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Culm anatomy of the genus *Otatea* (Poaceae, Bambusoideae, Bambuseae, Guaduinae) as a contribution to Mexican species identification

MIGUEL A. GARCÍA-MARTÍNEZ[1,2,6], TERESA TERRAZAS[3,7], DANIEL SÁNCHEZ[4,5,8], PABLO CARRILLO-REYES[4,9] & EDUARDO RUIZ-SANCHEZ[2,4,10]*

[1] *Doctorado en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas, Universidad de Guadalajara, Camino Ing. Ramón Padilla Sánchez 2000, Nextipac, Zapopan, Jalisco, 45200, Mexico.*
[2] *Laboratorio Nacional de Identificación y Caracterización Vegetal (LaniVeg), Instituto de Botánica, Universidad de Guadalajara, Camino Ing. Ramón Padilla Sánchez 2000, Nextipac, Zapopan, Jalisco, 45200, Mexico.*
[3] *Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, Mexico City, Mexico.*
[4] *Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Camino Ing. Ramón Padilla Sánchez 2000, Nextipac, Zapopan, Jalisco, 45200, Mexico.*
[5] *Investigador por México, Consejo Nacional de Humanidades Ciencias y Tecnologías (CONAHCYT), Laboratorio Nacional de Identificación y Caracterización Vegetal, Departamento Botánica y Zoología, Universidad de Guadalajara, Camino Ing. Ramón Padilla Sánchez 2000, Nextipac, Zapopan, Jalisco, 45200, Mexico.*
[6] *miguel.gmartinez@alumnos.udg.mx; https://orcid.org/0000-0002-8472-7295*
[7] *tterrazas@ib.unam.mx; https://orcid.org/0000-0001-7749-5126*
[8] *dsanchezc@conahcyt.mx; https://orcid.org/0000-0002-8621-0222*
[9] *pablo.creyes@academicos.udg.mx; https://orcid.org/0000-0001-9278-0208*
[10] *ruizsanchez.eduardo@gmail.com; https://orcid.org/0000-0002-7981-4490*
**Corresponding author*

Abstract

Otatea is a Neotropical woody bamboo genus with thirteen described species. It is distributed from Mexico to Central America and northeastern Colombia. In Mexico, the archaeological evidence suggests that the culms of *Otatea* have been used for centuries by some ethnic groups. It is important to explore morphological and anatomical characters for species identification in woody bamboos as consequence of its monocarpic nature. Most studies on culm anatomy are centered on commercially used species describing their physical and mechanical properties. The aim of this work was to find culm anatomical characters with taxonomic value. To perform this, we collected internode samples from adult plants at mid culm of 15 specimens that represent 10 described species and two putative new species of *Otatea.* Our results include descriptions of culm anatomy at a cross section accompanied with images that detail all the structures measured and described. Additionally, we elaborate a key based on anatomical characters. This contribution is the first to explore culm anatomy of the genus *Otatea* in search for characters with taxonomic value, providing useful evidence for species identification, including samples from archaeological sites.

Key words: Archaeobotany, cross section, Mexico, neotropical woody bamboos, vascular bundles

Introduction

Bamboos belong to one of the major clades of the grass family Poaceae, specifically subfamily Bambusoideae. They grow in forests and can be found in tropical, subtropical, and temperate regions around the world (Clark *et al.* 2015, Soreng *et al*. 2017). Bambusoideae is a monophyletic lineage that is divided into two groups: herbaceous bamboos (tribe Olyreae) and woody bamboos (tribes Arundinarieae and Bambuseae) (Kelchner & BPG 2013, Clark *et al*. 2015, Wysocki *et al.* 2015, 2016, Soreng *et al*. 2017, Ruiz-Sanchez *et al*. 2021). Due to their abundance, fast growth rate, and ease of handling, woody bamboos have been used in countless ways, including as living ornamentals, for soil rehabilitation on degraded land, and to prevent erosion (Akinlabi *et al*. 2017). Different parts of the plant, such as roots, shoots, culms, leaves, and fruits, have various uses including edible products, paper and textile production, handicrafts, furniture fabrication, building construction, and even the generation of engineered bamboo products (Liese *et al*. 2015, Akinlabi *et al*. 2017).

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The culm is the above-ground stem of the bamboo plant and has the most well-known uses, its usefulness being determined by its structural properties. Culms are segmented into nodes and internodes. The nodes are the transverse connections between internodes, and the internodes can be hollow (the lacuna) or solid (Liese *et al*. 2015). Anatomically, the culm is composed of vascular bundles, numerous sclerenchyma cells, fibers or sclereids as support tissue, and parenchyma cells as ground tissue. The vascular bundles are formed by one or two protoxylem cells, two round-shaped metaxylem vessels, and phloem consisting of sieve tubes and companion cells. Fibers surround the metaxylem and phloem tissues. Vascular bundles vary in shape and abundance from the periphery towards the center, and they can be categorized into three sections: peripheral, transitional, and central (Grosser & Liese 1971). In the peripheral section, the vascular bundles are small, and the sclerenchyma sheaths are large and compacted. In the transitional section, the vascular bundles are larger and more widely spaced. The central vascular bundles again become smaller and are sparse, with xylem and phloem areas larger than the peripheral ones. Species with solid culms have parenchyma with small and sparse vascular bundles, and the phloem is oriented in different directions in the central region of the culm. Variation in the shape, size, and density of vascular bundles would determine its overall appearance at a cross-section (Liese & Weiner 1996, Liese 1998, Liese *et al*. 2015).

Members of the Bambusoideae subfamily (excluding the Olyreae tribe) grow for several years without flowering (3 to 150), after which they rapidly produce large quantities of flowers, release fruits, and die (Janzen 1976, Judziewicz *et al*. 1999, Zheng *et al*. 2020). Therefore, it is crucial to investigate morphological features that aid in identifying bamboo species during their vegetative stage. Grass taxonomists have explored the usefulness of leaf anatomy and micromorphology as tools for species or group classification (Brown 1958, Ellis 1987, Soderstrom *et al*. 1987); however, culm anatomy has received less attention for this purpose. Additionally, most studies on culm anatomy have focused on commercially used species, describing their physical and mechanical properties for industrial applications (e.g. Zaragoza-Hernández *et al*. 2014, Abdullah-Siam *et al*. 2019, Yormann *et al*. 2020).

The Neotropical woody bamboo clade is one of the four major clades within Bambusoideae. It has a geographical distribution that extends from Mexico through Central and South America (Kelchner & BPG 2013, Clark *et al.* 2015). Culm anatomical studies have been carried out on some Neotropical woody bamboo species from South America, including *Aulonemia* Goudot (1846: 75), *Colanthelia* McClure & E. W. Sm. in McClure (1973: 77), *Chusquea* Kunth (1822: 151), *Guadua* Kunth (1822: 150), *Merostachys* Sprengel (1825: 132), and *Rhipidocladum* McClure (1973: 101), as well as *Guadua angustifolia* Kunth (1822: 253), the most popular bamboo species used as construction material in Central and South America (Londoño *et al*. 2002, Rúgolo de Agrasar & Rodríguez 2003, Guerreiro *et al*. 2013). These studies were conducted to describe culm cell characteristics and to use that information to identify woody bamboo species found in archaeological sites in South America. There is only one study of the culm anatomy in *Guadua aculeata* Ruprecht ex Fournier (1886: 130) from Mexico, which was conducted by Zaragoza-Hernández *et al*. (2014) to determine the anatomical structure and fiber size of this species.

The genus *Otatea* (McClure & E.W. Sm. in McClure (1973: 116)) Calderón & Soderstrom (1980: 21) belongs to the subtribe Guaduinae, with its geographical distribution ranging from Mexico to Central America and northeastern Colombia. There are thirteen described species in this genus, with Mexico having the greatest diversity, including twelve species, eleven of which are endemic. One undescribed species was found in Chiapas during fieldwork for this study (Ruiz-Sanchez *et al*. 2019, Ruiz-Sanchez *et al*. 2021). The *Otatea* species inhabit various vegetation types such as tropical dry forests, xerophilous scrub, oak forests, humid pine-oak forests, and cloud forests, at elevations of 50–2100 m (Ruiz-Sanchez *et al*. 2011). In Mexico, *Otatea* species are known as "otate", which derives from the Nahuatl word "otatl" meaning "cane". The culms of *Otatea* are used to construct bajareque walls, roofs, doors, and fences for rural house building. The most common use is the fabrication of baskets and walking sticks. In the agricultural industry, the culms are used as a support and guide pole and have the advantage of being a reusable source (Guzmán *et al*. 1984, Vázquez-López 1995).

Mexico has 193 public archaeological sites (INAH 2023). Fragments or impressions of woody bamboos used as bajareque have been found in some of these sites. According to Shaffer (1993), bajareque is a type of wattle and daub architecture. This involves constructing walls with frames made of wooden or bamboo members (wattles) that are lashed and/or interwoven. Puddled, clayey soil (daub) is then applied as a thick plaster. Archaeological evidence suggests that the culms of *Otatea* have been used for centuries by some ethnic groups (Juárez & Márquez 1992, Ruiz-Sanchez & Clark 2018). Rests of bajareque have been found at Guachimontones archaeological site in Jalisco, Mexico, dating back from BC 400 to AD 500 years (Guerrero *et al*. 2016). A fragment of bajareque impression, which probably corresponds to *O. acuminata* (Munro 1868: 25) Calderón & Soderstrom (1980: 21), is exhibited at the Teuchitlán *in situ* museum (Ruiz-Sanchez *et al*., unpublished). Trabanino & Núñez (2014) found turned-to-charcoal wood fragments at Chinikiná archaeological site in Chiapas, Mexico, dating back from AD 650 to 800 years. These fragments were found as a mortuary item in a Maya burial and correspond to some *Guadua* species. Impressions of bajareque, corresponding to *O. acuminata*, were also found by Juárez & Márquez (1992) in the Loma Iguana archaeological site in Veracruz, Mexico, dating back from AD 800 to 890 years. Other archaeological evidence of the use of *O. acuminata* was found in the elaboration of "chimalli," a Nahuatl shield (Mejía-Saules 2022).

In this study, we examined the anatomical structure of culms from 10 previously described species and two potential new species of *Otatea*, all native to Mexico. Our objective was to identify differences in these structures that could have taxonomic value, and to provide evidence that would be useful in identifying samples from archaeological sites. We also created an identification key based on culm anatomical characteristics in cross-section.

Materials and methods

Sample collection:—We collected culm material from 15 specimens of *Otatea* during our field work between 2020 and 2022 (Fig. 1). Internode samples were taken from mid culm of adult plants with at least three years of development. *Otatea acuminata* is endemic to Mexico and has the widest geographical distribution and morphological variation. A delimitation species study is currently in progress. For this reason, we labeled four *O. acuminata* samples using the state of collection (*O. acuminata* AGS from Aguascalientes; *O. acuminata* JAL from Jalisco; *O. acuminata* OAX from Oaxaca; *O. acuminata* VER from Veracruz). During field work in Chiapas, we collected material from a putative new species labeled here as *Otatea sp. nov.* 1. We also collected material from a flowering population of *Otatea* in Jalisco state that has been identified as *O. reynosoana* Ruiz-Sanchez & Clark in Ruiz-Sanchez *et al*. (2011: 328). However, the reproductive and vegetative morphology did not match with the type specimen. Therefore, we labeled it as *Otatea sp. nov.* 2. The other species examined (*Otatea carrilloi* Ruiz-Sanchez, Sosa & Mejía-Saulés in Ruiz-Sanchez *et al*. (2011: 324), *O. fimbriata* Soderstrom in McVaugh (1983: 280), *O. glauca* Clark & Cortés (2004: 3), *O. ramirezii* Ruiz-Sanchez (2012: 25), *O. rzedowskiorum* Ruiz-Sanchez (2015: 265), *O. victoriae* Ruiz-Sanchez (2015: 267), and *O. ximenae* Ruiz-Sanchez & Clark in Ruiz-Sanchez *et al*. (2011: 330) were collected near or at their type locality, except *O. transvolcanica* Ruiz-Sanchez & Clark in Ruiz-Sanchez *et al*. (2011: 330) and *O. reynosoana*.

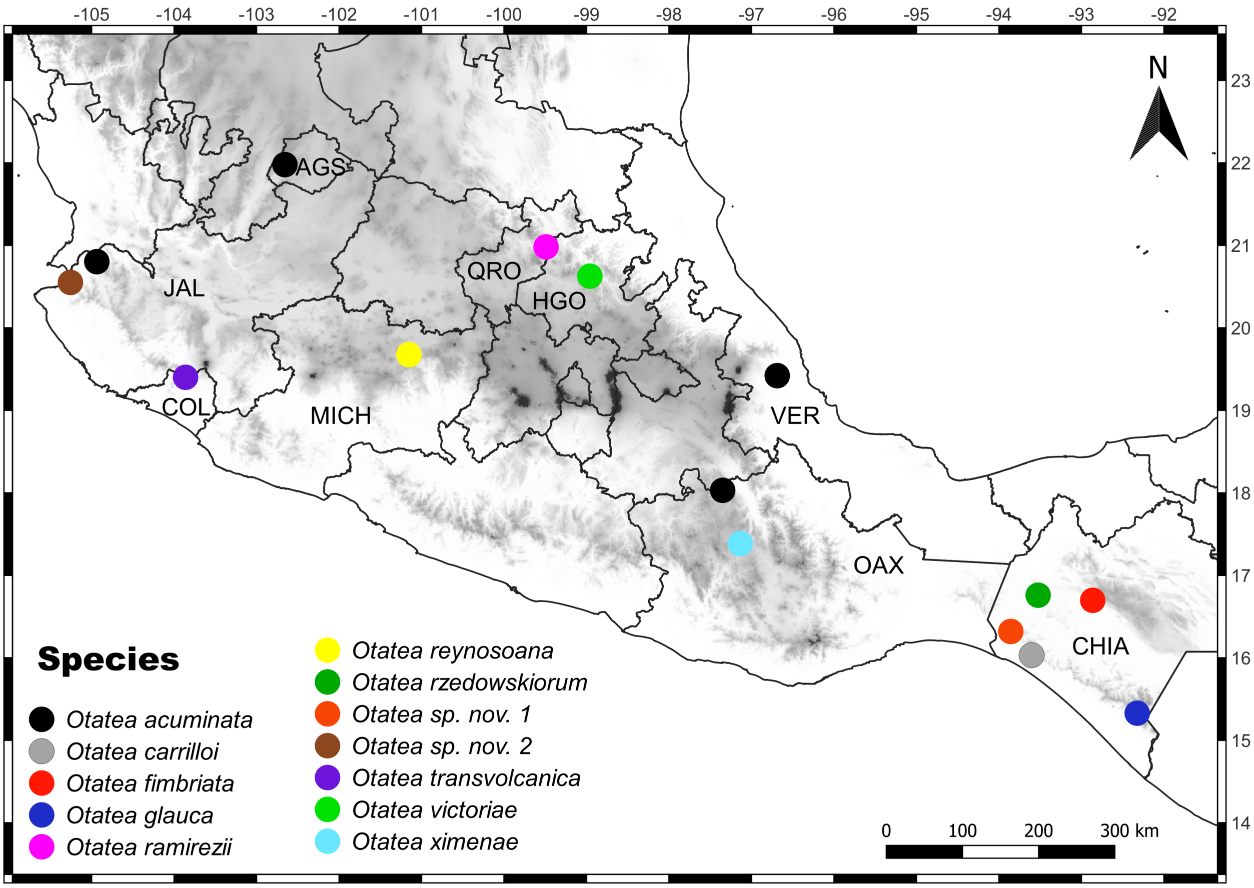

FIGURE 1. Locations of the *Otatea* used for culm sampling in the present study. AGS = Aguascalientes, CHIA = Chiapas, COL = Colima, HGO = Hidalgo, JAL = Jalisco, MICH = Michoacán, OAX = Oaxaca, QRO = Querétaro, VER = Veracruz.

In the field, samples were cut to around 3–5 cm at mid-culm and stored in FAA solution (which comprises five parts formaldehyde, five parts glacial acetic acid, and 90 parts 50% ethanol) for 24 hours. They were then dehydrated in 70% ethanol (Johansen, 1940) and stored until processing (Calderón & Soderstrom, 1973; Soderstrom & Young, 1983).

Laboratory procedure:—Culm fragments were affixed to wooden blocks using instant glue, which served as a support during the microtome procedure. Cross-sections of 20 µm thickness were cut using the sliding microtome American Optical AO Spencer 860, resulting in 6 to 12 sections per specimen. Half of the sections were treated with 50% hypochlorite for 30 minutes. All samples, including both the treated and untreated sections, were dehydrated with an increasing ethanol series of 50%, 70%, and 95%, with each solution being applied for 3 minutes. Finally, the bleached and unbleached sections were double-stained with safranin and fast green (Ruzin 1999) and mounted with synthetic resin.

Structural observations and data analysis:—Culm cross sections were observed using an Olympus BX51 light microscope and photographed using a Lumera Infinity1 digital camera with a resolution of 3.1 megapixels. A bar scale was included in all photographs to ensure accurate measurements with ImageJ software version 1.53k (Schindelin *et al*. 2015). The epidermis, cortex, and vascular bundles in the ground tissue were observed, measured, and described. We do not follow Liese's (1998) terminology, who recommended to use the term hypodermis for bamboos because in some species it was difficult to differentiate the hypodermis from the other cortical cells. We studied mature culms, hence it was not possible to know the origin of the hypodermis, which commonly derives from ground tissue since it is the outer layer of the cortex. To avoid confusion, we decided to name this area cortex and describe its differences. To describe the vascular bundles, the number of cycles, their position, and shape were taken into consideration. The size of central vascular bundles was measured by determining the contour shape in accordance with Stearn's (1983) terminology. Another important feature described was the shape and size of silica bodies, which were identified by their lack of birefringence when viewed using light polarization (Carlquist, 2001).

Results

The appearance of the culm internode at a cross-section is determined by the numerous vascular bundles embedded in the parenchymatous ground tissue. The size, shape, spatial arrangement of the vascular bundles, as well as the epidermal and cortical cells and silica bodies, differ among species, as reported below.

Otatea acuminata AGS (Fig. 2)

Material examined:—Aguascalientes: Calvillo, Jagúey canyon, 3 km E of Piedras Chinas, 1.5 km N of El Zapote de la Labor, 21°58'46.99"N, 102°39'23.2"W, 1969 m, 9 June 2020, *E. Ruiz-Sanchez & M.A. García-Martínez 652* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with axially elongated silica bodies (Fig. 2B). Cortex with 2 layers of narrow thick-walled cells followed by 3–4 layers of thinner-walled rounded cells (5–6 layers in total, 70 µm length; Fig. 2B). Vascular bundles in 8–9 alternating cycles (Fig. 2A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 2C–E). Elliptical central vascular bundles, 488 × 444 µm; two round shaped metaxylem vessels, 98 × 106 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 119 ×136 µm; sieve tubes 24 µm × 23 µm (Fig. 2E). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 70 μm^2. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 30 μm^2.

Otatea acuminata JAL (Fig. 3)

Material examined:—MEXICO. Jalisco: San Sebastián del Oeste, Mascota-Puerto Vallarta road, near arch bridge, 20°48'11"N, 104°56'13"W, 725 m, 28 July 2020, *E. Ruiz-Sanchez 672* (IBUG!).

Culm anatomy description:—Culm solid (Figs. 3A, B). Epidermis with a layer of rounded lignified cells, 10 µm length, with frequent squared to rectangular silica bodies on top (Fig. 3C). Cortex with 3–4 layers of thick-walled rounded cells, followed by 1–2 layers of thinner-walled rounded cells (5–6 layers in total, 41 µm length; Fig. 3C).

Vascular bundles in 9–10 alternating cycles (Fig. 3A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 3D–F). Ovate central vascular bundles, 287 × 288 µm; two round shaped metaxylem vessels, 69 × 73 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 63 × 75 µm; sieve tubes 19 × 18 µm (Fig. 3F). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 86 µm^2.

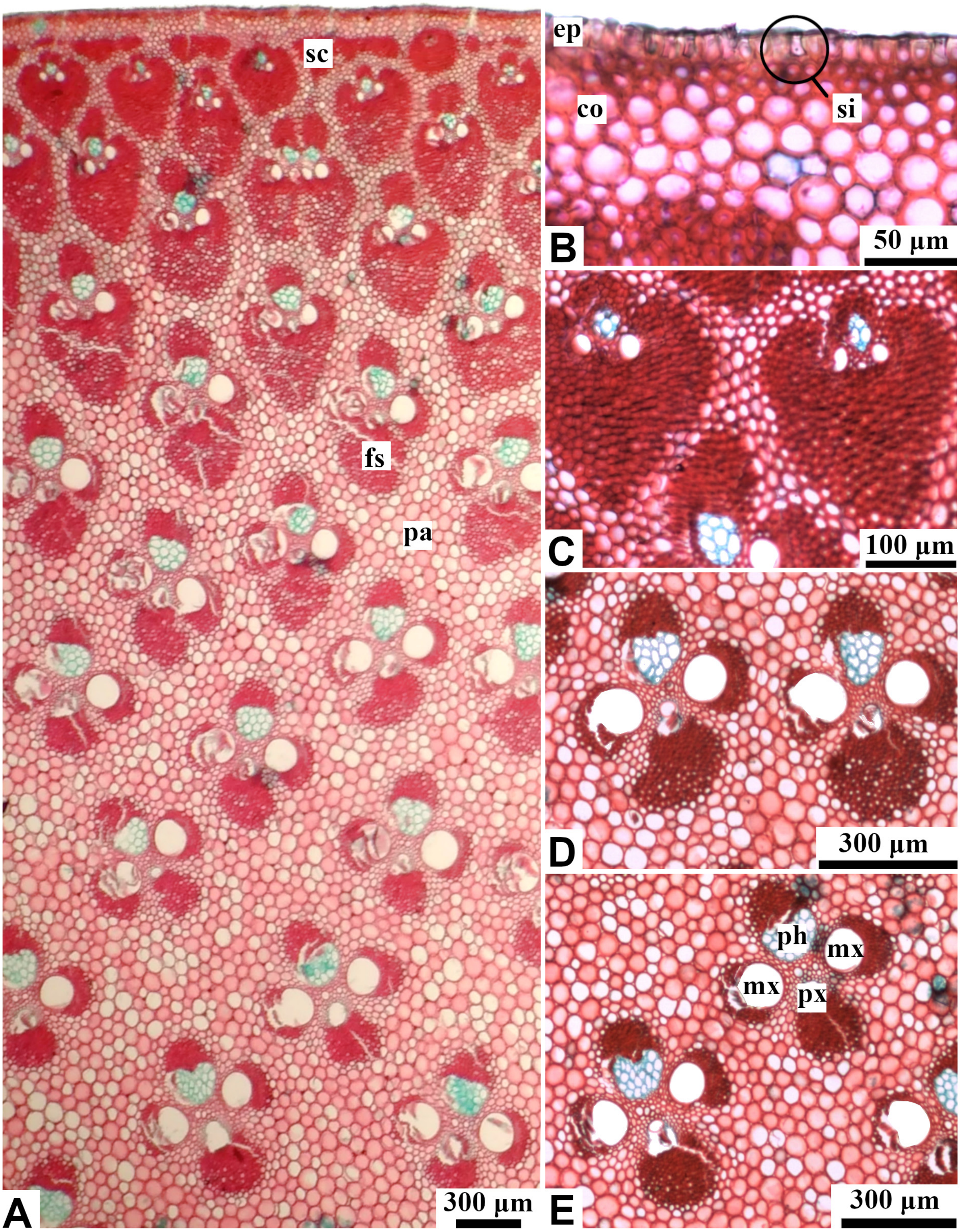

FIGURE 2. Culm cross section of *Otatea acuminata* AGS. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

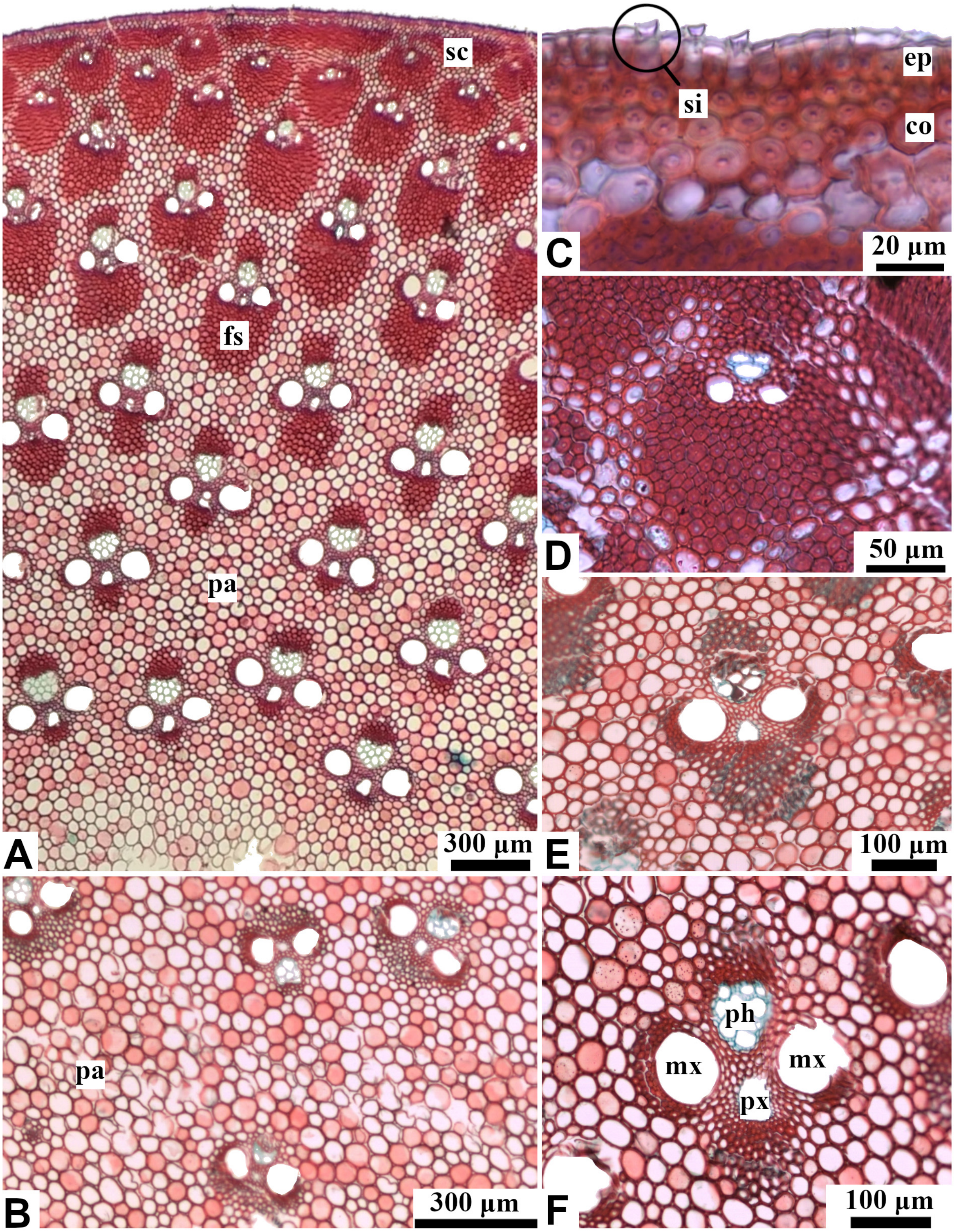

FIGURE 3. Culm cross section of *Otatea acuminata* JAL. **A**. Cross section general view. **B**. Central area with vascular bundles with different orientations. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Otatea acuminata OAX (Fig. 4)

Material examined:—Oaxaca: Tepelmeme Villa de Morelos, Km 107, Puebla-Oaxaca toll road, Puente Los Otates, 18°1’53.2”N, 97°20’58.7”W, 1961 m, 1 February 2020, *E. Ruiz-Sanchez & M.A. García-Martínez 644* (IBUG!).

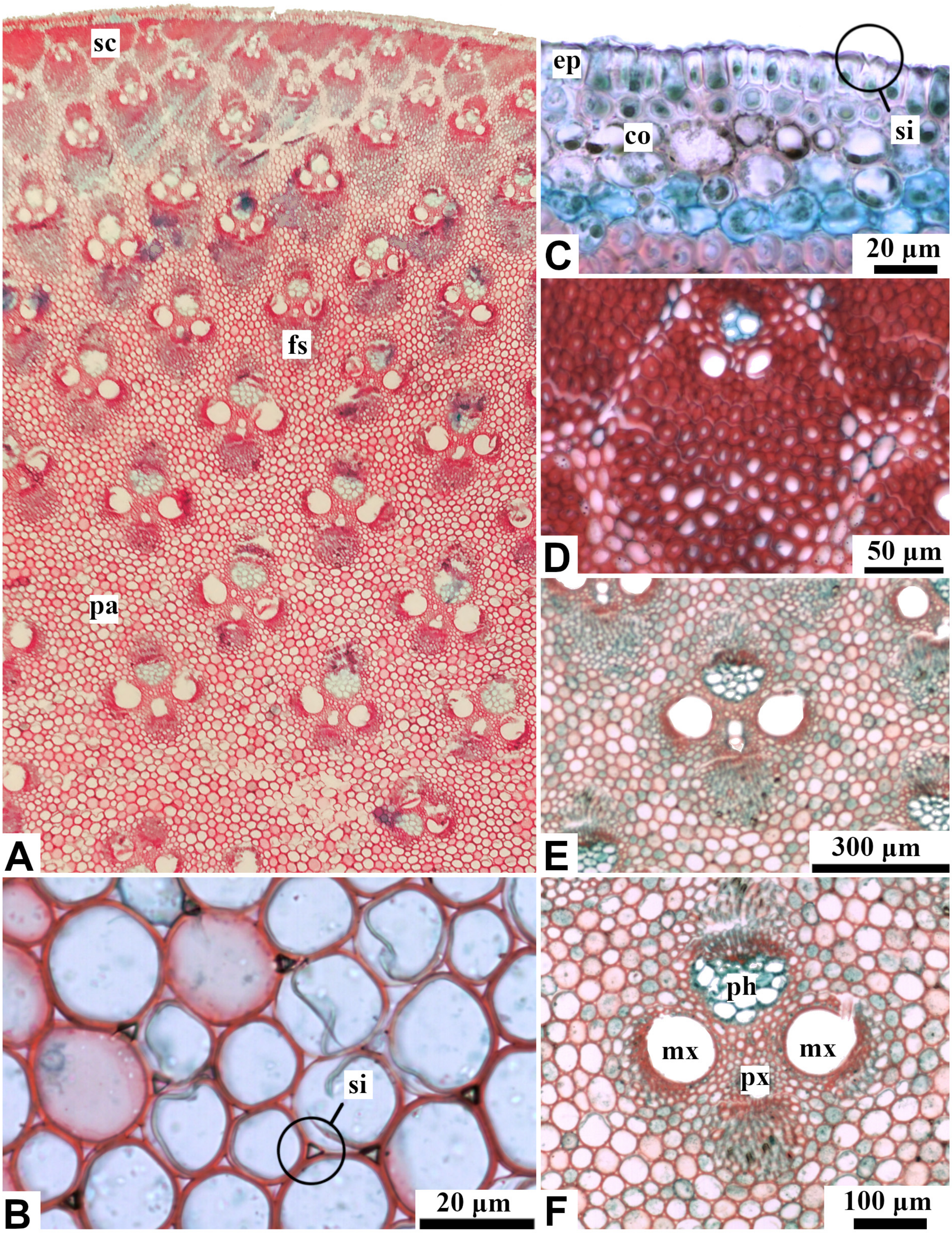

FIGURE 4. Culm cross section of *Otatea acuminata* OAX. **A**. Cross section general view. **B**. Parenchymatous ground tissue with sporadic conical silica bodies. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with frequent silica bodies on top (Fig. 4C). Cortex with one layer of narrow thick-walled rounded cells followed by 3–4 layers of wider thin-walled rounded cells (4–5 layers in total, 43 µm wide), the inner ones only with primary walls, chloroplasts and dark staining deposits in some cells (Fig. 4C). Vascular bundles in 11–12 alternating cycles (Fig. 4A). Peripheral, transitional, and central vascular bundles surrounded by a lignified sclerenchyma sheath defining their contour (Figs. 4D–F). Cell wall of fiber sheath weakly lignified. Ovate central vascular bundles, 426 × 392 µm; two round shaped metaxylem vessels, 93 × 89 µm, phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 93 × 136 µm; sieve tubes 17 × 21 µm (Fig. 4F). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 49 µm^2. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 17 µm^2(Fig. 4B).

Otatea acuminata VER (Fig. 5)

Material examined:—MEXICO. Veracruz: Emiliano Zapata, 2 km south of Cerro Gordo, 19°25'17.3"N, 96°41'17.1"W, 525 m, 31 January 2020, *E. Ruiz-Sanchez & M.A. García-Martínez 643* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 11 µm length, with frequent squared to rectangular silica bodies on top (Fig. 5C). Cortex with one layer of thick-walled rounded cells followed by 2–3 layers of thinner-walled oval to rounded cells (3–4 layers in total, 34 µm length; Fig. 5C). Vascular bundles in 10–11 alternating cycles (Fig. 5A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 5D–F). Elliptical central vascular bundles, 484 × 466 µm; two round shaped metaxylem vessels, 104 × 106 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 66 × 81 µm; sieve tubes 18 µm × 25 µm (Fig. 5F). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 122 µm^2. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 17 µm^2 (Fig. 5B).

Otatea carrilloi (Fig. 6)

Material examined:—MEXICO. Chiapas: Tonalá, La Sepultura, vicinity of Raymundo Flores, 16°1'57.86"N, 93°36'12.20"W, 649 m, 15 February 2022, *E. Ruiz-Sanchez et al. 730* (IBUG!).

Culm anatomy description:—Culm hollow, the lacuna occupying > 50% of the total diameter. Epidermis with a layer of axially elongated lignified cells, 14 µm length, with frequent squared to rectangular silica bodies on top (Fig. 6B). Cortex with 2 layers of narrow thick-walled rounded cells, followed by 3–4 layers of wider thinner-walled rounded cells (in total, 5–6 layers, 61 µm length; Fig. 6B). Vascular bundles in 10–11 alternating cycles (Fig. 6A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 6C–E). Cell wall of fiber sheath weakly lignified. Ovate central vascular bundles, 439 × 451 µm; two round shaped metaxylem vessels, 100 × 110 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 91 × 120 µm; sieve tubes 18 × 23 µm (Fig. 6E). Frequent amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 90 µm^2.

Otatea fimbriata (Fig. 7)

Material examined:—MEXICO. Chiapas: Ixtapa, Km 20 toll road Tuxtla Gutiérrez-San Cristóbal de las Casas, 16°41'53.31"N, 92°51'48.14"W, 1300 m, 15 February 2022, *E. Ruiz-Sanchez et al. 732* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with frequent squared to rectangular silica bodies on top (Fig. 7B). Cortex with 1–2 layers of narrow thick-walled rounded cells followed by 4–5 layers of wider and thinner-walled rounded cells (6–7 layers in total, 73 µm length; Fig. 7B). Vascular bundles in 11–12 alternating cycles (Fig. 7A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 7C–E). Elliptical central vascular bundles, 556 × 615 µm; two round shaped metaxylem vessels, 119 × 117 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 115 × 195 µm; sieve tubes 20 × 27 µm (Fig. 7E). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 127 µm^2. Frequent conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 20 µm^2.

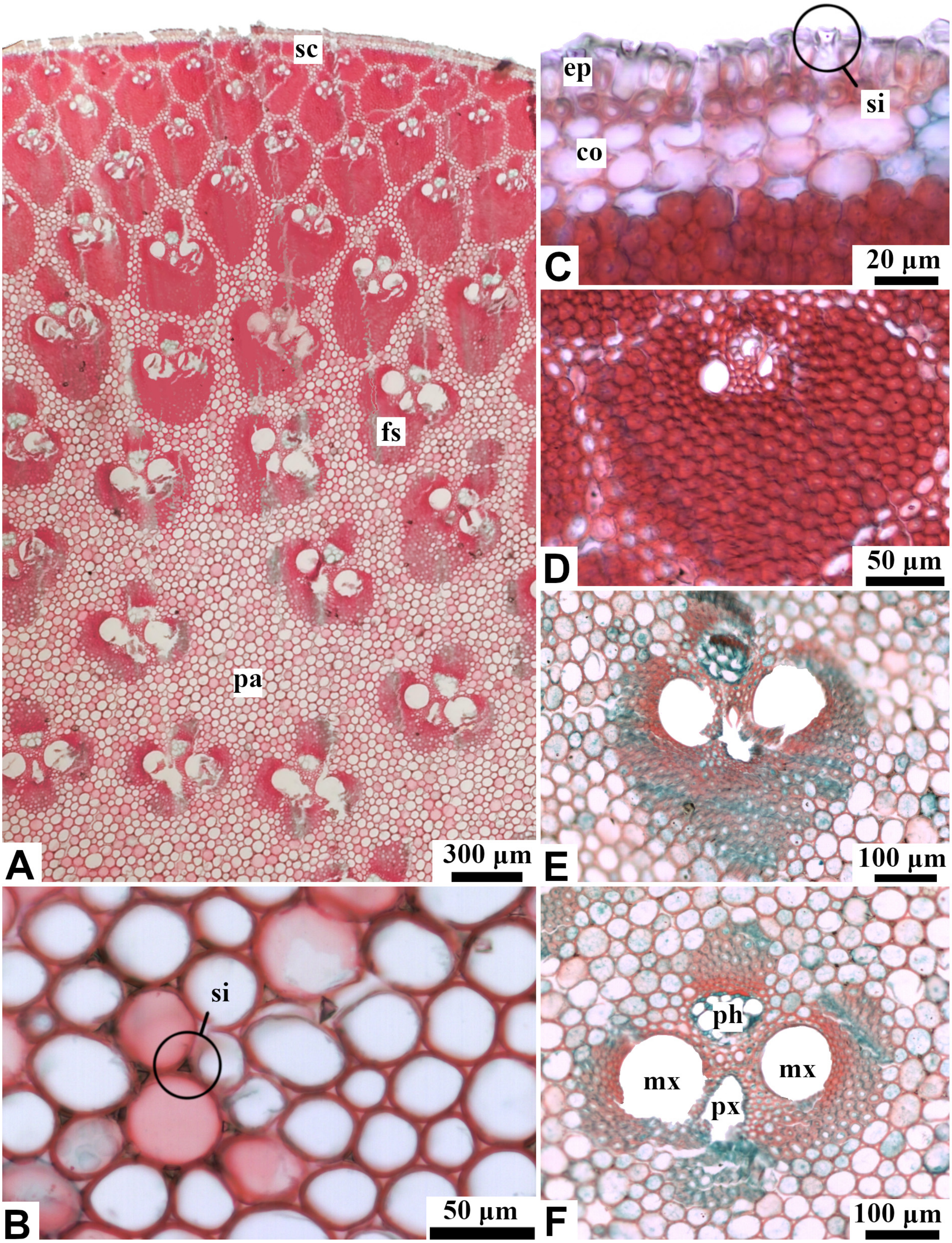

FIGURE 5. Culm cross section of *Otatea acuminata* VER. **A**. Cross section general view. **B**. Conical silica bodies occupying intercellular spaces. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

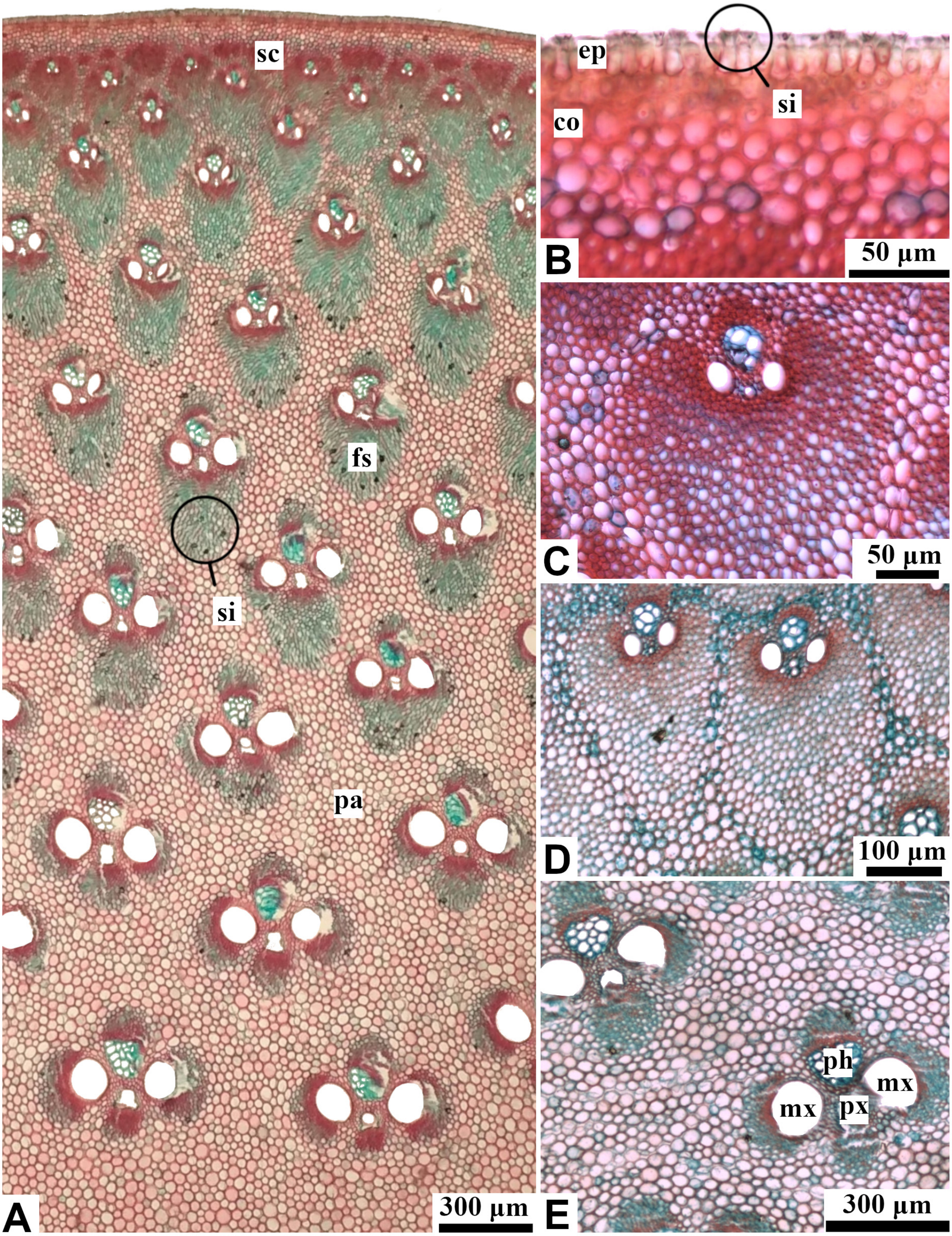

FIGURE 6. Culm cross section of *Otatea carrilloi*. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

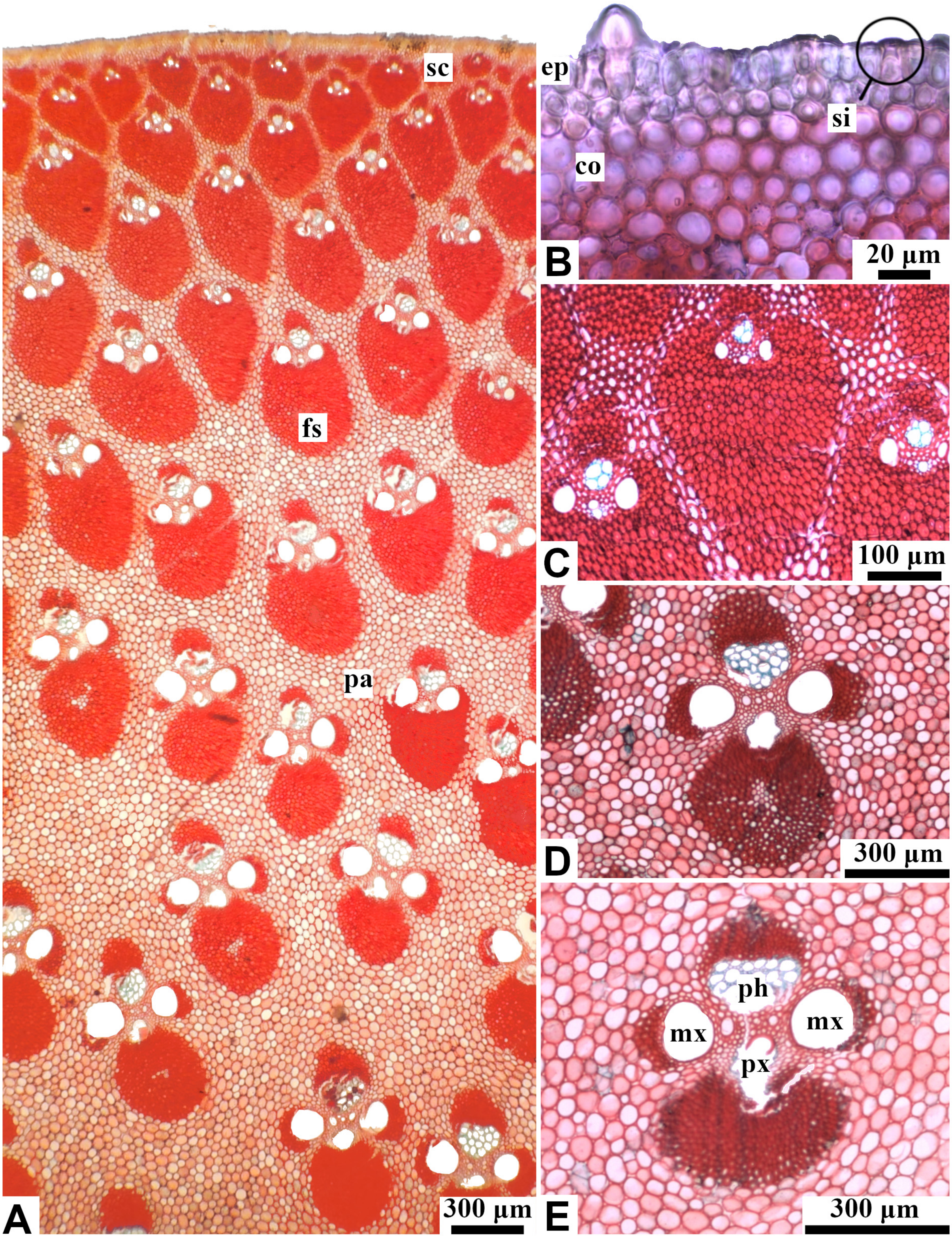

FIGURE 7. Culm cross section of *Otatea fimbriata*. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Otatea glauca (Fig. 8)

Material examined:—MEXICO. Chiapas: Motozintla, Km 37 of the Huixtla-Motozintla highway, 15°19'46.58"N, 92°19'25.72"W, 1141 m, 14 February 2022, *E. Ruiz-Sanchez et al. 726* (IBUG!).

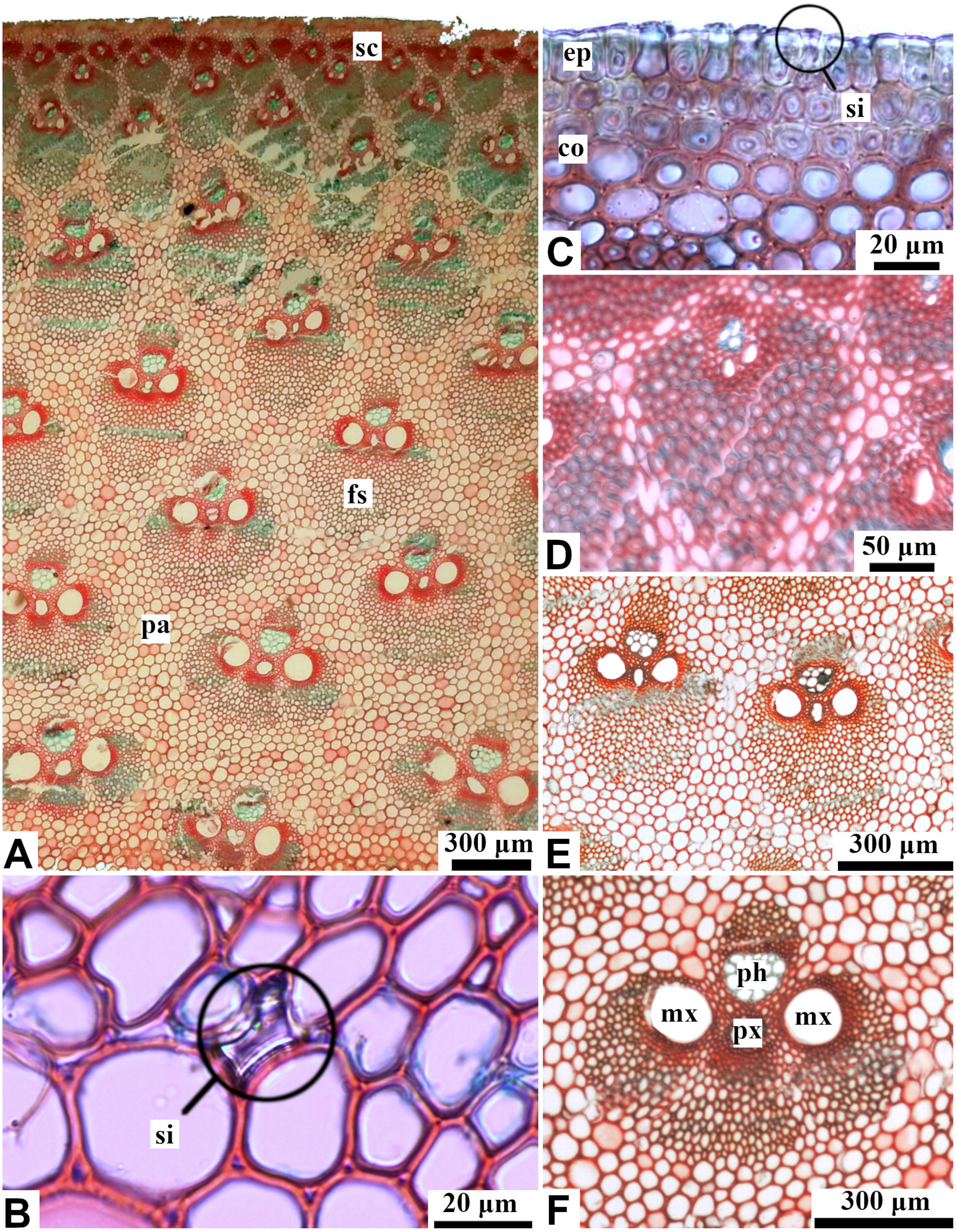

FIGURE 8. Culm cross section of *Otatea glauca*. **A**. Cross section general view. **B**. Fiber sheath with an amorphous silica body occupying cellular space. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Culm anatomy description:—Culm hollow, the lacuna occupying > 50% of the total diameter. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with frequent rectangular silica bodies on top (Fig. 8C). Cortex with 2 layers of narrow thick-walled cells followed by 2–3 layers of wider, thinner-walled cells (in total, 4–5 layers, 52 µm length; Fig. 8C). Vascular bundles in 8–9 alternating cycles (Fig. 8A). Peripheral, transitional, and central vascular bundles surrounded by a lignified sclerenchyma sheath defining their contour (Figs. 8D–F). Cell wall of fiber sheath weakly lignified. Oval central vascular bundles, 462 × 362 µm; two round shaped metaxylem vessels, 89 × 92 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 82 × 104 µm; sieve tubes 18 × 21 µm (Fig. 8F). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 187 µm^2 (Fig. 8B). Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 6 µm^2.

Otatea ramirezii (Fig. 9)

Material examined:—MEXICO. Querétaro: San Joaquín, along dirt road from Apartadero to San Juan Tetla, 20°58'56"N, 99°29'28"W, 1285 m, 19 February 2022, *J.P. Ortíz-Brunel et al.1395* (IBUG!).

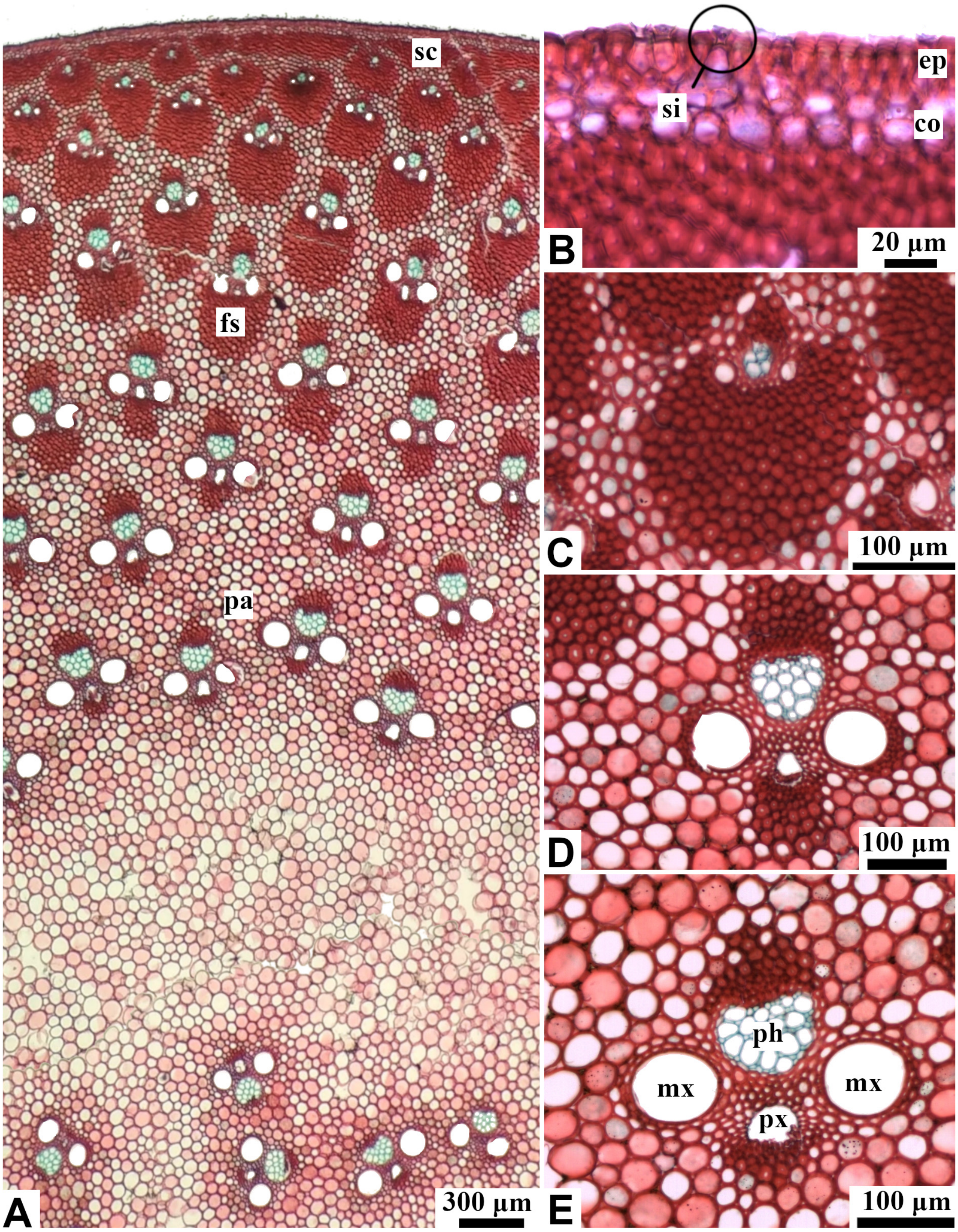

FIGURE 9. Culm cross section of *Otatea ramirezii*. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 11 µm length, with frequent squared to rectangular silica bodies on top (Fig. 9B). Cortex with one layer of narrow thick-walled cells followed by 2–3 layers of wider thinner-walled rounded cells (in total, 3–4 layers, 28 µm length; Fig. 9B). Vascular bundles in 9–10 alternating cycles. Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 9C–E). Oval central vascular bundles, 275 × 297 µm; two round shaped metaxylem vessels, 77 × 81 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 73 × 97 µm; sieve tubes 20 × 20 µm (Fig. 9E). Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 26 $µm^2$.

Otatea reynosoana (Fig. 10)

Material examined:—MEXICO. Michoacán: Morelia, Filtros Viejos, on the banks of the Chiquito river, 19°40'35.2"N, 101°9'13.7"W, 1947 m, 3 February 2020, *E. Ruiz-Sanchez & M.A. García-Martínez 649* (IBUG!).

Culm anatomy description:—Culm hollow, the lacuna occupying < 50% of the total diameter. Epidermis with a layer of square to rectangular thick-walled cells, 11 µm length, with occasional rectangular silica bodies on top (Fig. 10C). Cortex with 3–4 layers of thick-walled rounded cells, the outer narrower, 27 µm length, with dark staining deposit (Fig. 10C). Vascular bundles in 5–6 alternating cycles (Fig. 10C). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 10D–F). Depressed central vascular bundles, 249 × 351 µm; two round shaped metaxylem vessels, 80 × 88 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 56 × 91 µm; sieve tubes 19 × 21 µm (Fig. 10F). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 78 $µm^2$. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 11 $µm^2$ (Fig. 10B).

Otatea rzedowskiorum (Fig. 11)

Material examined:—MEXICO. Chiapas: Ocozocoautla, El Aguacero waterfall, 16°45'35.34"N, 93°31'29.32"W, 535 m, 15 February 2022, *E. Ruiz-Sanchez et al. 731* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 9 µm length, with occasional rectangular silica bodies on top (Fig. 11C). Cortex with a layer of thick-walled axially elongated cells followed by 2–3 layers of wider thinner-walled rounded cells (in total, 3–4 layers, 23 µm length; Fig. 11C). Vascular bundles in 5–6 alternating cycles (Fig. 11A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 11D–F). Central vascular bundles broadly depressed, 180 × 260 µm; two round shaped metaxylem vessels, 63 × 64 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 49 × 60 µm, sieve tubes 15 × 14 µm (Fig. 11F). Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 8 $µm^2$ (Fig. 11B). Abundant starch grains in the ground cells (Fig. 11B).

Otatea transvolcanica (Fig. 12)

Material examined:—MEXICO. Colima: Comala, dirt road Campo Cuatro to El Terrero, 19°24'05"N, 103°51'39"W, 1709 m, 25 July 2020, *J.P. Ortíz-Brunel et al. 831* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with frequent squared to rectangular silica bodies on top (Fig. 14B). Cortex with one layer of narrow thick-walled rounded cells followed by 4–5 layers of wider, thinner-walled rounded cells (6–7 layers in total, 72 µm length; Fig. 14B). Vascular bundles in 10–11 alternating cycles (Fig. 14A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 14C–E). Cell wall of fiber sheath weakly lignified. Elliptical central vascular bundles, 468 × 269 µm; two round shaped metaxylem vessels, 77 × 79 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 101 × 106 µm; sieve tubes 17 × 24 µm (Fig. 14E). Frequent amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 54 $µm^2$.

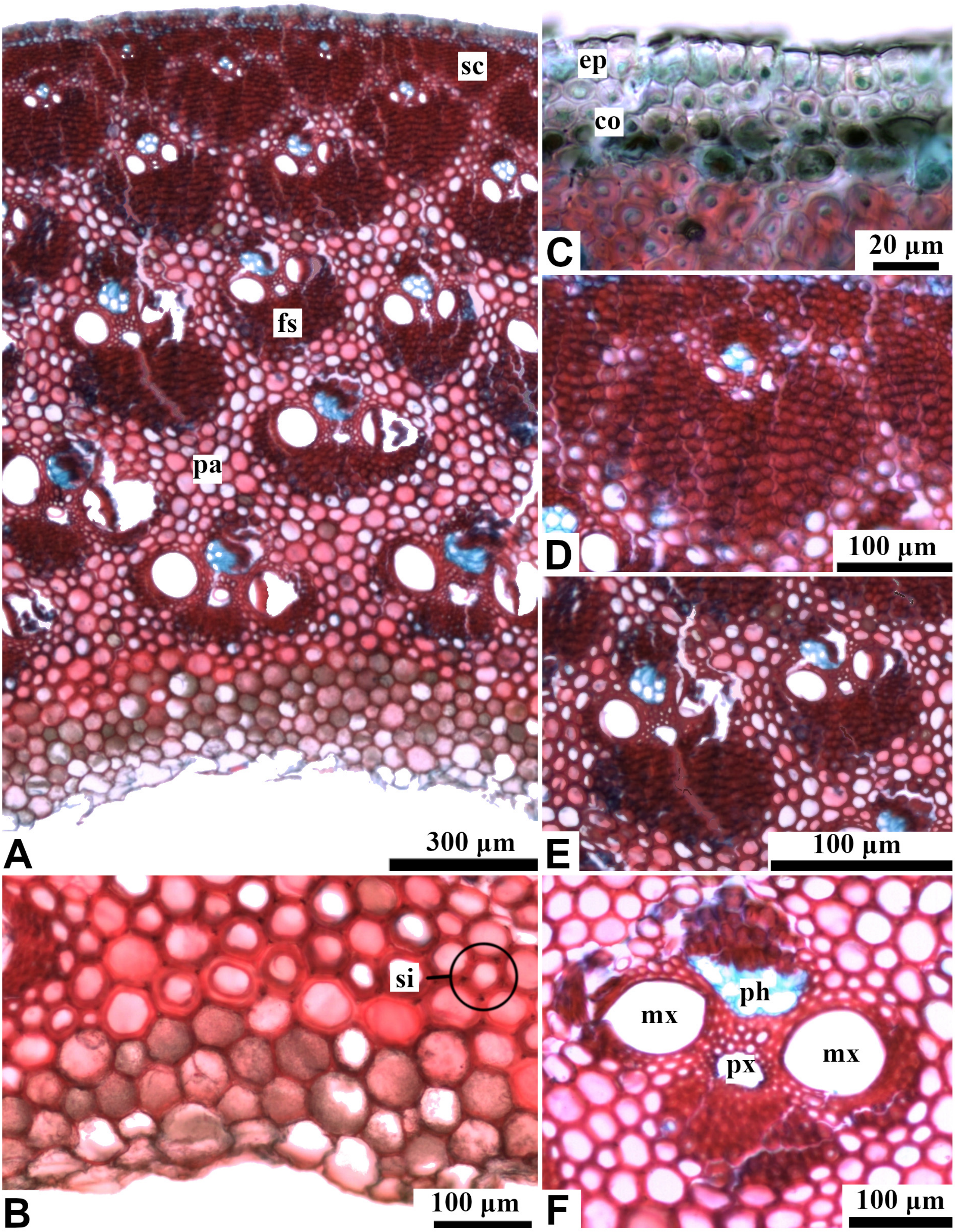

FIGURE 10. Culm cross section of *Otatea reynosoana*. **A**. Cross section general view. **B**. Parenchymatous ground tissue with sporadic conical silica bodies. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

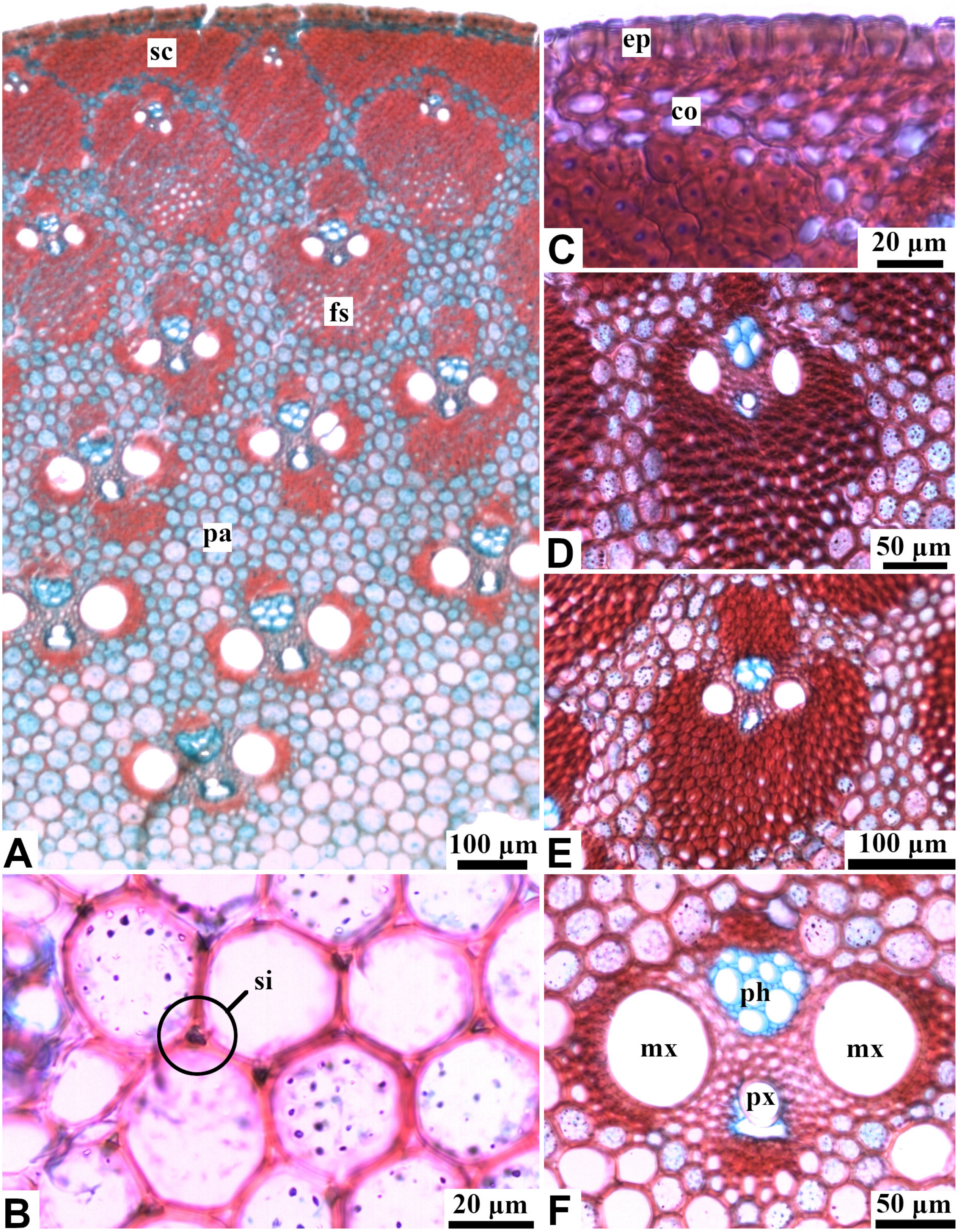

FIGURE 11. Culm cross section of *Otatea rzedowskiorum*. **A**. Cross section general view. **B**. Conical silica bodies occupying intercellular spaces. **C**. Epidermis and cortex. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

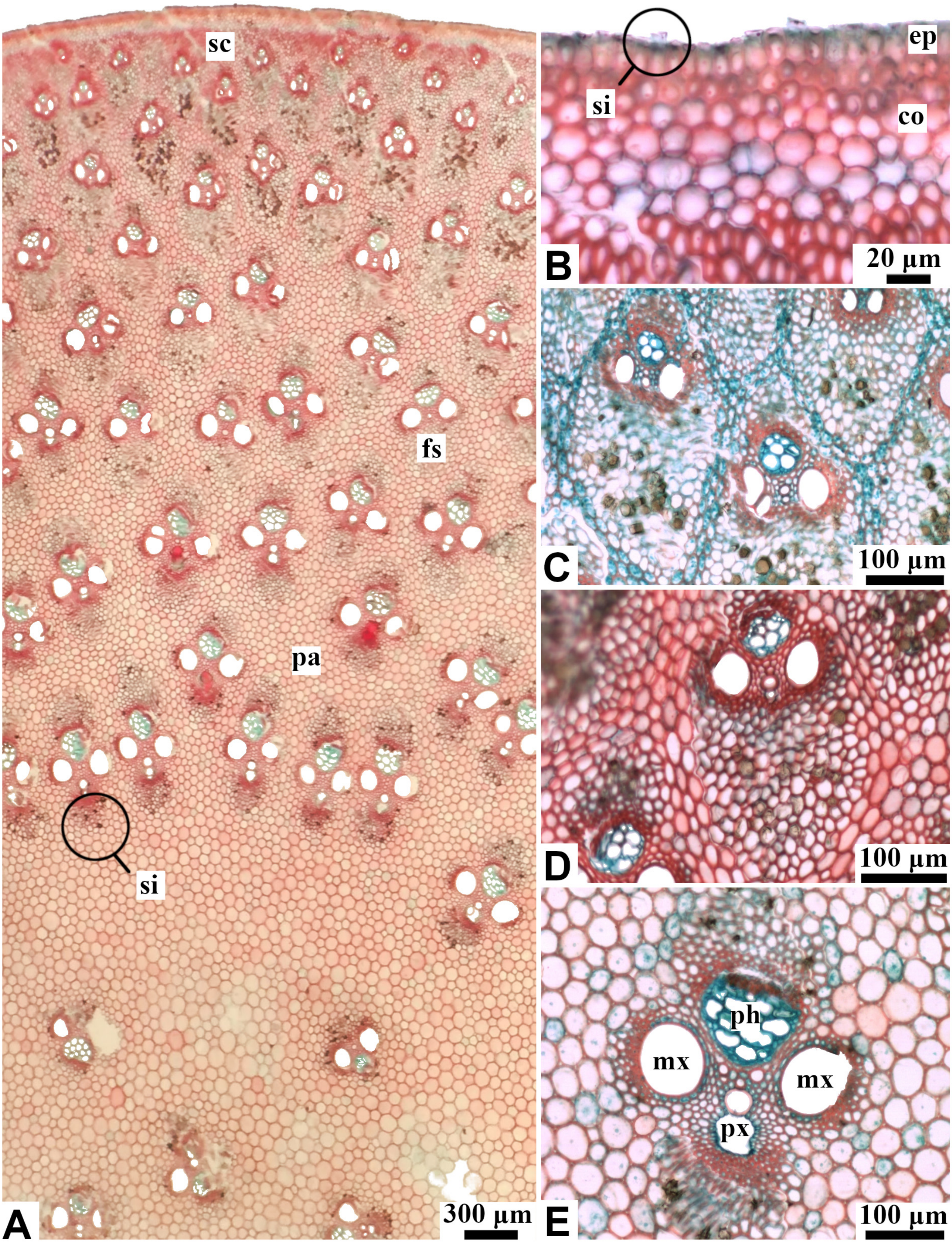

FIGURE 12. Culm cross section of *Otatea transvolcanica*. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Otatea victoriae (Fig. 13)

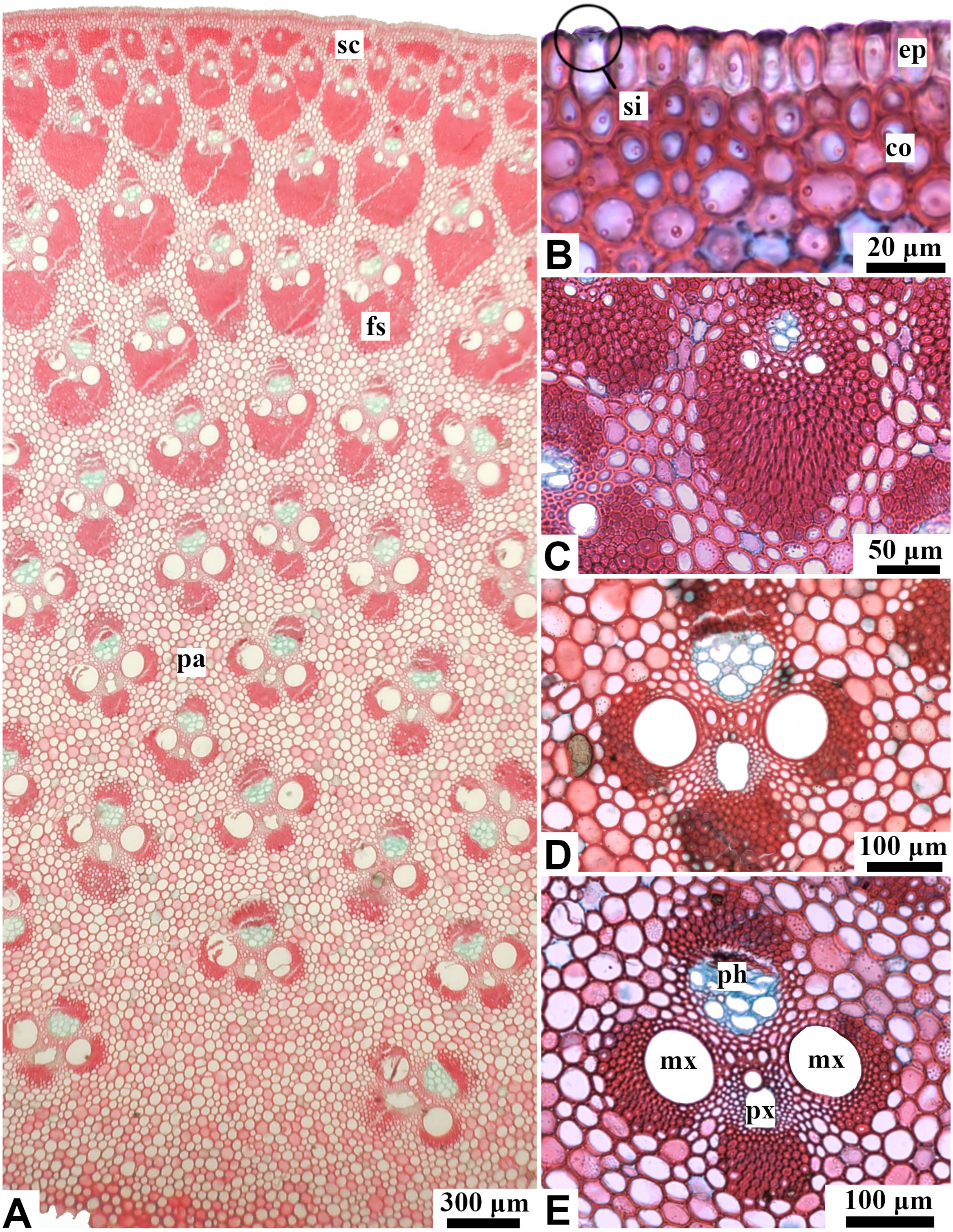

FIGURE 13. Culm cross section of *Otatea victoriae*. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Material examined:—MEXICO. Hidalgo: Cardonal, Tolantongo-Metztitlán dirt road, 5 km east of the junction to the road that leads to Tolantongo, 20°37'38.1"N, 98°57'31.3"W, 1775 m, 2 February 2020, *E. Ruiz-Sanchez & M.A. García-Martínez 647* (IBUG!).

Culm anatomy description:—Culm hollow with wide lacuna occupying > 50% of the total diameter. Epidermis with a layer of axially elongated lignified cells, 15 µm length, with frequent squared to rectangular silica bodies on top (Fig. 15B). Cortex with 3–5 layers of thick-walled rounded cells, the outer layer with slightly narrower cells(48 µm length; Fig. 15B). Vascular bundles in 9–10 alternating cycles. Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 15C–E). Ovate central vascular bundles, 357 × 353 µm; two round shaped metaxylem vessels, 81 × 87 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 87 × 104 µm; sieve tubes 22 × 21 µm (Fig. 15A). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 58 μm^2. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 9 μm^2.

Otatea ximenae (Fig. 14)

Material examined:—MEXICO. Oaxaca: Asunción Nochixtlán, Km 190 of the Puebla-Oaxaca highway, 17°23'9.1"N, 97°8'8.9"W 1928 m, 2 February 2020, *Ruiz-Sanchez & M.A. García-Martínez 645* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 12 µm length, with occasional rectangular silica bodies on top (Fig. 16C). Cortex with one layer of thick-walled cells followed by 2 layers of wider, thinner-walled ovoidal cells (3 layers in total, 30 µm length; Fig. 16C). Vascular bundles in 5–6 alternating cycles (Fig. 16A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 16D–F). Ovate central vascular bundles, 309 ×304 µm; two round shaped metaxylem vessels, 75 × 83 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 78 × 98 µm; sieve tubes 19 × 20 µm (Fig. 16F). Ground parenchyma with abundant starch grains. Frequent conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 10 μm^2 (Fig. 16B).

***Otatea sp. nov.* 1** (Fig. 15)

Material examined:—Chiapas: Arriaga, Km 13 toll road Tuxtla-Arriaga, 16°19'13.50"N, 93°51'22.8"W, 564 m, 14 February 2022, *E. Ruiz-Sanchez et al. 724* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated thick-walled cells, 17 µm length, with frequent squared to rectangular silica bodies on top (Fig. 12B). Cortex with a layer of narrow thick-walled rounded cells followed by 2 layers of wider, thinner-walled ovoidal cells (3 layers in total, 27 µm length; Fig. 12B). Vascular bundles in 10–11 alternating cycles (Fig. 12A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 12C–E). Ovate central vascular bundles, 336 × 331 µm; two round shaped metaxylem vessels, 89 × 98 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 73 × 98 µm; sieve tubes 23 × 21 µm (Fig. 12E). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 168 μm^2.

***Otatea sp. nov.* 2** (Fig. 16)

Material examined:—MEXICO. Jalisco: Puerto Vallarta, 100 m upstream of Palo María river, 20°33'4.14"N, 105°15'24.48"W, 50 m, 10 December 2021, *E. Ruiz-Sanchez 723* (IBUG!).

Culm anatomy description:—Culm solid. Epidermis with a layer of axially elongated lignified cells, 22 µm length, with frequent squared to rectangular silica bodies on top (Fig. 13B). Cortex with 3–4 layers of strongly thicker-walled rounded cells, 84 µm length (Fig. 13B). Vascular bundles in 9–10 alternating cycles (Fig. 13A). Peripheral, transitional, and central vascular bundles surrounded by a sclerenchyma sheath defining their contour (Figs. 13C–E). Elliptical central vascular bundles 445 × 315 µm; two round shaped metaxylem vessels, 118 × 105 µm; phloem with sieve tubes and companion cells located between two metaxylem vessels on the upper level, 108 × 104 µm; sieve tubes 16 × 28 µm (Fig. 13E). Scarce amorphous silica bodies embedded along the periphery of vascular bundles occupying cellular spaces covering a mean area of 173 μm^2. Sporadic conical silica bodies across the ground tissue occupying intercellular spaces covering a mean area of 26 μm^2.

We here present a key to Mexican *Otatea* species based on anatomical cross-section characters.

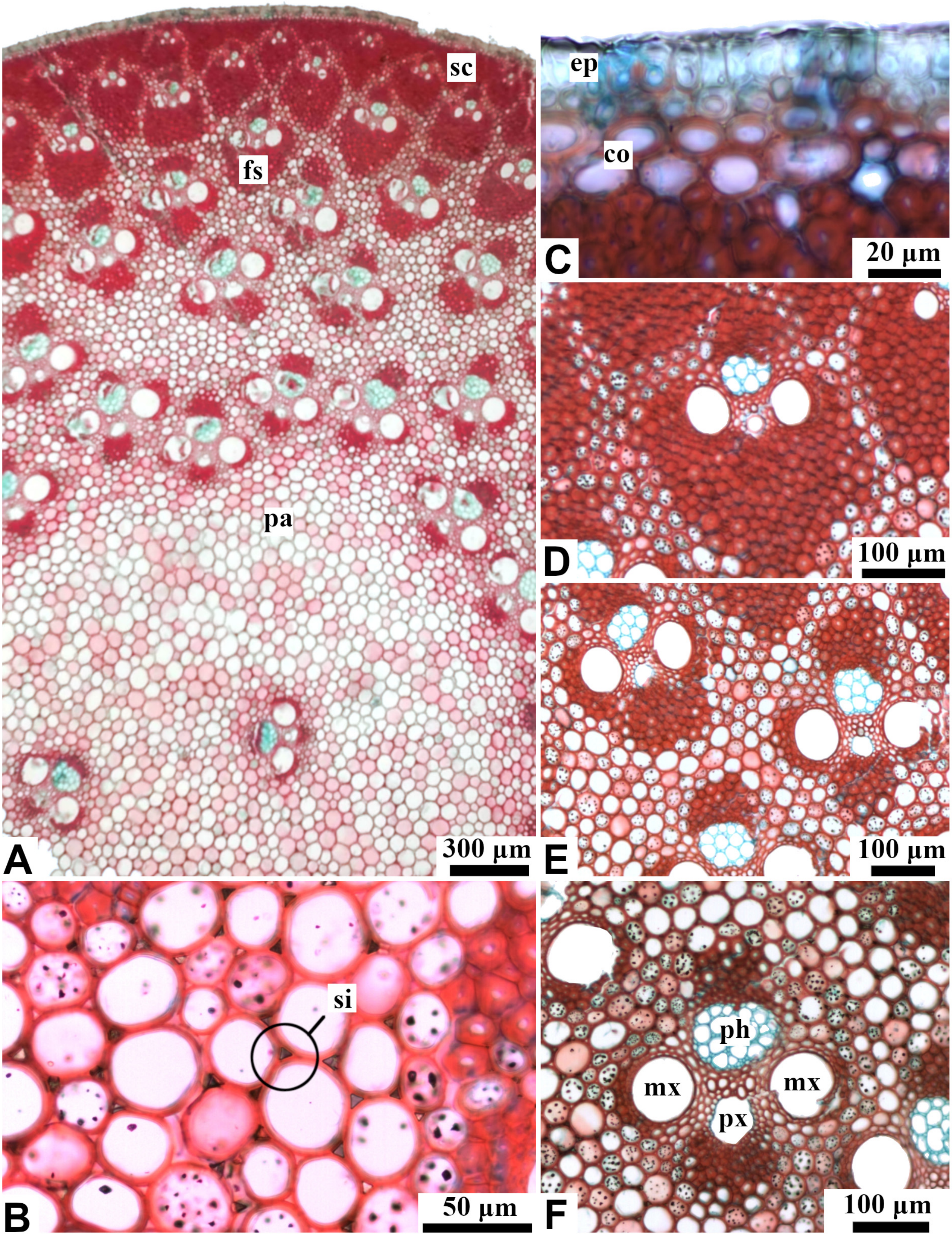

FIGURE 14. Culm cross section of *Otatea ximenae*. **A**. Cross section general view. **B**. Central area with vascular bundles following different orientations. **C**. Epidermis and hypodermis. **D**. Peripheral vascular bundle. **E**. Transitional vascular bundle. **F**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

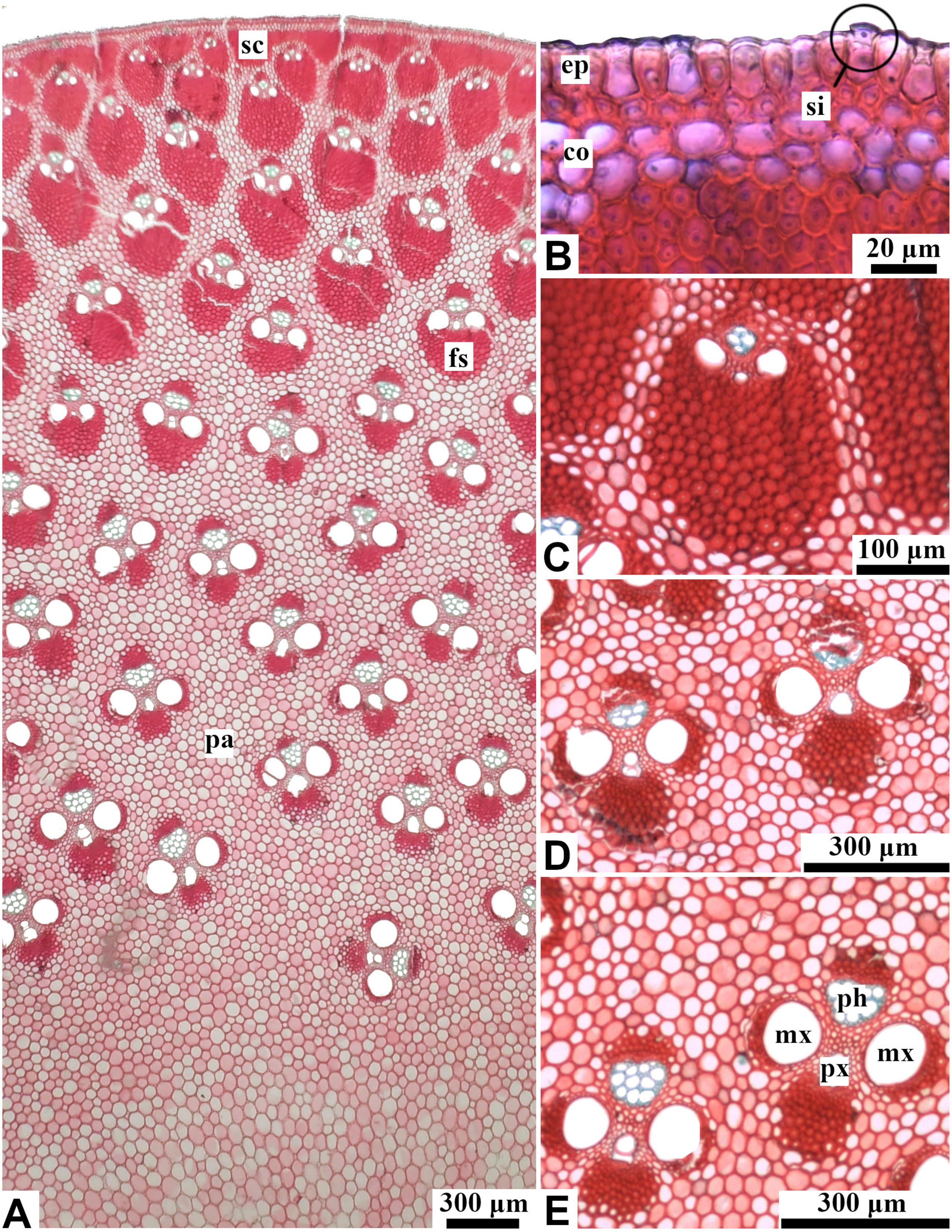

FIGURE 15. Culm cross section of *Otatea sp. nov*. 1. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

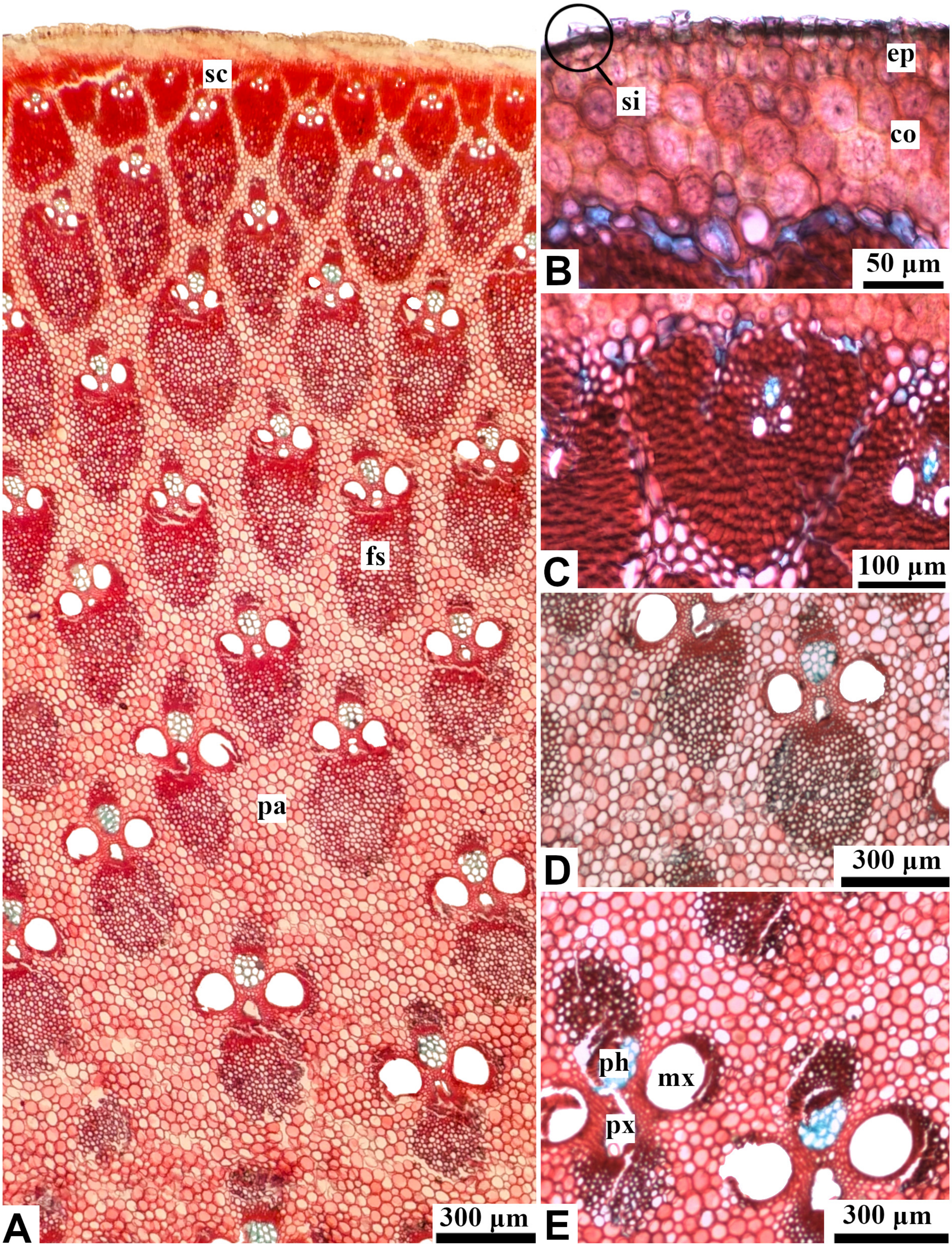

FIGURE 16. Culm cross section of *Otatea sp. nov*. 2. **A**. Cross section general view. **B**. Epidermis and cortex. **C**. Peripheral vascular bundle. **D**. Transitional vascular bundle. **E**. Central vascular bundle. Abbreviations: **co**, cortex; **ep**, epidermis; **fs**, fiber sheath; **mx**, metaxylem; **pa**, parenchyma; **ph**, phloem; **px**, protoxylem; **sc**, sclerenchyma; **si**, silica bodies.

Key to Mexican *Otatea* species based on culm characters in cross section

1. Culm hollow 2
- Culm solid 5

2. Transitional and central vascular bundles with fiber sheath composed of cells with strongly lignified walls....3
- Transitional and central vascular bundles with fiber sheath composed of cells with weakly lignified walls....4
3. Vascular cycles 5–6; depressed central vascular bundles.... *O. reynosoana*
- Vascular cycles 9–10; ovate central vascular bundles.... *O. victoriae*
4. Vascular cycles 10–11; frequent amorphous silica bodies occupying cellular spaces on fiber sheath; conical silica bodies absent... .... *O. carrilloi*
- Vascular cycles 8–9; sporadic amorphous silica bodies occupying cellular spaces on fiber sheath; intercellular conical silica bodies on ground tissue occupying and area of 6 (4–7) µm^2.... *O. glauca*
5. Transitional and central vascular bundles with fibr sheath composed of cells with strongly lignified walls....6
- Transitional and central vascular bundles with fiber sheath composed of cells with weakly lignified walls...... *O. acuminata* OAX
6. Thick epidermis 22 (20–23) µm and thick cortex 84 (73–93) µm and these two structures strongly and equally lignified .... *Otatea sp. nov.* 2
- Thin epidermis (< 20 µm) and variable size cortex (≤ 77 µm) and these two layers with different patterns of lignification 7
7. Absence of conical silica bodies in ground tissue....8
- Presence of conical silica bodies in ground tissue....10
8. Elliptical central vascular bundles with frequent amorphous silica bodies occupying cellular spaces on fiber sheaths .... *O. transvolcanica*
- Ovate central vascular bundles with sporadic amorphous silica bodies occupying cellular spaces on fiber sheaths....9
9. Cortex with 3–4 layers of thick-walled rounded cells, followed by 1–2 layers of thinner-walled rounded cells (5–6 layers in total, 41 (37–45) µm length).... *O. acuminata* JAL
- Thin cortex with a layer of narrow thick-walled rounded cells followed by 2 layers of wider, thinner-walled, ovoidal cells (3 layers in total, 27 (23–31) µm length) .... *Otatea sp. nov. 1*
10. Thick cortex > 65µm....11
- Thin cortex ≤ 40 µm....12
11. Vascular bundles in 8–9 cycles; epidermis with axially elongated silica bodies .... *O. acuminata* AGS
- Vascular bundles in 11–12 cycles; epidermis with frequent squared to rectangular silica bodies and prickle hairs on top .... *O. fimbriata*
12. Absence of amorphous silica bodies occupying cellular spaces on fiber sheaths; vascular bundles in 5–6 cycles....13
- Presence of amorphous silica bodies occupying cellular spaces on fiber sheaths; vascular bundles in 9–11 cycles....14
13. Broadly depressed central vascular bundles, 180 (167–198) × 260 (243–281) µm.... *O. rzedowskiorum*
- Ovate central vascular bundles; 309 (280–325) × 304 (261–330) µm.... *O. ximenae*
14. Ovate central vascular bundles, 275 (245–309) × 297 (256–317) µm.... *O. ramirezii*
- Elliptical central vascular bundles; 484 (477–488) × 446 (427–466) µm.... *O. acuminata* VER

Discussion

The internal part of the culms of *Otatea* can either be solid or hollow (Ruiz-Sanchez *et al*. 2011). Our results show that 11 out of 15 *Otatea* specimens have solid internodes (Table 1). Liese (1998) mentioned that culms are rarely solid in bamboo species. However, this condition is frequently found in Neotropical woody bamboos, particularly in Chusqueinae (Clark *et al*. 2015), as reported by Ruiz-Sanchez *et al*. (2021). Rúgolo de Agrasar & Rodríguez (2003) examined the culm anatomy of 15 species belonging to three subtribes: Arthrostylidiinae, Chusqueinae, and Guaduinae. They found that five out of six species of Chusqueinae and one Guaduinae species have solid culms. Guerreiro *et al*. (2013) analyzed eight species from the subtribes Arthrostylidiinae and Chusqueinae and also reported solid culms in all six Chusqueinae species examined. According to Liese *et al*. (2015), solid culms can be found at the basal portion of the culm and the rest of the culm can be hollow. However, to date, there is no single hypothesis explaining why some bamboos have solid culms, as this condition is less common in Bambusoideae species.

Although culms can be solid or hollow, they are protected by the epidermis, an unstratified layer composed of lignified cells (Liese 1998). Our results have shown that this layer can be formed by rounded or axially elongated cells that differ in size among *Otatea* species (Table 1). The epidermis of *Otatea sp. nov.* 2 is the widest with 22 µm, while the narrowest was found in *O. rzedowskiorum* with 9 µm. Below the epidermis lies the cortex, consisting of several layers of rounded to ovoidal, thin- or thick-walled cells. Although Liese (1998) differentiated hypodermis from other cortical cells, we were unable to do it since only mature culms were studied; and in some species, these cells have the same shape and wall thickness. Thus, the term cortex is used for the layers of cells between the epidermis and vascular system. Our results indicate that the widest cortex is also found in *Otatea sp. nov.* 2 with 84 µm, while the thinnest was found in *O. rzedowskiorum* with 23 µm. Species may have all cortical cells of the same shape, but the outer layer is commonly narrower, as observed in *Otatea sp. nov.* 2. The size of the epidermis and cortex in other species is highly variable (Table 1). We do not know if the size of these layers is related to abiotic variables such as precipitation, elevation, soil type, etc. Correlation studies are needed to elucidate possible anatomical traits developed or influenced by environmental factors.

TABLE 1. Comparison of selected culm cross section characters of *Otatea*.

Species	Culm	Epidermis	Cortex			Central vascular bundles		Silica bodies (area, μm^2)	
		Length, μm	Length, μm	Layers	Vascular cycles	size (μm, width × length)	Shape	Amorphous	Conical
O. acuminata AGS	Solid	12 (11–12)	70 (66–77)	5–6	8–9	488 (456–509) × 444 (432–463)	Elliptical	70 (33–97)	30 (17–43)
O. acuminata JAL	Solid	10 (8–12)	41 (37–45)	5–6	9–10	287 (255–318) × 288 (255–301)	Ovate	86 (61–115)	-
O. acuminata OAX	Solid	12 (10–14)	43 (39–47)	4–5	11–12	426 (410–458) × 392 (380–406)	Ovate	49 (27–65)	17 (13–26)
O. acuminata VER	Solid	11 (10–11)	34 (30–40)	3–4	10–11	484 (477–488) × 446 (427–466)	Elliptical	122 (64–184)	17 (12–22)
O. carrilloi	Hollow	14 (11–16)	61 (58–67)	5–6	10–11	439 (418–475) × 451 (411–504)	Ovate	90 (75–115)	-
O. fimbriata	Solid	12 (11–13)	73 (69–76)	6–7	11–12	556 (511–615) × 615 (561–669)	Elliptical	127 (108–147)	20 (13–23)
O. glauca	Hollow	12 (10–15)	52 (49–57)	4–5	8–9	462 (300–570) × 362 (300–432)	Ovate	187 (178–195)	6 (4–7)
O. ramirezii	Solid	11 (10–13)	28 (25–31)	3–4	9–10	275 (245–309) × 297 (256–317)	Ovate	-	26 (20–35)
O. reynosoana	Hollow	11 (10–12)	27 (24–30)	3–4	5–6	249 (218–279) × 351 (346–355)	Depressed	78 (61–94)	11 (9–13)
O. rzedowskiorum	Solid	9 (8–10)	23 (19–26)	3–4	5–6	180 (167–198) × 260 (243–281)	Depressed	-	8 (7–9)
O. transvolcanica	Solid	12 (12–13)	72 (65–81)	6–7	10–11	468 (443–485) × 269 (237–294)	Elliptical	54 (26–68)	-
O. victoriae	Hollow	15 (13–16)	48 (42–55)	4–5	9–10	357 (321–396) × 353 (321–384)	Ovate	58 (57–60)	9 (7–10)
O. ximenae	Solid	12 (9–13)	30 (27–33)	3	5–6	309 (280–325) × 304 (261–330)	Ovate	-	10 (6–12)
O. sp. nov. 1	Solid	17 (14–19)	27 (23–31)	3	10–11	336 (303–369) × 331 (303–360)	Ovate	168 (155–180)	-
O. sp. nov. 2	Solid	22 (20–23)	84 (73–93)	3–4	9–10	445 (413–482) × 315 (298–342)	Elliptical	173 (120–232)	26 (22–32)

Undoubtedly, the most obvious characteristic of culm cross sections is the presence of numerous vascular bundles embedded in the ground tissue. Grosser and Liese (1971) proposed four major types of vascular bundles based on the distribution of associated fibres. According to this classification, the vascular bundles found in *Otatea* species correspond to type I, which consists of one part (central vascular strand) serving as supporting tissue only, enclosed in a sclerenchyma sheath. This type of vascular bundle has also been reported in *Arundinaria* Michaux (1803:73), *Phyllostachys* Siebold and Zucc. (1843:745), *Bambusa* Schreber (1789:236) (Liese 1980), and in the Neotropical woody bamboo genera *Aulonemia*, *Colanthelia*, *Chusquea*, and *Merostachys*, as well as in *Rhipidocladum* (Rúgolo de Agrasar & Rodríguez 2003), but not in *Guadua aculeata* (Zaragoza-Hernández *et al*. 2014).

While all the analyzed species shared the same type of vascular bundles, the fibers surrounding the conducting elements had different levels of lignification. The fiber sheaths around each vascular bundle of *O. acuminata* OAX, *O. carrilloi*, *O. glauca*, and *O. transvolcanica* were constituted by weakly lignified cells, in contrast to the rest of the species studied, which had strongly lignified cells. *Otatea transvolcanica* and *O. acuminata* OAX have solid culms, but a key characteristic that distinguishes *O. glauca* is its hollow, thin-walled culm with a lacuna greater than 50% of the internode diameter (Clark & Cortez, 2004). These two characteristics, weakly lignified fiber sheaths of vascular bundles and a thin-walled culm, make *O. glauca* the most fragile of all the genus (García-Martínez pers. obs.).

Silica bodies are a common component found on bamboo culms (Liese 1987, Prychard *et al*. 2003, Lybeer 2006). According to Liese (1998), most of the silica appears to be deposited in the epidermis, and the frequency of silica bodies can influence the hardness of culms, as reported in *Guadua angustifolia* (Londoño *et al*. 2002). In the present study, we observed square to rectangular silica bodies on top of the epidermal layer when viewed in cross section. On a surface view, Guerreiro *et al*. (2013) reported silica bodies of various shapes, including square, rectangular, dumb-bell, saddle, and round, on *Chusquea* species. To complement the descriptions presented in this work, studies on the culm epidermal surface view of *Otatea* species need special attention to describe the shape of silica bodies, as well as ribs and furrows, long cells, papillae, stomatal apparatus, prickle hairs, microhairs, and macrohairs.

In addition to observing silica bodies in the epidermis, we observed these inclusions embedded along with fibers in the periphery of the vascular bundles (except in *O. ramirezii*, *O. rzedowskiorum* and *O. ximenae*) and in the intercellular spaces between parenchyma cells in the ground tissue (except in *O. acuminata* JAL, *O. carrilloi*, *Otatea sp. nov.* 1 and *O. transvolcanica*). Gritsch *et al*. (2004) reported the same distribution of silica bodies in *Guadua angustifolia*. The silica bodies found in the periphery of the vascular bundles had no symmetry. For this reason, we refer to them as amorphous. On the other hand, the silica bodies found in the intercellular spaces were always conical and significantly smaller than the amorphous ones. Piperno & Pearsall (1998) reported the existence of these inclusions in the leaves and culms of *O. fimbriata*. The shape of the silica bodies found in culms was bilobate, rondel, and conical. However, they indicated that most of these inclusions have only three indentations at a cross-section view. A new study in detail of the shape, location, and content of silica bodies can have a positive impact on taxonomic identification.

As we illustrated in our results and discussed earlier, we observed differences among the specimens of *Otatea* that we analyzed, including within the four specimens of *O. acuminata*. The fiber sheaths around the transitional and central vascular bundles of *O. acuminata* OAX are constituted by weakly lignified cells, in contrast to the other *O. acuminata* specimens we studied, which have strongly lignified cells. *Otatea acuminata* AGS has a thick cortex that is greater than 66 µm, while the other species have a maximum length of 47 µm. *Otatea acuminata* JAL has the smallest vascular bundles in size, which are almost half the size of the other specimens (287 × 288 µm versus >410 × 427 µm). *Otatea acuminata* VER and *O. acuminata* AGS share elliptical-shaped vascular bundles; however, *O. acuminata* VER has vascular bundles in 9–10 cycles and a thin cortex of 34 (30–40) µm, whereas *O. acuminata* AGS has 6–7 cycles with a thick cortex of 70 (66–77) µm. These character differences support our hypothesis that the name *O. acuminata* encompasses populations with distinct characters that can be segregated into different species. However, more characters from anatomical, morphological, and molecular studies need to be compiled, analyzed, and compared before a taxonomic decision can be made.

Through detailed analysis, we have identified taxonomically significant anatomical differences in *Otatea* culms cross sections that can be used for species identification. This knowledge is valuable in archaeological research, where the presence of bamboo fragments or impressions, especially bajareque, is frequently encountered. Accurate identification of these bamboo remainders enhances our understanding of past human activities, construction techniques, and cultural practices. This study provides an essential tool for the identification and classification of bamboo samples from archaeological contexts, facilitating more accurate reconstructions of past societies and enriching our understanding of Mexico's cultural heritage.

Conclusion

We studied the culm anatomy of *Otatea* to identify character differences that have taxonomic value. As a result, we obtained useful evidence for species identification and generated a key at the species level based on anatomical characters. This key, along with detailed descriptions and images, will be a valuable tool for identifying species, including samples of culms from archaeological sites.

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