

×*PHLEBOSIA* (POLYPODIACEAE), A NEW FERN NOTHOGENUS  
WITH A NEW CULTIVAR ‘NICOLAS DIAMOND’

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**ABSTRACT**

In 2014 James J. Georgusis reported that a chance hybrid between *Phlebodium aureum* ‘Mandaianum’ and *Pyrrosia lingua* ‘Cristata’ had originated in his greenhouse in Metairie, Louisiana (USA). Though we initially were inclined to consider it a bizarre mutation of one of its putative progenitors, further study of its morphology and its nuclear 2C DNA content revealed it to be intermediate between its putative parents. We thus accept its proposed ancestry and hence describe this plant here in the new nothogenus ×*Phlebosia*.

**INTRODUCTION**

This article describes the discovery and research on an accidental and surprising hybrid between two (sub)tropical species, one from *Phlebodium* and the other a *Pyrrosia*, both belonging to the Polypodiaceae.

Direct crossings or hybrids between different fern species are not rare, but generally sterile (Reichstein, 1981). Usually, hybrids originate within a genus, and close relatives cross more easily. In the wild and within their own genus, sterile hybrids are formed between many fern species, but are best known and described in the northern temperate floras of Europe, North America, and Japan (Reichstein, 1981; 1984; Tutin et al., 1993; Iwatsuki et al., 1995). Because of their unbalanced genome, often a lack of homologous chromosomes, these primary hybrids (usually) cannot form viable spores and are sterile. However, disturbances during mitosis (Rasbach et al., 1991; Schneller, 1983) or meiosis may lead to a doubling of the number of chromosomes within a cell, a process usually referred to as “whole-genome duplication” or “polyploidisation”. If this process occurs in a sterile hybrid, such a plant can create viable spores that could lead to a new self-reproducing population, reproductively isolated from its progenitors, i.e., a new species (e.g., Dauphin et al., 2017a; 2017b; Sigel, 2016). Hybrids are thus not always “dead ends” in evolution, but may be sources for new evolutionary lines as shown by extensive cytological research in the second half of the twentieth century (e.g., Lovis, 1977; Reichstein, 1981; Kramer et al., 1995; Sigel, 2016). About half of all European fern species are polyploid (Reichstein, 1981; 1984; Kramer, 1984; Kramer et al., 1995, Tutin et al. 1993), and just under half have arisen, sometimes even repeatedly, from a sterile cross between two (diploid) species (Schneller, 1996; Sessa et al., 2018; Vogel et al., 1999). Multiple origins of fern complexes were often postulated in the literature cited above and have now been demonstrated using molecular techniques (e.g., Soltis et al., 1987; Ranker et al., 1989; Schneller, 1996; Thomson & Alonso-Amelot, 2002; Beck et

al., 2012; Sigel et al., 2014; Sigel, 2016; Williams et al., 2016; Yahaya et al., 2016; Dauphin et al., 2017a; 2017b); similar evolutionary patterns are known from higher plants (Alix et al., 2017). Hybrids between species belonging to different genera are very rare (Alston, 1940; Reichstein, 1981; Schneller, 1981; Wagner et al., 1992; Rothfels et al. 2015; Engels & Canestraro, 2017; Lehtonen, 2018) and are ascribed to a nothogenus of their own. The existence of intergenetic hybrids often constitutes one of the arguments either to (re)unite relatively smaller genera, e.g., *Asplenium*, *Ceterach*, *Pleurosorus*, and *Phyllitis* to a single genus: *Asplenium* (Kramer & Viane, 1990), or to keep species within one and the same genus (Tejero-Diaz et al., 2009). According to Turland et al. (2018) a hybrid genus must be indicated by writing the symbol “×” before and without space against the generic name, e.g., ×*Asplenosorus*, ×*Dryostichum*, and ×*Pleopodium*. According to Brickell et al. (2016, art. 14.1) cultivar status has to be indicated by enclosing the cultivar epithet within single quotation marks.

In 2014, James J. Georgusis from Metairie, Louisiana, USA, found separate strange juvenile plants in his greenhouse, which he suspected to be the result of a spontaneous cross between the only two fern species he grew at the time: *Phlebodium aureum* ‘Mandaianum’ and *Pyrrosia lingua* ‘Cristata’ (Hoshizaki & Moran, 2001). From the hybrid sporelings he selected a single plant considered worthy of commercialization and to be introduced into the trade. The putative parents of the hybrid belong to different genera that may have been separated for at least 35 million years (Wei et al., 2017). Given the rather unlikely emergence of this hybrid, we first considered that it might be a bizarre mutation of one of its putative ancestors and thus sought to confirm its parentage.

### MATERIAL AND METHODS

In 2015, the parent plants and their hybrid were delivered to the research department of the fern nursery “Vitro Plus, B.V.”, located in Burgh-Haamstede (NL), with the intention to propagate the hybrid vegetatively. After two years, in 2017, enough material for further research had been obtained.

We studied the general morphology, the micro morphology of the leaf indument and rhizome scales, and the DNA quantity. Only fully-grown scales at a short distance from the apex were compared because they are often damaged on older parts. Because microscopic hairs on the rachis and the costae are often deciduous, or shrivel and become difficult to observe on older fronds of both *Phlebodium* and ×*Phlebodia*, their indument was studied on young, uncurling fiddleheads at a comparable stage. Unfortunately, no spore characterizations could be done because these were not available for *Pyrrosia lingua* ‘Cristata’, nor for ×*Phlebodia*, which at present remains sterile.

Sections of the rhizome, the rhizome scales, and the leaf hairs were photographed with a Canon MP-E 65mm f/2.8 1-5x Macro Photo lens on a Canon EOS 5DSR camera. Flow cytometry with propidium iodide (PI) staining was used to compare 2C DNA values (the content of DNA from the nucleus of vegetative cells). Nuclei were obtained from thin slices of the rhizome or the petiole finely chopped together with a comparable slice of *Agave* ‘Shaka Zulu’ (2C = 8.97 pg) as internal standard, and stained with the ‘CyStain PI absolute P’ nuclei extraction and staining kit (Partec GmbH, Münster) according to the manufacturer’s instructions. Samples were analyzed with a Becton Dickinson BD Accuri™ C6 flow cytometer (Franklin Lakes, USA). At least 5,000 nuclei per sample were analysed, with five replicates per accession. The 2C value was calculated based on the ratios of the G1 peak means of the sample and the standard.

Terminology follows Stearn’s *Botanical Latin* (1992) or Hoshizaki & Moran (2001).

## RESULTS AND DISCUSSION

Numerical results, based on at least twenty measurements, are summarized in Table 1. Both the overall morphology and the size (see Table 1) of the hybrid are more or less intermediate between those of the putative parents, although the average values of the hybrid are shifted slightly more towards those of *Pyrrrosia*. See Figures 1 to 3.

### Rhizome

All plants have a widely creeping aboveground rhizome. The diameter of the rhizome of  $\times$ *Phlebosia* (4 to 8.5 mm) is intermediate between that of *Pyrrrosia* (2.5 to 3.5 mm) and that of *Phlebodium* (10 to 20 mm; Table 1).

### Rhizome scales

The rhizome of *Pyrrrosia* carries appressed, narrowly triangular to lanceolate, rusty to golden brown, peltate scales (Fig. 2A), set apically and along the margin with extremely thin hairs (cilia sensu Hovenkamp, 1986). Scales on the rhizomes of *Phlebodium* and  $\times$ *Phlebosia* have a similar shape but lack the hairy apex (Fig. 2B-C) and are less appressed. The size of the rhizome scales of  $\times$ *Phlebosia* lies between that of the putative parents (Figure 2; Table 1).

### Fronds

The mature leaf of  $\times$ *Phlebosia* is reasonably intermediate (somewhat more shifted towards *Pyrrrosia*) between that of the suspected parents: it is clearly smaller (40 to 75 cm) than that of *Phlebodium* (115 to 135 cm) but much larger than that of *Pyrrrosia* (19 to 32 cm). Furthermore, it is also pinnatipartite but not pinnatisect as in *Phlebodium*, medium to dark green, shiny and often laciniate or crested (cristate) at the apex of the frond and/or of its segments.

### Frond surface

The characteristic stellate hairs of *Pyrrrosia* (Figure 3D) are absent from the leaf surface of  $\times$ *Phlebosia*. However, we found microscales next to unicellular-to-multicellular glands (Figure 3, B-C) typical of prothallia and juvenile leaves of *Pyrrrosia* (Nayar & Chandra 1965: 37; Hovenkamp 1986: 45), and not found in *Phlebodium*. These hairs are either



**Figure 1.** Rhizome cross sections (scales removed). A. *Pyrrrosia* (R.Viane 14240); B.  $\times$ *Phlebosia* (R.Viane 14238); C. *Phlebodium* (R.Viane 14239) (scale bar: 10 mm).

**Table 1.** Summary of morphological characters studied.

	<i>Pyrrosia</i>			× <i>Phlebosia</i>			<i>Phlebodium</i>		
	min	Mean	max	Min	mean	max	min	mean	Max
Rhizome	creeping			creeping			creeping		
Rhizome ø (mm)	2.5	<b>3.2</b>	3.5	4.0	<b>6.9</b>	8.5	10.0	<b>15.4</b>	20.3
Scale length (mm)	3.9	<b>5.9</b>	8.9	6.2	<b>9.9</b>	11.0	12.9	<b>14.4</b>	15.0
Scale width (mm)	0.3	<b>0.7</b>	1.1	0.9	<b>1.0</b>	1.2	1.5	<b>2.0</b>	2.4
Stipe base ø (mm)	2.0	<b>2.4</b>	3.0	3.2	<b>3.6</b>	4.5	7.0	<b>8.0</b>	9.0
Stipe length (cm)	4	<b>6</b>	7	20	<b>28</b>	35	40	<b>46</b>	50
Lamina length (cm)	15	<b>20</b>	25	20	<b>33</b>	40	75	<b>80</b>	85
Lamina width (cm)	6	<b>9</b>	16	17	<b>22</b>	32	30	<b>32</b>	34
Fronde length (cm)	19	<b>26</b>	32	40	<b>59</b>	75	115	<b>126</b>	135
Lamina/Stipe length	<b>3.3</b>				<b>1.1</b>			<b>1.7</b>	
Fronde incision	simple to pinnatifid near the apex			pinnatifid			pinnatifid to almost pinnatisect		
Segment incision	—			lacinate to crested			lacinate to squarrose-lacinate		
Fronde apex	acute or crested			crested			acute		
2C DNA (pg)	14.7	<b>15.2</b>	17.7	25.4	<b>26.3</b>	27.3	35.3	<b>38.0</b>	39.9

unicellular (c. 0.07 to 0.09 mm long), or 2 to 6-cellular and uniseriate, or 3-cellular and forked (Figure 3C) with individual cells usually less than 0.1 mm long.

### DNA content

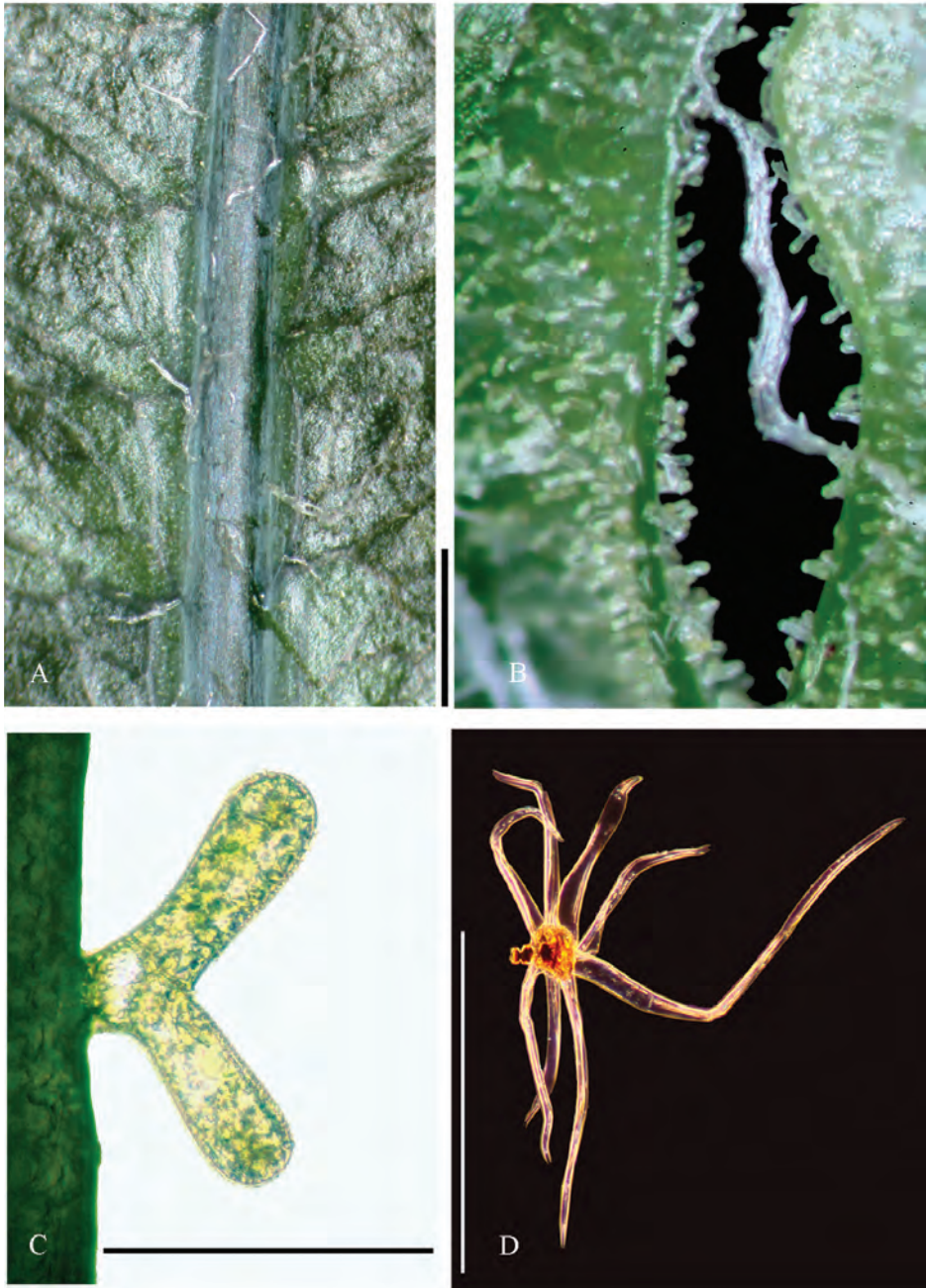
The 2C DNA content of  $\times$ *Phlebosia* is about intermediate (Table 1) between that of its putative parents.

### Discussion and conclusion

*Phlebodium aureum* is an allotetraploid species (Walker, 1985; Tejero-Diez, 2009) with



**Figure 2.** Rhizome scales. A. *Pyrrosia* (R.Viane 14240), note hairs at apex; B.  $\times$ *Phlebosia* (R.Viane 14238); C. *Phlebodium* (R.Viane 14239) (scale bar: 2 mm).



**Figure 3.** Lamina indument. A. *Phlebodium* (R.Viane 14239), sparsely hairy young leaf; B-C, *Phlebosia* (R.Viane 14238), densely hairy young leaf; C. central detail of B; D. stellate hair of *Pyrrosia* (R.Viane 14240), note short brownish stalk directed to left (scale bar: A, B, D: 0.5 mm; C: 0.1 mm).

$2n=4x=148$  chromosomes (e.g., Evans, 1963; Walker, 1985), while *Pyrrosia lingua* is diploid with  $2n=2x=72$  chromosomes (Takei, 1969 Wang et al., 2011), which is clearly manifested in the c. double DNA content of the first. In the putative hybrid we expected that half of the *Pyrrosia* genome ( $1C = 7.6$  pg) would be united with half of the *Phlebodium* genome ( $1C = 19$  pg) which would result in an average total weight of  $2C = \sim 26.6$  pg. The  $2C$  DNA content found for  $\times$ *Phlebosia* fits well with this expectation (Table 1 and Figure 4). In *Pyrrosia*, both the basic chromosome number ( $x=36$ ) and the basic DNA content ( $Cx = c. 7.6$  pg) are different from those in *Phlebodium* ( $x = 37$  and  $Cx = 9.5$  pg).

### Description

We first considered the possibility that the novelty might be a bizarre mutation of one of its putative ancestors. However, because its general and micromorphology, its size characters, as well as its nuclear  $2C$  DNA content are about intermediate between those of its putative parents, we accept the ancestry proposed by the breeder and hence describe this plant here in the new nothogenus  $\times$ *Phlebosia*.

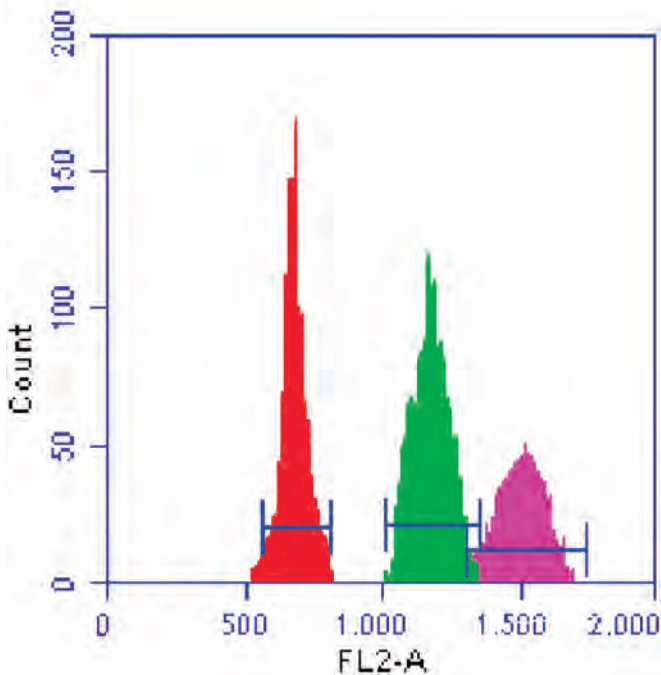
$\times$ *Phlebosia* Viane & Pompe, nothogen. nov. (*Phlebodium* J.Sm.  $\times$  *Pyrrosia* Mirbel).

Genus hybridum inter *Phlebodium* J.Sm. et *Pyrrosiam* Mirbel intermedium.

Since other species from these genera could also form similar hybrids, the present hybrid between *Ph. aureum* and *P. lingua* is given a specific epithet referring to the state of Louisiana (USA) where it originated in cultivation.

$\times$ *Phlebosia louisiana* Viane & Pompe, nothosp. nov.

Hybrida inter *Phlebodium aureum* 'Mandaianum' et *Pyrrosiam linguam* 'Cristatam' plus



**Figure 4.** Flow histogram, with colours added, from combined leaves of *Pyrrosia lingua* 'Cristata' (red),  $\times$ *Phlebosia* (green), and *Phlebodium aureum* 'Mandaianum' (pink), following staining with propidium iodide. FL-2: propidium iodide fluorescent channel.

minusve intermedia.

Rhizome creeping, 4-9 mm in diameter, densely covered with imbricate, rusty to golden brown scales, 6-12 x 0.9-1.2 mm. Fronds erect to arching, 40-75 x 17-32 cm; stipes terete with a ventral groove, greenish to dark brown, 3-5 mm in diameter at the base, 20-35 cm long; uncurling lamina set with forked, multicellular hairs becoming subglabrous at maturity, lamina 20-40 x 17-32 cm, texture papery, dark green, brilliant, pinnatipartite; segments narrowly triangular, acuminate, often forked or crested at the apex. No fertile structures present yet on adult plant. DNA weight  $2C=25.4 - 27.3$  pg.

Type: herbarium material taken from progeny of the original hybrid of J.J. Georgusis (Metairie, New Orleans, Louisiana, USA), raised and multiplied by “Vitro Plus, B.V.” (Burgh-Haamstede, NL), obtained by the author in 2019 for flow cytometric analysis, 2 July 2019, R. Viane 14238 (holo-: L; iso-: BM; P; pers. herb. R. Viane).

Progeny of the original hybrid between *Phlebodium aureum* ‘Mandaianum’ and *Pyrrosia lingua* ‘Cristata’ have been introduced into the trade as a new cultivar called ‘Nicolas Diamond’ (US plant patent: US 2019/01116710P1).

Specimens of the putative parents *Phlebodium aureum* ‘Mandaianum’ (R. Viane 14239) and *Phlebodium aureum* ‘Mandaianum’ (R. Viane 14240) will also be deposited in Leiden (L).

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