in the distinguishing between cuticles of ordinary epidermal cells, subsidiary cells, guard cells and hypodermal cells of the stomatal apparatus. A three dimensional reconstruction of the cuticle ultrastructure was obtained. *Pseudofrenelopsis gansuensis* is the second species of this genus for which the cuticle ultrastructure has been statistically examined with 30 measurements and estimated confidence interval values. The close comparison of the cuticle ultrastructure characters, including statistical data, among Cheirolepidiaceae and other fossil conifers provides potential evidence of the taxonomic significance of this genus: ten characters are potentially valuable for specific separation, eleven parameters for generic separation and three parameters seem also to be useful for Family determination. The differences in the chemical composition according to preliminary statistical element analyses of the cuticles based on three ratios in two species of *Pseudofrenelopsis*, *P. dalatzensis* and *P. gansuensis*, should also be examined in future studies.

1. Introduction

The application of transmission electron microscopy (TEM) in the study of Cheirolepidiaceae (Archangelsky and Taylor, 1986; Archangelsky and Del Fueyo, 1989; Guignard et al., 1998; Villar de Seoane, 1998; Axsmith et al., 2004; Del Fueyo et al., 2008; Yang et al., 2009; Mairot et al., 2014) has achieved fruitful results since the eve of the 21st century. Recently, generation of detailed statistical data and a reconstruction of the cuticle ultrastructure of cheirolepidiaceous conifers have been provided (Yang et al., 2009; Mairot et al., 2014). *Pseudofrenelopsis dalatzensis* and *Suturovagina intermedia* are a few of the taxa that have been studied in detail. There are significant differences between *Suturovagina* and *Pseudofrenelopsis* in four cuticle ultrastructural characters, and the proportions of different layers also markedly differed (Mairot et al., 2014). The proportions of the cuticle proper and cuticular layer in the epidermal cell cuticular membranes and wavy and polylamellate and granular layers in the cuticle proper differed among cheirolepidiaceous conifers, but few statistical data are available.

The present study describes the cuticle ultrastructure of another cheirolepidiaceous conifer, *Pseudofrenelopsis gansuensis*, to provide further anatomical details and elucidate the potential taxonomic and palaeoecological significance of the ultrastructural features observed using transmission electron microscopy. In addition, trial element analyses were also applied to examine the cuticles of two species of *Pseudofrenelopsis* for similar purposes.

2. Material and methods

Samples were collected from Luozigou, Wangqing County, Jilin Province, northeastern China (43°53′48″N, 130°03′26″E, Fig. 1). The plant-bearing strata in Luozigou Basin belong to the Lower Cretaceous Dalazi Formation (Oishi, 1941; Zhou et al., 1980; Bureau of Geology and Mineral Resources of Jilin Province, 1997). The type section of the Dalazi Formation is well developed in Dalazi village, Zhixin Town, Longjing County in Yanji Basin, approximately 150 km to the southwest of Luozigou Basin in eastern Jilin Province, China

* Corresponding author. Fax: +86 25 83357026.
E-mail addresses: guignard@univ-lyon1.fr (G. Guignard), xjyang@nigpas.ac.cn (X.-J. Yang).

http://dx.doi.org/10.1016/j.cretres.2016.11.006
0195-6671/

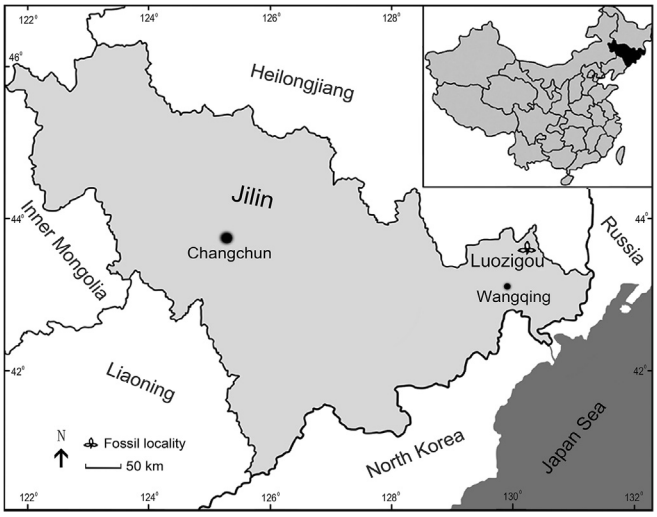

Fig. 1. Sketch map showing the fossil locality in Wangqing, Jilin Province, China (indicated by ✈).

(Li et al., 2016). Until recently, several cheirolepidiaceous conifers have been reported in Yanji Basin (Chow and Tsao, 1977; Zhang et al., 1980; Zhang, 1986; Zhou, 1995), but only one conifer species has been reported in Luozigou (Yang and Deng, 2007).

After several field trips to Luozigou, Yang XJ collected abundant shoots of cheirolepidiaceous conifers belonging to *Pseudofrenelopsis*, and some of these conifers were branched. Further examination showed that the cuticle is not similar to *Pseudofrenelopsis dalatzensis* (Chow et Tsao) Cao ex Zhou (Zhou, 1995), but resembled *Pseudofrenelopsis gansuensis* based on a fragmentary leafy shoot from the Cretaceous in Jiuquan Basin, Gansu Province, Northwest China (Deng et al., 2005). Interestingly, *Pseudofrenelopsis dalatzensis* occurs rather frequently in the Dalazi Formation of Yanji Basin, while *P. gansuensis* is the most common plant from Luozigou Basin. Although the two cherolepidiaceaeous conifers from the two localities are generally similar in gross morphology, the cuticles of these plants are markedly different (Yang and Deng, 2007).

In the present study, the cuticle ultrastructure of *P. gansuensis* was examined using SEM and TEM. Pieces of cuticles were removed from the specimens, treated with hydrofluoric acid (HF) for 18 h, and subsequently macerated in Schulze's solution (nitric acid and potassium chlorate). The time for oxidation depended upon the degree of coalification of the compressed specimen, typically approximately 5–8 h. For the next step (see also Kerp, 1990), after the cuticles became yellow and translucent, the samples were rinsed with water and treated with dilute ammonia (5%) for a few seconds to half a minute, followed by thorough rinsing with water. The samples for scanning electron microscopy (SEM) were mounted on stubs using double-sided adhesive tape, coated with gold, observed and photographed using a Hitachi S-4300 SEM at the SEM Laboratory of the Swedish Museum of Natural History, Stockholm, Sweden. The samples for transmission electron microscopy (TEM) were prepared according to Lugardon (1971), a technique that is also used for fossil pollen and spores and living plant cuticles (conifers and angiosperms; Bartiromo et al., 2012, 2013). A total of 12 cuticle samples were obtained from the specimens (2 Epon resin blocks were obtained from slightly treated material PB21041 and 10 blocks were obtained from treated material PB21045). Among these blocks, 220 ultrathin 60–70 nm sections (70 sections from specimen PB21041 and 150 sections from specimen PB21045) were obtained and collected on uncoated 300 Mesh copper grids (200 transversal sections, i.e., perpendicular to the leaf length; 20 longitudinal sections, i.e., parallel to the leaf length). Ultrathin sections were selected, observed and photographed using a Philips CM 120 at 80 kV at the Centre de Technologie des Microstructures (CTμ) of Lyon-1 University, France; and a few sections were examined using a Hitachi H-7650 at 80 kV at the Life Sciences Laboratory Center of Nanjing Agricultural University in China.

To obtain information on the approximate stomatal and cuticle structures, 100 transversal and 10 longitudinal 1-μm sections were mounted on 22 glass slides and subsequently examined. These sections were stained according to Richardson et al. (1960). Consistent with Richardson et al. (1960), the samples were stained with methylene blue, Azur II solution (½–½), prior to obtaining micrographs under a Zeiss axioscope 2 light microscope using Zen 1.0.1.0 software.

EDS analysis was performed on TEM images using SIRIUS SD ENSOTECH and IDFIX software, with an acceleration voltage of 120 kV, 1–3 spot sizes, and 60–120 s processing time at a constant time of 4 μseconds. For each of the two *Pseudofrenelopsis* species compared in the present study, 10 copper 300 Mesh uncoated grids were used, devoid of uranyl acetate and lead citrate staining. Among the available elements, Cu and Al were eliminated from the results as components of the grid, Os was eliminated as a component of the embedding technique, Si was eliminated as a component of the oils used in the TEM, and C and O were also eliminated as major components of the EPON embedding resin. Element analyses of the matrix containing fossil plants were undertaken using an energy dispersive X-ray system attached to LEO-1530VP in Nanjing.

Specimens (PB21041–21045), SEM stubs and negatives were housed at Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences in China. TEM material and negatives were stored at Lyon-1 University, Villeurbanne, France.

The following terms and abbreviations are used in the text, tables, figures and appendices: OEC = ordinary epidermal cell cuticle; SC and GC = subsidiary and guard cell, respectively, of the cuticle of the stomatal apparatus; CM = cuticular membrane (CP + CL); CP = cuticle proper (A = A1 + A2); A1 = outer polylamellate layer of the cuticle proper; A2 = inner mainly granular layer; CL = cuticular layer (B); B1 = outer fibrilous layer; B2 = inner most granular layer; OL = opaque lamellae of the polylamellate layer (A1); and TL = translucent lamellae of the polylamellate layer (A1).

3. Results

3.1. Gross morphology and cuticle structure

There are numerous vegetative shoots in the collection. Most of these structures are unbranched shoots, up to 110 mm long and 3–8 mm wide (Fig. 2). The internodes are typically 7–14 mm long. The leaves are adpressed on the axis, loosely arranged in a simple spiral. The free area is triangular, approximately 0.5–1 mm long with an acute apex and decurrent base that encircles the entire internode. The outer surface bares distinct fine, parallel, longitudinal ridges and grooves that converge towards the leaf apex.

The abaxial cuticle of the leaf is approximately 8–28 μm thick, with well-defined longitudinal stomatal and non-stomatal files (approximately 7–9 in stomatal files per mm) that are clearly visible on both outer and inner surfaces (Fig. 3, A, B). The outer surface is normally smooth without hairs or papillae (Fig. 3, A). The epidermal cells have special periclinal walls that irregularly split to form fissures along the anticlinal wall on the outer surface, except at the cell corner (Fig. 3, A). In the stomatal files, epidermal cells are more or less square in outline or isodiametrically polygonal, approximately 12–30 μm × 14–25 μm in size; in the non-stomatal