Naphthoquinones in Pygmy Sundews (*Drosera* sect. *Bryastrum*)

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Abstract: A detailed study of 44 accessions representing 38 taxa (76% of the diversity known at present) of pygmy sundews (*Drosera* sect. *Bryastrum*) reveals the first naphthoquinone patterns in this lineage, in which previous studies have not yielded reliable evidence for naphthoquinones. While most samples do not display detectable amounts of naphthoquinones as previously reported for the group, ramentaceone is detected in three mutually related taxa, and both ramentaceone and its regio-isomer plumbagin is present in all accessions investigated of *D. pulchella*.

Introduction

Pygmy sundews (*Drosera* sect. *Bryastrum*) are a well-defined lineage of ca. 50 species almost entirely confined to southwestern Western Australia (Fleischmann *et al.* 2018; Robinson *et al.* 2018). Only the more widespread *D. pygmaea* (reaching New Zealand) and the curiously disjunct *D. meristocaulis* (endemic to southernmost Venezuela) occur elsewhere. Almost all species (except the tropical *D. meristocaulis*) are known to form gemmae that are vegetative propagules from modified leaves (Goebel 1908; Rivadavia *et al.* 2013; Lowrie 2014: 44-58 & 116-151).

While naphthoquinones like plumbagin (2-methyljuglone, **P**) and ramentaceone (7-methyljuglone, **M**) have been known for a long time as characteristic, chemotaxonomically informative metabolites in the genus *Drosera*, they have not been detected with any certainty in pygmy sundews yet. A single, dubious report (reference to unpublished data without any experimental details) of **P** in *D. pygmaea* (Krenn in Länger *et al.* 1995) stands in clear contrast to negative results from a previous, comprehensive screening (Culham and Gornall 1994).

More recently, the re-investigation of species formerly believed devoid of naphthoquinones has yielded some unexpected, positive results (**P** in *D. menziesii*, Schlauer *et al.* 2018, **M** in *D. arcturi*, Schlauer *et al.* 2019a, and in *D. schizandra*, Schlauer *et al.* 2019b). The removal of ripe gemmae does not damage the rosettes that produced them, so this material was investigated for the first time in the present study.

Among numerous confirmed and new negative results, a few species are reliably found to contain **M**, and *D. pulchella*, previously considered devoid of naphthoquinones whatsoever, even contains both **M** and **P**.

Materials and methods

All plants used in the present study were raised from gemmae and cultivated at Andreas Fleischmann's greenhouse in southern Germany (Figs. 1 & 2). The geographic origin of all accessions was traced as far as possible (see Table 1). Ten to twenty fresh, ripe gemmae (ca. 10 mg) of each accession were harvested and investigated under the same conditions applying the same methods as



Figure 1: Flower, habit, and gemmae of the three M-positive members of *Drosera* sect. *Bryastrum*: A. *D. minutiflora*. B. *D. pedicellaris*. C. *D. sargentii*. Flower and habit images not at the same scale. Plants cultivated by A. Fleischmann, photo vouchers were made for the material used in this study.

reported previously for the investigation of leaves (Schlauer *et al.* 2017). Additionally, fresh leaves were investigated for some species or as control of the gemmae results.

Results

Naphthoquinones were detected in the investigated samples as summarized (together with previous results) in Table 1.

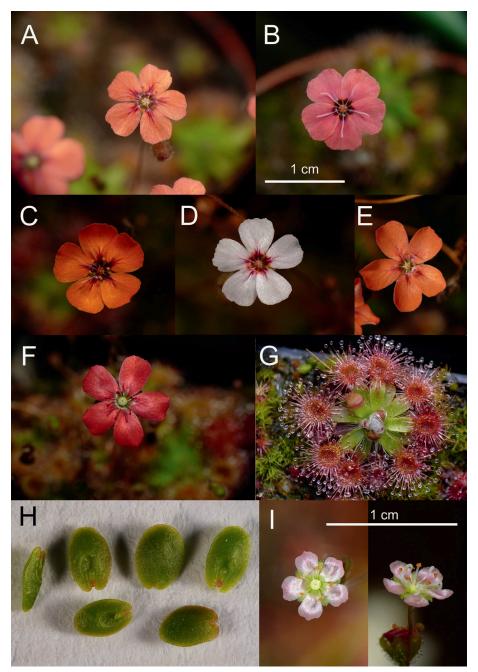


Figure 2: Drosera pulchella (A-H) and D. depauperata (I). Accessions of D. pulchella from Scott River, Western Australia (A, flower; H, gemmae), clone 52-A (B); Baker's Junction, Western Australia (C, flower; G, habit), white flower, red center (D), Mount Cooke, Western Australia (E), red flower (F). Images not at the same scale. Plants grown and photographed by A. Fleischmann.

Table 1. Taxa of <i>Drosera</i> sect. <i>Bryastrum</i> and quinones found.						
Taxon ^a	Accessions	Provenance	Quinone(s) b	References c		
D. androsacea	1	Western Australia (WA)	0	*		
D. (occidentalis subsp.) australis	1	WA	0	*		
D. barbigera	1		0	1		
D. bindoon	1	Bindoon, WA	0	*		
D. callistos	1	Brookton, WA	0	*		
D. citrina	1	Regans Ford, WA	0	*		
D. closterostigma	1	(type form), WA	0	*		
D. coomallo	1	Coomallo, WA	0	*		
D. depauperata	1	locus classicus, WA	0	*		
D. echinoblastus	1	WA	0	*		
D. enneabba	2	Moore River NP, WA; (red from), WA	0	*		
D. gibsonii	1	WA	0	*		
D. grievei	1	WA	0	*		
D. helodes	2	Bullsbrook, WA; Great Northern Highway, WA	0	*		
D. hyperostigma	1	WA	0	*		
D. lasiantha	1	Porongorup NP, WA	0	*		
D. leucoblasta	1		0	1		
D. (nitidula var.) leucostigma	1	WA	0	*		
D. mannii	1	WA	0	*		
D. meristocaulis	1	Sierra de la Neblina, Venezuela	0	2		
D. (occidentalis var.) microscapa	1	locus classicus, WA	0	*		
D. miniata	1	(type form), WA	0	*		
D. nitidula	1		0	1		
D. (citrina var.) nivea	1	WA	0	*		
D. occidentalis	3		0	1		
D. (nitidula subsp.) omissa	1		0	1		
D. (nitidula subsp.) omissa	1	(pink flower), WA	M(+P-trace)	*		
D. oreopodion	1	locus classicus, WA	0	*		
D. micrantha	1		0	1 (as "D. paleacea")		

Table 1. Continued.						
Taxon ^a	Accessions	Provenance	Quinone(s) b	References c		
D. micrantha	1	WA	0	*		
D. paleacea	1	WA	0	*		
D. minutiflora (?)	1		0	1 (as "D. parvula")		
D. minutiflora	1	WA	M	*		
D. (nitidula var.) patens	1	WA	0	*		
D. (parvula var.) pedicellaris	1	locus classicus, WA	M	*		
D. platystigma	1		0	1		
D. pulchella	2		0	1		
D. pulchella	3	Scott River, WA; Baker's Junction, WA; (white flower, red center), WA	P+M	*		
D. pulchella × nitidula	1		0	1		
D. pulchella × occidentalis	1		0	1		
D. pycnoblasta	1		0	1		
D. pygmaea	1		"P"	3		
D. pygmaea	2		0	1		
D. pygmaea	2	Windmill Lagoon, Tasmania; New Zealand	0	*		
D. (paleacea subsp.) roseana	1	WA	0	*		
D. (parvula subsp.) sargentii	1	WA	М	*		
D. scorpioides	2		0	1		
D. scorpioides	1	WA	0	*		
D. sewelliae	1	(reddish form) WA	0	*		
D. (barbigera subsp.) silvicola	1	WA	0	*		
D. (paleacea subsp.) stelliflora	1	WA	0	*		
D. (paleacea subsp.) trichocaulis	1	WA	0	*		
D. verrucata	1		0	1 (as "D. dichrosepala")		
D. verrucata	1	Fishtrack Road, WA	0	*		
D. walyunga	1	Walyunga NP, WA	0	*		

^aNomenclature following Lowrie (2014), in parentheses alternative classification following Schlauer (1996).

^bP: plumbagin, M: ramentaceone, 0: no quinone detected

cReferences

^{*} New/additional data from this study

¹ Culham & Gornall 1994

² Schlauer 2012

³ Länger et al. 1995

Fresh leaves of *D. roseana*, *D. scorpioides*, and *D. pygmaea* did not yield quinones, confirming gemmae and/or literature results. No quinones were likewise detected in leaf samples of *D. minutiflora* and *D. pedicellaris*, of which gemmae contained **M**. Quinones (**M** and **P**) in trace amounts were detected in leaves of *D. pulchella* (Mt. Cooke, red flower, Scott River).

Discussion

Although using gemmae instead of leaves should initially be only a test if less invasive sampling methods can be applied to delicate species, this study produced some noteworthy and chemotaxonomically relevant results. Previously, also seeds were found to contain naphthoquinones (unpublished results) but the amount required to yield reliable signals (usually the entire crop of an individual) cannot be expected to be available from most pygmy sundews, of which several taxa are more or less seedless for various reasons (e.g., self-sterility of the majority of species and usually only single clones available in cultivation, but also hybridity or aneuploidy, Kondo and Lavarack 1984).

The comparison between fresh leaves vs. gemmae confirm our assumption that gemmae are suitable for the investigation of quinone patterns. Especially where only tiny amounts of leaf material were available, gemmae provided more reliable data than leaves ("no detection" is just absence of evidence, not evidence of absence).

The discovery of **M** in *D. minutiflora* (syn. *D. parvula*: Lowrie 2014: 620), *D. pedicellaris*, and *D. sargentii* suggests a close phylogenetic relationship between these taxa as deduced from morphological similarity before (Schlauer 1996; Lowrie 2014: 700 & 796).

It is rather surprising that no quinone was found in *D. pulchella* and its hybrids before, as both **M** and **P** were clearly detected in the gemmae of all three accessions investigated in the present study. This widespread species is fairly isolated within *Drosera* sect. *Bryastrum* (Fleischmann, unpublished data) and none of the related taxa yielded any quinone so far. This likewise makes a hybrid origin of *D. pulchella* (that could be assumed from the chemotaxonomic pattern containing both quinones; see Schlauer and Fleischmann 2016) less probable.

The hypothesis that *D. depauperata* is conspecific with *D. pulchella* and just constitutes a depauperate form of it (Lowrie *et al.* 2017; Fleischmann *et al.* 2018) is not supported by the very different quinone content of both species, but it remains possible that the former is derived from a hybrid.

Drosera (nitidula subsp.) omissa is the only taxon in the D. nitidula group that contains quinones (predominantly M with traces of P) whatsoever. Only one accession was, however, investigated in the present study, and the group is remarkable for its well documented ability to hybridize even with phylogenetically more remote taxa (Lowrie 2014: 52-56), including the quinone-rich D. pulchella. Interestingly, Culham and Gornall (1994) did not detect quinones in any of the D. pulchella-hybrids investigated in their study. However, as pygmy Drosera were/are frequently misidentified in cultivation, also in botanical gardens and some carnivorous plant nurseries, any previously published data for this group from cultivated material (and without any voucher specimens) has to be taken with some care.

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