

## First Record of Sporangiasters in the Blechnaceae

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**ABSTRACT.**—Sporangiasters are an uncommon type of paraphyses, occurring in a handful of leptosporangiate fern genera. We report sporangiasters in *Parablechnum nesophilum*, which is their first recorded occurrence in the Blechnaceae. We provide evidence of their homology to sporangia and discuss their functional significance. The presence of sporangiasters in *P. nesophilum* is surprising because the species has a well-developed indusium, and paraphyses are generally most common in exindusiate ferns. We surmise that these sporangiasters act as a second stage of protection for the developing sporangia after the indusium retracts. Alternatively, it is possible that sporangiasters may influence the vagility of spores by inhibiting dehiscence and limiting the ability of the annulus to disperse its spores.

**KEY WORDS.**—Anatomy, morphology, paraphyses, sori, spore dispersal

Paraphyses of ferns are sterile structures that occur mixed among sporangia (Wagner, 1964; Parris, 1997). They may be borne either on the receptacle or on the sporangia themselves, such as on the sporangial stalks or capsules. Opinions have varied concerning whether sporangium-borne paraphyses should be considered paraphyses or given an alternate term, and whether the term paraphyses should also pertain to hairs that are like those elsewhere on the lamina (A. F. Tryon, 1965; R. Tryon, 1965; Wagner, 1964; 1965). In either case, the functional significance of paraphyses is thought to be of protection for the developing sporangia against desiccation, herbivory, or from each other as they grow in size (Wagner, 1964).

Paraphyses are widespread in leptosporangiate ferns (Copeland, 1947; Wilson, 1959), but they are unevenly distributed. They are common and diagnostic in groups such as *Pteris* (Bower, 1928), the Vittarioideae genera (Schuettpeltz *et al.*, 2016) and the Elaphoglossoideae (Moran, Labiak, and Sundue, 2010), where they occur upon the receptacle, and in many genera of Thelypteridaceae (Smith, 1990), Polypodiaceae (Baayen and Hennipman, 1987; Sundue, Islam, and Ranker, 2010) where they are prominent upon sporangial stalks and capsules. However, in other large groups such as Lindsaeaceae (Lehtonen *et al.*, 2010), Dennstaedtiaceae (Schwartzburd *et al.*, 2020), Aspleniaceae (Sundue and Rothfels, 2014), Athyriaceae (Wei *et al.*, 2018), Dryopteridoideae (Zhang *et al.*, 2013) and Tectariaceae (Holttum, 1991)

are absent or rare. In some instances, they are similar or identical to leaf indument. In other cases, they appear to be modified sporangia and have been termed sporangiasters (the suffix *-aster* indicating “incomplete likeness”; Stearn, 1992). Sporangiasters are less common than other types of paraphyses and occur only in a handful of leptosporangiate fern genera. They are best known in *Polypodium*, where they develop a typical sporangial stalk, but instead of forming a spore-bearing capsule, the apex develops into a clear vitreous sphere adorned with bulbous protrusions (Martens and Pirard, 1943). They develop precociously, well before the sporangia mature, and their rounded and enlarged apices form a roof over the young sporangia. When the sporangia mature, their stalks elongate, pushing the capsules above the sporangiasters, enabling subsequent dehiscence (Peterson and Kott, 1979).

Here, we report the occurrence of sporangiasters in *Parablechnum nesophilum* (T.C. Chambers & P.A. Farrant) Gasper & Salino. This is the first report of the occurrence of sporangiasters in the Blechnaceae. We provide evidence of their homology to sporangia and discuss their functional significance.

#### MATERIALS AND METHODS

This study is based on specimens of *Parablechnum nesophilum* (Fig. 1 A–E) collected in East New Britain, Papua New Guinea (*Sundue 4251*, BISH, LAE, MACB, VT). The species known primarily from the small islands of New Britain, New Ireland, and the Solomon Islands, and Santa Cruz Islands, but has also been recorded from mainland Papua New Guinea (Chambers, Edwards, and Johns, 2005; Chen *et al.*, 2017; Cámara-Leret *et al.*, 2020). The binomial means “island-loving.”

Plant microscopy protocols followed Ruzin (1999), and 1% TBO (Toluidine O Blue) and fluoroglycine were chosen for staining, which have been widely used in anatomical studies in ferns (*e.g.*, Prada *et al.*, 2016). The observations with light microscopy were taken with a Nikon Labophot-2 microscope with a Coolpix MDC camera. The same specimen was observed using scanning electron microscopy (SEM). The samples were mounted on a sample holder with carbon adhesive. Before observation, each sample was coated with a gold layer and observed on a JSM 6400 JEOL scanning electron microscope operating at 20 kv. The observations were made at the National Center for Electron Microscopy (CNME) of the Complutense University of Madrid, Spain. The nomenclature used for the sporangium characters is based on the combination of Lellinger (2002) and Wilson (1959) presented in Molino *et al.* (2020).

#### RESULTS

The sori of *Parablechnum nesophilum* are linear and occur on the lamina abaxially along both sides of the costa, forming a coenosorus. They are covered by continuous dark brown membranaceous indusia, formed by the recurved

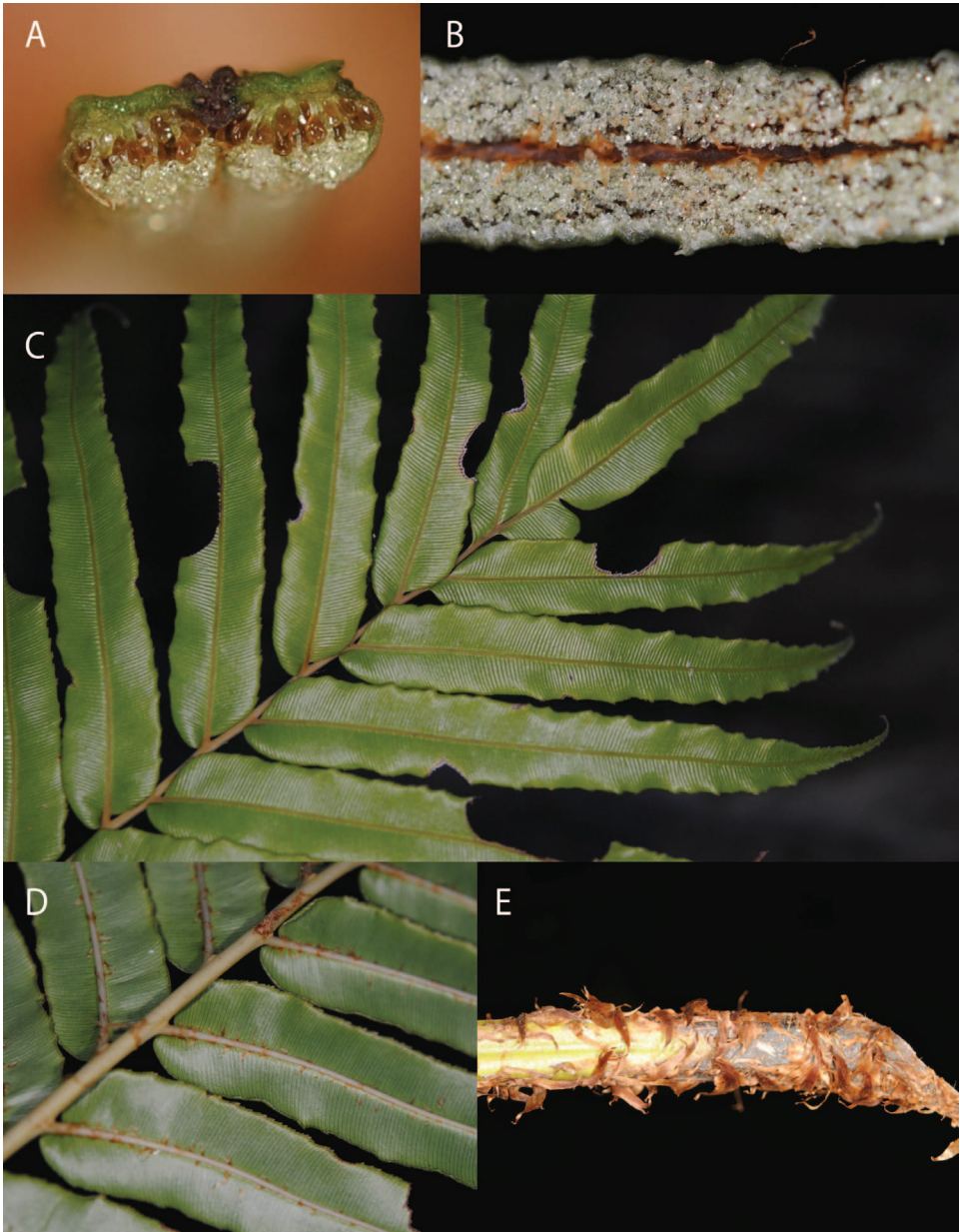


FIG. 1. *Parablechnum nesophilum* (Sundue 4251). A. Cross section of fertile pinna showing vitreous sporangiasters above brown sporangia. B. Abaxial fertile pinna showing vitreous sporangiasters. C. Adaxial surface of sterile lamina. D. Abaxial surface of sterile lamina. E. Petiole with scales.

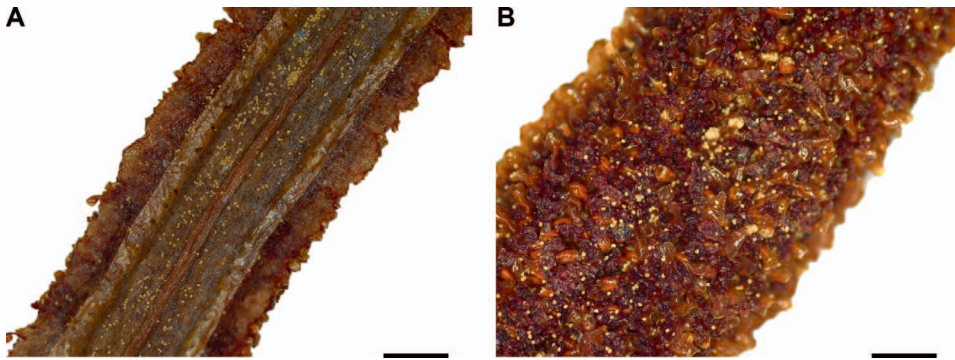


FIG. 2. Pinnae of *P. nesophilum* (Sundue 4251). A. Adaxial side showing remains of retracted indusium; B. Abaxial side, showing sporangia and sporangiasters concolorous after drying. Scalebar = 1.5 mm in A; 1 mm in B.

margin of the pinna. As in all Blechnaceae, the indusia open towards the costa (Fig. 2).

*Pinna anatomy*.—As in other *Parablechnum*, the receptacle is elongate, covering most of the abaxial surface of the pinnae. The costa is grooved adaxially and prominent abaxially, with sclerotic cells in the groove and the keel, which stain blue with TBO and bright pink with fluoroglycine (Fig 3. A, B). Three vascular bundles were visible in the costa; a small central bundle, and two larger lateral bundles. The largest ones were provided with an interrupted dark circumdermal sheath towards the abaxial side of the costa. This band remains black after the staining with both TBO and fluoroglycine (Fig. 3B). The indusium is composed of several layers of cells (Figs. 3A, 3C).

*Sporangia*.—Sporangia were thin-walled with a vertical annulus interrupted at the stalk, which is three cells wide, similar to most other Polypodiales (Fig. 3). Sporangia in various stages of development were present in the studied material (Fig. 3C). Fully developed sporangia had an annulus with 20–24 cells and capsules  $269.4 \pm 15.8 \mu\text{m}$  wide and  $410.2 \pm 33.9 \mu\text{m}$  long, with three (rarely five) lip cells, one above and two below (rarely two above and three below). The upper and lower lips were  $39.6 \pm 22.7 \mu\text{m}$  and  $47.5 \pm 0.2 \mu\text{m}$ , respectively. In most cases, four epistomial cells and one hypostomial cells are present (rarely three and two cells, respectively). The pedicel is  $311.6 \pm 125.6 \mu\text{m}$  long, with a three-cell rosette  $64.4 \pm 15.9 \mu\text{m}$  long.

*Sporangiasters*.—These occurred among the spore-bearing sporangia, and were  $1.3 \pm 0.2 \mu\text{m}$  long. Their stalks were identical to those of the sporangia, but their apices that developed into vitreous clavate heads of 6–10 subglobose cells (Figs. 3E, 4). Unlike spore-bearing capsules, the clavate heads lacked a central cavity and showed no signs of differentiation into an annulus or stomium. The sporangiasters occur at roughly the same density as the regular sporangia and are ca. 1.5x taller than the sporangia.

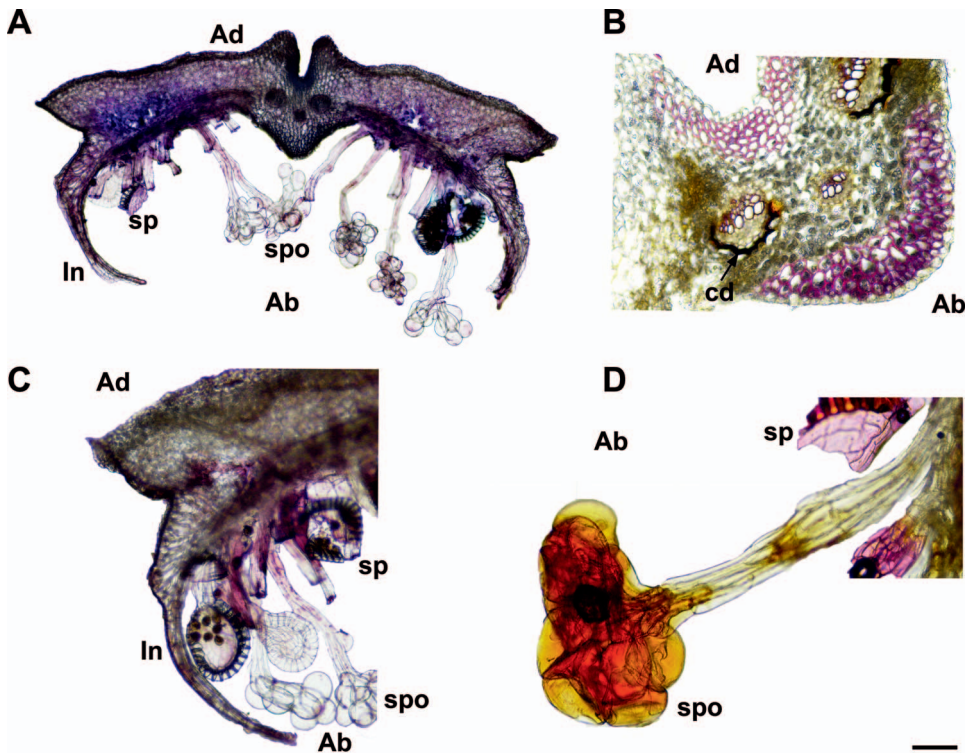


FIG. 3. Cross sections of *P. nesophilum* fertile pinnae (*Sundue 4251*). A. Complete cross section of the fertile pinnae stained with TBO; B. Detail of the costae cross section stained with fluoroglycine; C. Detail of the receptacle cross section stained with fluoroglycine; D. Detail of a sporangiaster. Ab= abaxial; Ad= adaxial; in= indusium; cd= circundermal sheath; sp= sporangia; spo= sporangia. Bar = 90  $\mu$ m in A; 225  $\mu$ m in B; 130  $\mu$ m in C; 100  $\mu$ m in D.

#### DISCUSSION

This report of sporangiasters in *Parablechnum nesophilum* represents the first recorded occurrence of them in the family. Neither sporangiasters nor any other type of paraphyses have been previously recorded in the Blechnaceae (Rolleri, Prada, and Passarelli, 2008; Rothfels *et al.*, 2012; Prada *et al.*, 2016). The sporangiasters were not previously overlooked because they are hard to observe; we immediately noticed the vitreous spherical heads during field work (Fig. 1A, 1B). In dried specimens, however, they darken and become less conspicuous, which probably accounts for their being previously overlooked.

Sporangiasters have also been reported in *Alsophila* (Janssen and Rakoton-drainibe, 2006), *Campyloneurum* (Wagner and Farrar, 1976), *Lecanopteris* (Hennipman, 1986), *Loxogramme* (Price, 1990), *Meniscium* (Fernandes and Salino, 2016), and *Vittaria* (Britton and Taylor, 1902). Within these examples, we note that the homology of the paraphyses to sporangia is not always clear; some of these may in fact be derived from hairs or scales rather than sporangia

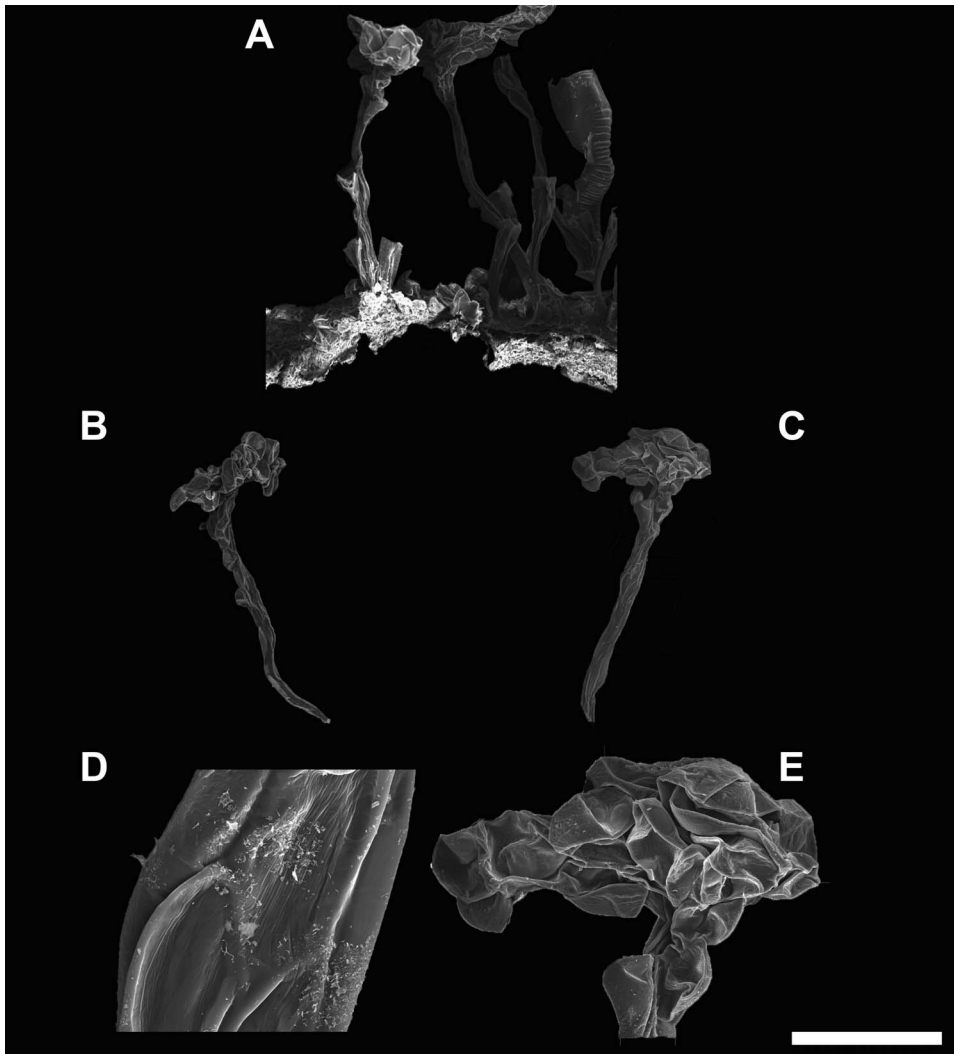


FIG. 4. Sporangiasters of *P. nesophilum* under SEM (Sundue 4251). A. Cross section of a fertile pinnae bearing attached sporangiasters; B–C. Sporangiasters; D. Detail of the sporangiaster pedicel; E. Detail of clavate head. scalebar = 530  $\mu$ m in A–C; 50  $\mu$ m in E; 200  $\mu$ m in E.

and would be better described using the general term paraphyses. Others may simply be aborted sporangia that are not otherwise modified. In those cases, an additional term does not seem helpful. Indehiscent sporangia such as those reported by Wang *et al.* (2012) are also different from sporangiasters in that they contain viable spores, but are not otherwise modified. We also acknowledge that some other groups of ferns such as *Acrostichum* (Adams and Tomlinson, 1979) and *Lomagramma* (Moran, Labiak, and Sundue, 2010) have paraphyses that bear a strong resemblance to sporangia; but the term

sporangiaster has never been applied, and more study is needed to establish homology.

As noted by Wagner (1964), paraphyses seem to occur most often in exindusiate ferns. This observation supports the idea that the primary function of paraphyses is to protect developing sporangia. Therefore, they are more likely to evolve in exindusiate plants. With this in mind, the presence of sporangiasters in *P. nesophilum* comes as a surprise, since the species also has a well-developed indusium (Fig. 2). We know very little about the timing of indusial opening in *P. nesophilum*, but it may be that it is early-opening. When *Sundue 4251* was collected, the indusium had already retracted and did not cover the sorus (Figs. 1A, 1B). It seems likely that its sporangiasters act as a second stage of protection for the developing sporangia after the indusium retracts. Alternatively, sporangiasters may influence the vagility of spores by inhibiting dehiscence and limiting the ability of the annulus to fling the spores away from the plant (Wagner *in* Carlquist, 1966). By limiting spore dispersal, the sporangiasters help maximize local recruitment and minimize loss of spores to potentially unfavorable conditions that may occur further away (Carlquist, 1966). The geographic distribution of *P. nesophilum* fits this scenario: it inhabits small islands of eastern Malesia and the western Pacific. Whether sporangiasters have any effect on the vagility of spores in this species remains unknown. Additional anatomical studies of species from the region will hopefully provide more insight.

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