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Styloid crystals in the genus *Libertia* (Iridaceae)

D. J. BLANCHON
School of Natural Sciences
Unitec
Private Bag 92025
Auckland 1142, New Zealand
dblanchon@unitec.ac.nz

J. E. BRAGGINS
Herbarium
The Auckland War Memorial Museum
Private Bag 92018
Auckland 1142, New Zealand

Abstract Styloid crystals are reported in the leaves of the New Zealand species *Libertia cranwelliae*, *L. edgariae*, *L. grandiflora*, *L. ixioides*, *L. micrantha*, *L. mooreae*, *L. peregrinans*, the Australian species *L. paniculata* and *L. pulchella*, and the South American species *L. chilensis* and *L. sessiliflora*. Styloid crystals appear to be absent from rhizomes examined in this study, except those of *L. micrantha*. The presence of styloids in *L. micrantha* and *L. pulchella* supports the retention of these species in *Libertia* rather than *Sisyrinchium*.

Keywords Iridaceae; *Libertia*; *L. chilensis*; *L. cranwelliae*; *L. edgariae*; *L. grandiflora*; *L. ixioides*; *L. micrantha*; *L. mooreae*; *L. paniculata*; *L. peregrinans*; *L. pulchella*; *L. sessiliflora*; *Sisyrinchium*; crystals; rhizomes; styloids

INTRODUCTION

Styloids (prismatic calcium oxalate crystals) are a diagnostic feature of certain families, including Agavaceae, Alliaceae, and Iridaceae (Prychid & Rudall 1999). In the Iridaceae, most genera have styloids, with the exception of some members of the tribe Sisyrinchiae (Goldblatt et al. 1990). Within the Sisyrinchiae, Goldblatt et al. (1990) recorded the presence of styloids in *Libertia*, *Orthosanthus*, *Tapeina*, and *Solenomelus*, but not *Olsynium* (including *Ona*, *Phaiophleps*, *Chamelum*) or *Sisyrinchium* s.s.

The generic limits of *Libertia* are largely uncontroversial, although some authors (e.g., Geerinck 1974) have considered that one species, *L. pulchella*, was better placed within *Sisyrinchium*. Rudall (1995), however, placed *L. pulchella* closer to *Libertia* than *Sisyrinchium* based on leaf margin and vascular bundle type.

In light of recent taxonomic changes (Blanchon et al. 2002) and with a view to assisting in the correct generic placement of *L. micrantha* and *L. pulchella*, a review has been done of styloids in the genus for Australasia (nine species), with the South American species *L. chilensis* and *L. sessiliflora* (Goldblatt & Celis 2005; species listed as *L. formosa* and *L. caerulescens*, respectively, in Blanchon et al. 2000, 2002) for comparison.

MATERIAL AND METHODS

Plant material was obtained for a range of species, from a variety of sources, including botanical gardens and field sites (Table 1). Plants were grown in uniform conditions outside, in a glasshouse, or in a shadehouse; where possible, voucher specimens were prepared and deposited in the Herbarium of the Auckland Institute and Museum (AK). Representative plant parts were also preserved in 70% ethanol or FAA (6.5 mL 37% formalin, 4.5 mL glacial acetic acid, made up to 100 mL with 70% ethanol) for later observation and/or sectioning. In some species there was insufficient fixed material for longitudinal sectioning (see Table 1). Fixed specimens were mechanically sectioned, stained in 0.05% toluidine blue, and permanently mounted as described in Blanchon et al. (2002). Slides were examined, using an Olympus BH-2 compound light microscope,

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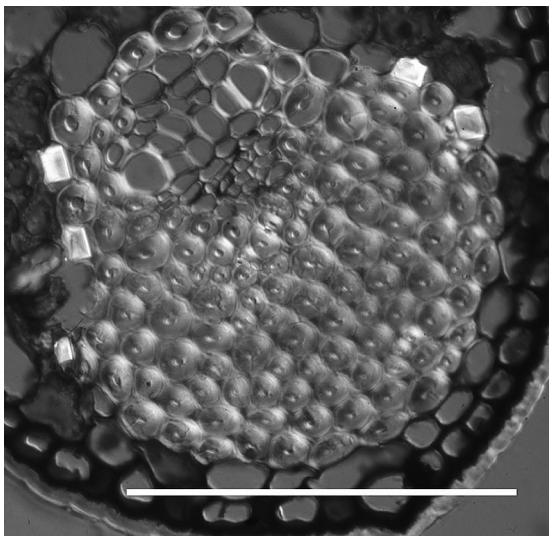

Fig. 1 Styloid crystals associated with vascular bundle in the leaf margin of *Libertia grandiflora* (transverse section, viewed under polarised light). Scale bar = 100 μm.

fitted with an eyepiece graticule calibrated against a slide micrometer, and Olympus polarising filters (CH3-AN and U-POT).

RESULTS

Styloid crystals were found in the leaves of all species examined (Table 1), and were found in elongate parenchyma cells throughout the leaf, with particular concentrations around vascular bundles (Fig. 1). Individual crystals were birefringent and were long and slender in shape with pointed ends (Fig. 2), with the longest axis of each crystal oriented in the same direction as the veins of the leaf. In transverse section, styloids appeared to be square or rectangular and ranged from 2.5 × 2.5 μm to 15 × 20 μm in size. In longitudinal section, styloids appeared to be long and thin, ranging from 60 to 190 μm in length. Styloids were generally absent from rhizomes where examined (Table 2), but were present in the aerial, leaf-bearing rhizome of *L. micrantha*, and very young shoots of *L. ixioides* where they were associated with the centre of the stem and around the periphery of vascular bundles.

Table 1 Styloid crystals from leaf material of *Libertia* species.

Species	Crystal size (transverse section, μm)	Crystal size (longitudinal section, μm)	Chromosome number*	Herbarium voucher
New Zealand				
L. cranwelliae (East Cape, ex Platt Nurseries)	5 × 5–10 × 10	80–190	$2n = 12x = 228$	AK 240225
L. edgariae (Palliser Bay, Wairarapa)	2.5 × 5–12.5 × 12.5		$2n = 6x = 114$	
L. grandiflora (Hicks Bay, East Cape)	2.5 × 2.5–12.5 × 12.5		$2n = 6x = 114$	AK 240212
L. grandiflora (Mt Manaia, Northland)	5 × 7.5–12.5 × 12.5			AK 240211
L. ixioides (Taranaki, ex Platt Nurseries)	5 × 5–15 × 20	75–125	$2n = 12x = 228$	AK 240224
L. ixioides (Hihi Manganui, Northland)	5 × 7.5–12.5 × 15			
L. micrantha (Mataraua Plateau, Northland)	2.5 × 5–12.5 × 12.5		$2n = 2x = 38$	AK 240209
L. mooreae (Mt Burnett, Nelson)	2.5 × 5–7.5 ×12.5	25–60	$2n = 6x = 114$	AK 240215
L. mooreae (Takaka Hill, Nelson)	5 × 5–10 × 10			AK 240214
L. mooreae (Pelorus Bridge, Marlborough)	2.5 × 2.5–2.5 × 5			
L. peregrinans (Foxton Beach)	2.5 × 5–10 × 12.5	25–145	$2n = 6x = 114$	AK 240218
L. peregrinans (Chatham Island)	5 × 5–12.5 × 12.5			
Australia				
L. paniculata (Blackheath, New South Wales)	5 × 7.5–7.5 × 10	60–190	$2n = 4x = 76$	
L. pulchella (Tasmania)	2.5 × 5–10 × 10		$2n = 2x = 38$	AK 240231
South America				
L. sessiliflora (cultivated)	2.5 × 2.5–7.5 × 7.5	30–115	$2n = 2x = 38$	AK 240227
L. chilensis (cultivated)	2.5 × 2.5–4 × 4	30–130	$2n = 2x = 76$	AK 240229

*From Blanchon et al. (2000).

Crystal size did not seem to substantially differ with species or ploidy level. The largest crystals were found in *Libertia ixioides* ($2n = 12x = 228$), but *L. cranwelliae*, with the same chromosome number, had smaller crystals, of similar size to hexaploid species. The smallest crystals were found in one population of the hexaploid *L. mooreae*, and these were smaller than in any of the three diploids examined. Crystal size varied widely within leaves and did not appear to be of use for separating species.

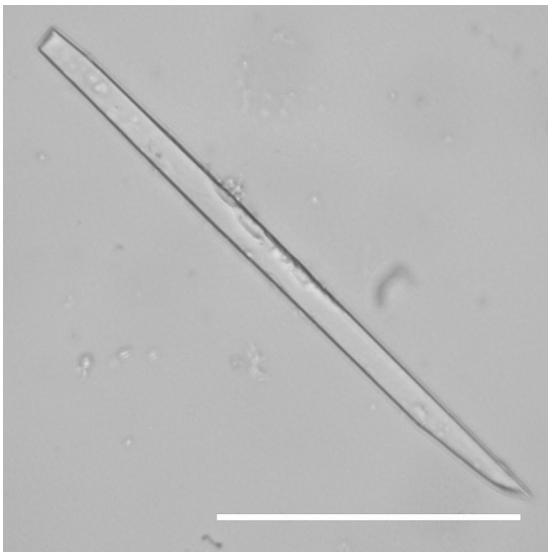

Fig. 2 Styloid crystal from leaf of *Libertia paniculata* (longitudinal view). Scale bar = 50 µm.

DISCUSSION

Results confirmed the presence of styloids for all seven New Zealand species, both Australian species, and the two South American species examined. This is consistent with, and expands on, the results of previous anatomical studies. Betts (1920) studied the anatomy of *L. ixioides* but did not note styloids. Reyes & Montes (1965) examined *L. sessiliflora* and reported calcium oxalate crystals in both the leaves and rhizomes. Goldblatt et al. (1984) examined seven species and found styloids in the leaves. Rudall (1995) also reported styloids from the leaves of *Libertia*, having studied 12 species including five Australasian species, and noted the absence of styloids from the rhizomes of *L. paniculata*. Unfortunately, the identity of the material of *L. paniculata* and *L. peregrinans* that was examined is not completely clear, as the geographical areas listed for the collections did not match the known distributions of the species and herbarium vouchers were not listed. Blanchon et al. (2002) revised *Libertia* for New Zealand, naming three new species, and separating New Zealand material of *L. pulchella* as *L. micrantha*, but did not discuss the presence of styloids. This research found that styloids are also absent from the mature rhizomes of *L. chilensis*, *L. edgariae*, *L. ixioides*, and *L. peregrinans*, but they are present in the rhizomes of *L. micrantha* and were present in the young shoots of *L. ixioides*.

Table 2 Styloid crystals from rhizome material of *Libertia* species.

Species	Crystal size (transverse section, µm)	Crystal size (longitudinal section, µm)	Herbarium voucher
New Zealand			
L. micrantha (Mataraua Plateau, Northland)	5 × 7.5–7.5 × 17.5		AK 240209
L. peregrinans (Omaui, Southland)	not present	not present	
L. edgariae (Palliser Bay, Wairarapa)	not present	not present	
L. ixioides (Taranaki, ex Platt Nurseries), mature rhizome	not present	not present	
L. ixioides (Taranaki, ex Platt Nurseries), young shoot	12.5 × 7.5		
South America			
L. chilensis (cultivated)	not present	not present	AK 240229

Unlike the underground horizontal rhizomes in most of the genus *Libertia*, those of *L. micrantha* are upright, leafy, and mostly above ground (Blanchon et al. 2002), although most of the stem is usually hidden in moss hummocks. Blanchon et al. (2002) considered *L. micrantha* (and the Australian *L. pulchella*) to be primitive and basal within the genus, based on morphology, anatomy, diploid chromosome number, and molecular data. Interestingly, the only other known diploid in the genus, the blue-flowered *L. sessiliflora*, also has styloids in the rhizome, perhaps suggesting that they have been lost from the other species with higher chromosome numbers.

These results do not support the contention (Geerinck 1974) that *L. micrantha* (as *L. pulchella*) is better placed within *Sisyrinchium*. Goldblatt et al. (1984) found that a representative sample of species of *Sisyrinchium* lack styloids in all plant organs. Both *L. micrantha* and *L. pulchella* have styloids in their tissues, which supports the retention of both species in the genus *Libertia*, agreeing with morphology, anatomy, base chromosome number, and molecular data (Blanchon et al. 2000, 2002).

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