



Plant species with extremely small populations (PSESP) in China: A seed and spore biology perspective



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ABSTRACT

Approximately one fifth of the world's plants are at risk of extinction. Of these, a significant number exist as populations of few individuals, with limited distribution ranges and under enormous pressure due to habitat destruction. In China, these most-at-risk species are described as 'plant species with extremely small populations' (PSESP). Implementing conservation action for such listed species is urgent. Storing seeds is one of the main means of *ex situ* conservation for flowering plants. Spore storage could provide a simple and economical method for fern *ex situ* conservation. Seed and spore germination in nature is a critical step in species regeneration and thus *in situ* conservation. But what is known about the seed and spore biology (storage and germination) of at-risk species? We have used China's PSESP (the first group listing) as a case study to understand the gaps in knowledge on propagule biology of threatened plant species. We found that whilst germination information is available for 28 species (23% of PSESP), storage characteristics are only known for 8% of PSESP (10 species). Moreover, we estimate that 60% of the listed species may require cryopreservation for long-term storage. We conclude that comparative biology studies are urgently needed on the world's most threatened taxa so that conservation action can progress beyond species listing.

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1. Introduction

China is one of the richest countries in the world in terms of plant diversity. Third only to Brazil and Columbia, China harbours over 30,000 plant species (Yang et al., 2005). However, at least 200 plant species have become extinct in the last 50 years and c.5000 more are currently threatened or on the brink of extinction, making China one of the highest priorities for global biodiversity conservation (Volis, 2016).

Among this rich diversity of plants in China, there are 120 wild plant species that were identified in 2012 as the first group for urgent protection nationally. These species have the following features: 1) estimated to have <5000 mature individuals in the wild; 2) distribution restricted to a limited range with a few

locations; 3) recognition as national or regional endemic species of China; and 4) potential for economic development or scientific value. Reflecting these attributes, a descriptor is now used for this cluster of species: 'Plant Species with Extremely Small Populations (PSESP)' (Ma et al., 2013; Sun, 2013). As a result of a new policy framework, several national and regional-level conservation strategies and actions for conserving China's PSESP are being implemented. Such an approach is particularly important as a recent spatial distribution analysis of 33 species from the 120 PSESP list revealed that only 12 of these are considered to be well protected in the National Nature Reserves (Wang et al., 2016a,b). Importantly, significant progress has been made to increase the coverage of threatened species in China's botanic gardens. For example, Xishuangbanna Tropical Botanical Garden, Yunnan has an extensive collection of nationally red-listed species. Nonetheless, only around 60 PSESP (i.e., about half) have been propagated and cultivated *ex situ* in China's botanic gardens (Sun, W–B., pers comm.).

The global need for botanical gardens to protect threatened species, e.g., trees, in dedicated conservation collections is

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recognised (Cavender et al., 2015). The broad aim of establishing and managing such living *ex situ* collections should be to maintain the greatest biodiversity at the greatest economic and logistic efficiency (Cibrian-Jaramillo et al., 2013). Yet the problem of limited genetic diversity of individual species in these collections is well known. Part of the solution is to establish a combined management strategy for the acquisition of living collections among botanic gardens and other organizations interested in plant conservation (Cibrian-Jaramillo et al., 2013). In the case of the cycad *Zamia decumbens*, collections can better conserve the genetic diversity of *in situ* populations as long as multiple accession are made over more than one year (Griffith et al., 2015).

Conservation efforts with the world's most threatened species is also hindered by gaps in fundamental biological information, e.g., on trees (Cavender et al., 2015) and on their seed biology (Pritchard et al., 2014). Two biological traits are of particular importance when considering the efficient and effective utilisation of seeds and fern spores in both the *ex situ* and *in situ* environment: desiccation tolerance and dormancy/germination. Storing seeds is one of the main means of *ex situ* conservation and involves drying as the first step in the preservation process. Tolerance of drying opens up the opportunity for reduced temperature storage, which is the main means of conserving millions of accessions of plant genetic resources (<http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/sow2/en/>), and underpins the global market for seeds, which was valued at US\$53.76 billion in 2014 (<http://www.marketsandmarkets.com/Market-Reports/seed-market-126130457.html>). Fern spores are a ready source of germplasm to aid re-establishment of waning fern populations (Pennisi, 2010). Since they tolerate high levels of desiccation (Ballesteros, 2010), dry storage of fern spores could provide simple and economical *ex situ* conservation of genetic diversity in a relatively small space (Pence, 2008; Ballesteros, 2010; Ibars and Estrelles, 2012). Germination timing, which is often strongly dependent on temperature (for dormancy loss and germination speed), is a key stage in species regeneration *in situ* and in the assessment of seed viability in *ex situ* collections (Smith et al., 2003; Suo et al., 2015).

Here we review what is known about the seed and fern spore biology (storage and germination) of at-risk species, using China's PSESP as a case study, and highlight some areas of future research that need addressing.

2. Materials and methods

The list of PSESP in Ma et al. (2013) and Sun (2013) was consulted and the plant name and authority checked against The Plant List (<http://www.theplantlist.org/>). Data searches were run on the 2008 Seed Information Database (<http://data.kew.org/sid/>), using the terms 'seed storage' and 'seed germination.' Individual species records from the published literature were summarised when available, particularly to bring the analysis up to date. Otherwise a seed biology perspective is given on either the genus or family basis, including some perceived scientific challenges.

3. Results and discussion

The taxonomic spread of the 120 PSESP extends to 76 genera from 33 families (Table 1) of mainly seed plants. Ferns (*sensu lato*, i.e. monilophytes) are represented by just one family and species. The species are not evenly spread among seed-bearing families with significant clustering in the Orchidaceae (37 species) and Cycadaceae (11 species). These two over-represented groups contribute 40% of PSESP species and should provide an urgent focus for future seed biology studies.

Of the 120 PSESP, there is readily available information on seed germination for 28 species (23% of PSESP), but storage

characteristics are accessible for only 8% of PSESP (i.e., 10 species), for which germination protocols are also known. These 28 species are profiled below. Our interpretation of the published literature for the listed species, or close relatives, suggests that storage under international gene bank standards (i.e., drying to 15% RH, plus hermetic storage at -20°C) may only be realistic for 40% of the PSESP (i.e. 48 species). The other 72 species (60%) may have to be cryopreserved for long-term storage, as spores, seeds or embryonic axes (Li and Pritchard, 2009; Walters et al., 2013).

3.1. Ferns in PSESP

There is only one fern species in PSESP for China, *Cystoathyrium chinense* Ching. This species was believed to be extinct in the wild but was recently rediscovered (Wei and Zhang, 2014). It is a critically endangered species endemic to China with no more than 40 extant individuals, closely related to North American taxa of the genus *Cystopteris* (Wei and Zhang, 2014).

To our knowledge there is no published information on the spore biology of *C. chinense*. However, fern spore storage and germination have been well studied (reviewed in Ballesteros, 2010; Suo et al., 2015) including related species, such as *Cystopteris fragilis* (e.g. Ballesteros et al., 2012). In terms of storage and conservation, spores of *C. fragilis* died at room conditions within 3 years, but initial germination percentage and rate was preserved after cryogenic storage at -80 and -196°C . In general, fern spores appear to be tolerant to desiccation, showing increased longevity as storage temperature decreases (e.g. Quintanilla et al., 2002; Aragón and Pangua, 2004; Li et al., 2010; Ballesteros et al., 2011, 2012; Li and Shi, 2014). However faster than expected ageing of fern spores from some species at temperatures between -10°C and -30°C (Ballesteros, 2010, and references therein; Li et al., 2010; Ballesteros et al., 2012; Li and Shi, 2014) have led to the suggestion that fern spores may have a storage physiology similar to intermediate seeds (Pence, 2008; Ballesteros, 2010). Cryopreservation is feasible for many fern spores and may be necessary to maximize spore longevity for long-term *ex situ* conservation (Pence, 2008; Ballesteros, 2010; Li et al., 2010; Ballesteros et al., 2011, 2012; Li and Shi, 2014; Mikula et al., 2015).

Most fern spores germinate on diverse mineral culture medium at temperatures between 20 and 25°C , however optimal light intensity and illumination time for spore germination are different among species (Suo et al., 2015). For *C. fragilis* spores, germination is successful in mineral culture medium, at 20°C with a 12 h photoperiod (common fluorescent tubes, photon irradiance $25\text{--}50\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ in the $400\text{--}700\ \text{nm}$ region). Additionally, some spores may require specific metal ions, pH, or other specificities during the *in vitro* culture.

3.2. Over-represented groups in PSESP

There are two over-represented groups in PSESP for China constituting 40% of the species: cycads and orchids.

3.3. Cycads

Of the 26 gymnosperms (22% of species) listed, 11 species (9%) are from a single genus, *Cycas*, in the family Cycadaceae. Globally cycads (Cycadaceae, Stangeriaceae, Zamiaceae) are the most threatened group of plant species (Donaldson, 2003), with 196 out of 339 taxa (58%) recently listed by IUCN (IUCN, 2015). Cycads are also included in Appendix I or II of CITES (Rutherford et al., 2013).

There appears to be no published information on the seed biology of the 11 *Cycas* species on the China PSESP list, other than Jian et al. (2006) noting that all five populations of *Cycas fairylakea*

Table 1

List of the top 120 PSESP (119 higher plants and one fern) in China for which greater seed biology knowledge could arrest the threat of extinction. All species are in CITES Appendix I or II. **Species in bold may have particular storage challenges, e.g., needing cryopreservation (Li and Pritchard, 2009).** Two clusters of species are highlighted that constitute 40% of the species: orchids (white text on a black background) and cycads (black text on a grey background). The 28 species marked * have only germination information available; the 10 species marked *# have both germination and storage information, sometimes extracted from the Seed Information Database (<http://data.kew.org/sid/>). All 28 species marked in this way are described under 'Species seed biology profiles.'

	Species	Family (-aceae)	Order	Possible seed biology challenges?
1	<i>Abies beshanzuensis</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
2	<i>Abies yuanbaoshanensis</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
3	<i>Abies ziyuanensis</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
4	<i>Acer catalpifolium</i>	Sapind- (formerly Acer-)	Sapindales	Expected to be orthodox
5	<i>Acer miaotaiense</i>	Sapind- (formerly Acer-)	Sapindales	Expected to be orthodox
6	<i>Acer yangjuechi</i>	Sapind- (formerly Acer-)	Sapindales	Expected to be orthodox
7	<i>Annamocarya sinensis</i> (=Carya)	Jugland-	Fagales	Oilseed cold stress in dry state
8	<i>Berchemiella wilsonii</i>	Rhamn-	Rosales	Often orthodox seeded species in the family
9	<i>Betula halophila</i>	Betul-	Fagales	Seeds expected to be orthodox
10	<i>Bhesa sinensis</i>	Celastr-	Celastrales	? No information
11	<i>Bulbophyllum hainanense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
12	<i>Calanthe sieboldii</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
13	<i>Calycopteris floribunda</i>	Combret-	Myrtales	Often orthodox in family
14	<i>Camellia impressinervis</i>	The-	Ericales	Will require cryopreservation if like <i>Camellia sinensis</i>
15	<i>Camellia pingguoensis</i> var <i>terminalis</i>	The-	Ericales	Will require cryopreservation if like <i>Camellia sinensis</i>
16	<i>Camellia pubipetala</i>	The-	Ericales	Will require cryopreservation if like <i>Camellia sinensis</i>
17	<i>Camptotheca acumate</i>	Nyss-	Cornales	? No information
18	<i>Carpinus putoensis</i>	Betul-	Fagales	Expected to be orthodox
19	<i>Carpinus tientaiensis</i>	Betul-	Fagales	Expected to be orthodox
*	<i>Cathaya argyrophylla</i> (=Tsuga)	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
21	<i>Ceratostylis hainanensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
22	<i>Changiostyrax dolichocarpa</i>	Styrac-	Ericales	Often orthodox in family
23	<i>Chieniodendron hainanense</i>	Annon-	Magnoliales	Family tends to orthodox but some recalcitrant
24	<i>Coptis quinquesecta</i>	Ranuncul-	Ranunculales	Short lived?
25	<i>Craigia yunnanensis</i>	Tili-	Malvales	Often orthodox in family
26	<i>Cycas bifida</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
27	<i>Cycas changjiangensis</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
28	<i>Cycas debaoensis</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
29	<i>Cycas diannanensis</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
30	<i>Cycas dolichophylla</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
31	<i>Cycas fairylakea</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
32	<i>Cycas hongheensis</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
33	<i>Cycas multipinnata</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo

34	<i>Cycas shiwandashanica</i>	Cycad-	Cycadales	cryopreservation may be needed. Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
35	<i>Cycas szechuanensis</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
36	<i>Cycas taiwaniana</i>	Cycad-	Cycadales	Possible slow germination and limited desiccation tolerance; embryo cryopreservation may be needed.
37	<i>Cymbidium eburnum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
38	<i>Cymbidium insigne</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
39	<i>Cymbidium wenshanense</i>	Orchid-*	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
40	<i>Cypripedium forrestii</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
41	<i>Cypripedium lichiangense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
42	<i>Cypripedium margaritaceum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
43	<i>Cypripedium micranthum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
44	<i>Cypripedium palangshanense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
45	<i>Cypripedium singchii</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
46	<i>Cystoathyrium chinense</i>	Cystopterid-	Polypodiales	Spores likely intermediate: high lipid content, relatively short lived, anomalous storage at -20°C, cryopreservation may be required
47	<i>Dayaoshania cotinifolia</i>	Gesneri-	Laminales	Orthodox to uncertain in family
48	<i>Dendrobium changjiangense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
49	<i>Dendrobium hainanense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
50	<i>Dendrobium huoshanensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
51	<i>Dendrobium sinense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
52	<i>Dendrobium strongylanthum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
53	<i>Dipteronia dyerana</i>	Sapind-*	Sapindales	Expected to be orthodox
54	<i>Doritis pulcherrima</i>	Orchid-*	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
55	<i>Eria quinquelamellosa</i>	Orchid-#	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
56	<i>Eria tomentosa</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
57	<i>Erythropsis kwangsiensis</i> (= <i>Firmiana</i>)	Sterculi-	Malvales	Orthodox and recalcitrant in family
58	<i>Euryodendron excelsum</i>	The-	Ericales	Will require cryopreservation if like <i>Camellia sinensis</i>
59	<i>Firmiana danxiaensis</i>	Sterculi-	Malvales	Orthodox and recalcitrant in family
60	<i>Gastrochilus acinacifolius</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
61	<i>Gastrochilus rantabunensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
62	<i>Geodorum eulophioides</i>	Orchid-*	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
63	<i>Gleditsia japonica</i> var <i>velutina</i>	Fab-*	Fabales	Expected to be orthodox
64	<i>Glyptostrobus pensilis</i>	Taxodi-#	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
65	<i>Holcoglossum omeiense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
66	<i>Holcoglossum rupestre</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
67	<i>Hoepa chinensis</i>	Dipterocarp-	Malvales	Likely recalcitrant

*				
68	<i>Hopea hainanensis</i>	Dipterocarp-	Malvales	Likely recalcitrant
*#				
69	<i>Horsfieldia hainanensis</i>	Myristic-	Magnoliales	Species in family tend to be recalcitrant
70	<i>Horsfieldia tetratropala</i>	Myristic-	Magnoliales	Species in family tend to be recalcitrant
71	<i>Ilex kaushue</i>	Aquifoli-	Aquifoliales	Possible seed immaturity and dormancy; may have orthodox seeds
72	<i>Kmeria septentrionalis</i>	Magnoli-	Magnoliales	Species in family tend to orthodox
*				
73	<i>Lumnitzera littorea</i>	Combret-	Myrtales	Often orthodox seeded species in this family
74	<i>Madhuca pasquieri</i>	Sapot-	Ericales	Other <i>Madhuca</i> recalcitrant
75	<i>Magnolia zenii</i>	Magnoli-	Magnoliales	Species in genus tend to be orthodox
76	<i>Manglietia decidua</i>	Magnoli-	Magnoliales	Recalcitrant species known in genus
77	<i>Manglietiastrum sinicum (=Magnolia)</i>	Magnoli-	Magnoliales	Species in family tend to be orthodox
*				
78	<i>Metasequoia glyptostroboides</i>	Taxodi-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
*#				
79	<i>Michelia wilsonii</i>	Magnoli-	Magnoliales	Mainly uncertain in genus
80	<i>Mussaenda anomala</i>	Rubi-	Gentianales	Orthodox to uncertain in family
81	<i>Myristica yunnanensis</i>	Myristic-	Magnoliales	Species in genus tend to be recalcitrant
82	<i>Nothodoritis zhejiangensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
83	<i>Nyssa yunnanensis</i>	Nyss-	Cornales	Tend towards orthodox in this family
*				
84	<i>Ostrya rehderiana</i>	Betul-	Fagales	Expected to be orthodox
*#				
85	<i>Paphiopedilum armeniacum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
*				
86	<i>Paphiopedilum emersonii</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
87	<i>Paphiopedilum gratrixianum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
*				
88	<i>Paphiopedilum helenae</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
89	<i>Paphiopedilum spicerianum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
90	<i>Paphiopedilum tranlienicum</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
91	<i>Paphiopedilum wenshanense</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
92	<i>Paraisometrum mileense</i>	Gesneri-	Laminales	Orthodox to uncertain in family
*				
93	<i>Parakmeria omeiensis</i>	Magnoli-	Magnoliales	Species in family tend to be orthodox
*				
94	<i>Paranephelium hainanensis</i>	Sapind-	Sapindales	Many orthodox and recalcitrant species in the family
95	<i>Parrotia subaequalis</i>	Hamamelid-	Saxifragales	Family tends to have species with orthodox seeds
*				
96	<i>Petrocosmea qinlingensis</i>	Gesneri-	Laminales	Orthodox to uncertain in family
97	<i>Phaius hainanensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
98	<i>Phalaenopsis lobbii</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
99	<i>Pinus dabeshanensis</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
*				
100	<i>Pinus squamata</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
101	<i>Pinus wangii</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
102	<i>Primulina tabacum</i>	Gesneri-	Laminales	Orthodox to many uncertain in family
103	<i>Pseudotsuga xichangensis</i>	Pin-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
104	<i>Pterocarpus indicus</i>	Fab-	Fabales	Expected to be orthodox

*#				
105	<i>Pterosperma kingtungense</i>	Sterculi-	Malvales	Orthodox and recalcitrant in family
106	<i>Pyrus hopeiensis</i>	Ros-	Rosales	Expected to be orthodox
107	<i>Rhododendron protistum</i> var <i>giganteum</i>	Eric-	Ericales	Mainly orthodox in genus
*#				
108	<i>Sinojackia huangmeiensis</i>	Styrac-	Ericales	Often orthodox in family
109	<i>Sinojackia microcarpa</i>	Styrac-	Ericales	Often orthodox in family
110	<i>Sonneratia hainanensis</i>	Lythr- (formerly Sonnerati-)	Myrtales	Possible orthodox species in this genus
*#				
111	<i>Sunipa hainanensis</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
112	<i>Taihangia rupestris</i> var <i>cilata</i>	Ros-	Rosales	Species in family tend to be orthodox
113	<i>Taxus cuspidata</i>	Tax-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
*#				
114	<i>Taxus fauna</i>	Tax-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
115	<i>Thrixspermum fragrans</i>	Orchid-	Asparagales	Likely short-lived, including at -20°C, requiring cryopreservation and germination <i>in vitro</i>
116	<i>Thuja koraiensis</i>	Cuppress-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
117	<i>Thuja sutchuenensis</i>	Cuppress-	Coniferales	Fibrous coat slows germination; empty seed fraction. Expected to be orthodox.
118	<i>Tsoongiodendron odorum</i> (= <i>Michelia</i>)	Magnoli-	Magnoliales	Family tends to orthodox
119	<i>Ulmus elongata</i>	Ulm-	Rosales	Expected to be orthodox
120	<i>Vatica guangxiensis</i>	Dipterocarp-	Malvales	Likely recalcitrant

'have an opposite pyramid age structure, few coning plants, little seed production, and a low level of seed germination rate or sterility.' Across the genus as a whole, i.e., just over 100 species, there is seed biology information for only four species. On the basis of the seeds tolerating sun-drying (Langkamp and Plaisted, 1987), *Cycas angulata* R.Br., *Cycas armstrongii* Miq. and *Cycas media* R.Br. are proposed to be orthodox for desiccation tolerance (<http://data.kew.org/sid/>). In contrast, the storage response of large seeds (8.2 g per seed) of *Cycas revoluta* Thunb is described as being uncertain. The seed is not likely to be recalcitrant (i.e., highly desiccation sensitive) as Dehgan and Schutzman (1989) found that about half the seeds lost germinability when stored open at 22 °C, compared to >90% after 6 months air-dry storage at 2 °C. Our recent assessment indicated that commercially-available *C. revoluta* seeds showed some desiccation tolerance with viability being lost around 15% moisture content, and opportunities for cryopreserving the isolated embryo is now being considered (Nadarajan, J., Pritchard, H.W., pers comm).

Seeds of most cycads require several months to germinate (Dehgan and Schutzman, 1989). There are at least three features that could contribute to delayed germination: a fleshy sarcotesta; a thick and hard sclerotesta; and, in some species, an immature embryo at the time of seed abscission. Again for the 'model' cycad, *C. revoluta*, germination has been stimulated by mechanical removal of the sarcotesta, scarification of the sclerotesta with H₂SO₄ and seed treatment with GA₃ as a means of reducing any physiological dormancy has also been attempted with varying success (Dehgan and Schutzman, 1989).

Although studies mainly on *C. revoluta* are informing future options for the handling of cycad seed and pollen, there is clearly a requirement for investigations on the seed storage and germination biology of PSESP cycads and other species in this important group of ancient plants.

3.4. Orchids

Seed storage can be challenging for some species of orchids. For the >90 orchid species' (and/or hybrids) seed storage responses

studied so far, c. 69%, 9% and 22% have been assigned, respectively, as orthodox, uncertain and intermediate, i.e., partially desiccation tolerant, but with sub-zero temperature sensitivity that appears to be species-specific (so-called Type II species (Pritchard, 2004)). Thus the challenge is not so much in relation to tolerance of drying (there are no known recalcitrant seeded orchids, to date) but with regards to storage lifespan. Since the first comparative assessments of dry seed storage noted that orchid seeds often have lifespans around 10% of the longest lived seeds under the same conditions (Pritchard et al., 1999; Pritchard and Dickie, 2003), confirmatory results have been presented on another >10 species in the family (Hay et al., 2010; Seaton et al., 2013). Overall, these studies indicate that about a third of the tested orchid species have seeds that undergo precipitous losses in germinability over relatively short times in dry, cold storage, a response that might relate to the physical transformation of the seed lipids (e.g. *Cattleya aurantiaca*; Pritchard and Seaton, 1993). However, cryopreservation of mature orchid seeds has been successful for a wide range of terrestrial and tropical species; 36 and 21 species respectively (see a listing for Popova et al., 2016). Such studies are limited usually to storage times of ≤2 years. Only longer-term studies at a range of seed moisture contents and storage temperatures will provide an adequate prospect for species *ex situ* conservation in this important and extremely diverse family (>25,000 species).

As the seeds are extremely small (dust-like and often weighing c. 3 µg), orchid seeds in nature rely on a symbiotic host to provide an external source of carbon for the completion of germination, protocorm formation and seedling growth (Arditti, 1992). This nutrient requirement can be replicated *in vitro* on asymbiotic media, with optimal composition varying with species. Readers are referred to Arditti (1984) as a starting point.

3.5. Species seed biology profiles

1) *Abies yuanbaoshanensis* Y.J.Lu & L.K.Fu (Pinaceae)

The natural regeneration ability of this species is poor, with variable cone production per plant, number of seeds per fruit, and seed

quality (Tang et al., 2001). Seeds absorb water readily and full seeds germinate better in the incubator (19%) than in the nursery/field garden (7%). Such low seed germination may make any assisted expansion of the population difficult and hinder natural regeneration. Seed storage information is not available.

2) *Acer catalpifolium* Rehder (Sapindaceae)

Seeds collected from two locations vary in seed mass from an average of 19–28 mg (Yu et al., 2008). Seeds readily absorb water (imbibe), and can germinate at 25 °C to 42–49%, depending on location. Germination is equally good in the light or the dark. A seed storage assessment is needed on this species, as variable levels of desiccation tolerance is known in species of this genus.

3) *Berchemiella wilsonii* (C.K.Schneid.) Nakai var *pubipetiolata* H. Qian (Rhamnaceae)

Seed quality in this species can be low, based on only 32% of seeds becoming red after tetrazolium chloride (viability) staining (Dang et al., 2005). Forty percent of full seeds did not stain and the rest (28%) were found to be empty. Treatment with 98% sulphuric acid increases the rate and total uptake of water, indicating some barrier to permeability. Germination inhibitors are present in the embryo and the pericarp, and the highest germination (14%) is achieved by soaking the seeds for 24 h in 300 mg⁻¹ GA₃ before sowing for germination. A seed storage assessment is yet to be made.

4) *Calanthe sieboldii* Decne. ex Regel (Orchidaceae)

For storage, information on this species is limited to the hybrid *Calanthe discolor* × *sieboldii* and it seems likely that this will not affect greatly the interpretation. The seed storage behaviour is thought to be intermediate, meaning that the seeds tolerate at least air-drying, but then store poorly at conventional seed bank temperature (i.e., -20 °C). This was the case for *C. discolor* × *sieboldii* seed as air-dry storage over a one year period at both 20 °C and -15 to -17 °C resulted in a germination decrease from 72% to c. 20%; non-dried seed failed to germinate after about 8 months storage (Hasegawa et al., 1978).

Immature seeds of *C. sieboldii* from unripe capsules at 80 days post pollination (compared to 120 DPP for full maturity) can be germinated on sterilized media (Park et al., 2000). Capsules can be surface-sterilized in 2% sodium hypochlorite for 20 min, washed several times with sterile, distilled water, and the capsules cut open and the seeds removed. The most appropriate medium for germination is modified Murashige and Skoog (MSH) with seed encapsulated in calcium alginate beads before incubation in the dark, resulting in 18% germination and protocorm formation in 8 weeks. Adding 1 mg l⁻¹ putrescine to the medium increases germination and protocorm formation in the dark to c. 44%. Supplementing MSH medium with 25 mg l⁻¹ adenine sulphate increases germination and protocorm formation to 66%. Generally, germination was lower in the light (16 h photoperiod) than the dark (Park et al., 2000). However, for seeds of *C. discolor* × *sieboldii* isolated from capsules nearly 6 months after pollination, germination on a Hyponex-Bactopeptone medium reaches 72% at 25 °C on a 16 h photoperiod (Hasegawa et al., 1978). The physiological needs for *in vitro* germination rates for terrestrial orchids vary among species and response levels can be low, possibly as a result of seed dormancy (Rasmussen, 1996).

5) *Cathaya argyrophylla* Chun & Kuang (Pinaceae)

The mean seed weight is 19.7 mg, but varies from 12 to 28 mg with mother trees (Xie and Li, 2000). Seeds stained with

tetrazolium chloride indicate 50% viability (Cao et al., 2010), with about 16% empty seed fraction (Xie and Li, 2000). But to achieve this level of germination, seeds stored moist at 4 °C for 30 days require soaking for 24 h in 100 mg⁻¹ of NAA or IAA, yielding 55% germination. Moist storage at 4 °C over a longer period of 90 days results in decreasing germination, accompanied by a fall in enzyme activity, e.g. superoxide dismutase (SOD). Nonetheless, refrigerated storage of moisture seed has been recommended for these seeds. The tolerance of the seeds to drying is not clear, although seeds at 11% moisture content retain 50% viability.

6) *Cycas debaoensis* Y.C.Zhong & C.J.Chen (Cycadaceae)

Seeds of *C. debaoensis* can germinate between temperatures of 20 and 35 °C, with 87–93% germinating at 25–30 °C (Wang et al., 2014). Peeled seeds have a higher germination potential, and overall the germination rate is quick, suggesting that germination factors are not the main cause of endangerment in this species. Seed storage information appears not to be available.

7) *Cymbidium wenshanense* YS Wu & FY Liu (Orchidaceae)

The *in vitro* germination of young seed of this orchid is low, but prominently higher when seed is harvested from 6 month old capsules (Ding et al., 2005). Although seeds can germinate on a basic nutrient medium, adding coconut milk stimulates germination. Plantlets development is relatively slow, taking 7 months from seed sowing. A seed storage assessment is yet to be made.

8) *Dipteronia dyeriana* Henry (Sapindaceae)

With the testa removed, germination at 6 °C reaches 98% in 5 months, perhaps indicative of the presence of some physiological dormancy. Seeds sown in the wild germinate to 17% after the first winter, increasing to 58% after two winters and one summer when kept in pots. Warmer (15–30 °C) temperatures result in no germination and there is no light requirement for germination. Based on tetrazolium chloride (vital) staining, seed tolerates storage at 4 °C for 510 days with 96% vitality (Ouyang et al., 2006), but a full assessment of seed storage response is needed.

9) *Doritis pulcherrima* Lindl. (Orchidaceae)

The seed storage behaviour for this species is thought to be intermediate, as <20% germination was observed after 2 months hermetic storage at -10 °C (Bowling and Thompson, 1972). The seeds of 3-month-old pods can, however, tolerate cryopreservation by plunging into liquid nitrogen (Thammasiri, 2000). After chemical dehydration in plant vitrification solution 2 (PVS2) at 25 °C for 50 min, cryopreservation and rapid warming, 62% of seeds can develop into normal seedlings. Germination is achieved on Vacin and Went medium. Similarly, *D. pulcherrima* seeds 90 days after pollination containing 95% globular embryos germinate to 90% on asymbiotic medium (Wu et al., 2005). Adding the phytohormones NAA (0.5 mg l⁻¹) greatly improve germination. Glutamine (400 mg l⁻¹) supports protocorm growth and peptone (2000 mg l⁻¹) promotes seedlings formation (Wu et al., 2005).

10) *Geodorum eulophioides* (Lam.) Schltr. (Orchidaceae)

The optimal cultural medium for seed germination of *G. eulophioides* is 1.0 g l⁻¹ Hyponex1 + 1 g l⁻¹ Hyponex2 + 1 mg l⁻¹ 6-benzylaminopurine + 0.2 mg l⁻¹ zeatin + 100 mg l⁻¹ coconut milk + 20 g l⁻¹ sucrose + 1 g l⁻¹ activated charcoal (Lan et al., 2014). Optimal culture media have also been devised for protocorm

proliferation and plantlet rooting, providing a rapid propagation technology for this endangered species. Nothing is known about seed storage in this species.

11) *Gleditsia japonica* Miq.¹ (Fabaceae).

As with the majority of Fabaceae species' seeds, those of *G. japonica* tolerate drying at 15% RH and 15 °C and storage at c. –20 °C, with survival recorded after at least four years in the Royal Botanic Gardens, Kew, Millennium Seed Bank (<http://data.kew.org/sid/>). Since the seed coat of dried *G. japonica* is extremely hard, few animal species are thought able to feed on it. However, observations in Japan have identified two specialist seed predators in the field, which cause physical damage to >99% of the seeds (Takakura, 2002). Indeed, all germinating seeds found in the field contained bean weevil larvae, indicating that the physical damage facilitated germination. Germination at 25 °C (16 h photoperiod) can be enabled through artificially damaging the seeds by drilling a hole c. 1 mm in diameter in the seed coats, subsequently resulting in c. 80% germination (Takakura, 2002). Seeds can also be germinated by chipping with a scalpel (scarifying) and sowing on 1% agar-water at 21 °C (12 h photoperiod), resulting in 86% germination (<http://data.kew.org/sid/>). The hard seed coat can also be scarified to enable water uptake, and germination, using hot water (75 °C) for 10 min or concentrated sulphuric acid for 60 min (Liu, 2012).

12) *Hopea chinensis* (Merr.) Hand.-Mazz. (Dipterocarpaceae)

The viable seed rain from *H. chinensis* is 51%; the remaining seeds suffering from necrosis (36%), damage by insect larvae (10%) or immaturity (3%) (Tang et al., 2009). No seed dormancy is evident, germination reaches 94%, with larger seeds germinating better. Nothing is known about seed storage in this species, although other species in the genus tend to be highly desiccation sensitive (recalcitrant).

13) *Hopea hainanensis* Merr. & Chun (Dipterocarpaceae)

Seeds of this species take about 170 days from anthesis to maturity (Lan et al., 2007), at which point the seed is c. 34% moisture content, has a mean seed weight of 0.97 ± 0.20 g and an initial germination of 97% (Wang et al., 2004). Seed germination is efficient at 15–20 °C, and does not require light as there is no obvious difference in germination between seeds sown in full darkness and under light (2800 lx, 14 h photoperiod) (Wen et al., 2002). Although viability measurements and electrical conductivity analyses after drying treatment show gradually increasing desiccation tolerance of seeds/embryonic axes from 122 days after anthesis (Lan et al., 2007), these changes are relative and the seed retains a high level of desiccation sensitivity. Seeds held under room conditions in South Yunnan lose germination ability completely within 3 weeks (Wen et al., 2002). Moreover, seeds are inviable on drying to 20% MC (Song et al., 1984, 1986), with a mid-point moisture content for viability loss of 23–25% (Wang et al., 2004). Moist storage of the seeds (fruits) is possible for many months, but is temperature dependent. At best, 80% germination is possible after 1 year's moist storage (35–38% MC) in coconut dust at 18 °C (Song et al., 1984, 1986), or about 30% germination after 9 months at 15 °C and c. 34% MC (Wang et al., 2004). The period for viability to fall to 50% (P_{50}) is c. 7, 4 and 3 months for the wet

storage of seeds (fruits) at 15 °C, 5 °C and 25 °C, respectively (Wang et al., 2004). Such moist storage lifespan is quite similar to the recalcitrant seeds of other species in the Dipterocarpaceae, e.g., 3–5 months at 16 °C for *Hopea odorata*, *Neobalanocarpus heimii*, *Shorea assamica*, *Shorea henryana*, *Shorea leprosula*, *Shorea macroptera*, *Shorea roxburghii* and *Vatica astrotricha* (Pritchard et al., 2004).

14) *Kmeria septentrionalis* Dandy (Magnoliaceae)

The red aril may inhibit seed germination and should be rubbed off before sowing (Lai et al., 2007). Seeds germinate well in sand. Cold storage in wet sand for 130 days results in 59% of seed being able to germinate; but placing seeds at room temperature with dry sand decreases germination to 12% (Wang et al., 2012). A seed storage assessment is yet to be made.

15) *Manglietiastrum sinicum* Y.W.Law [is a synonym of *Magnolia sinica* (Y.W.Law) Noot.] (Magnoliaceae)

Seeds of this species are dormant (Zheng and Sun, 2009). Pre-treating seeds with 500 mg l⁻¹ GA₃ seeds for 24 h results in 66% germination subsequently at 25 °C (12 h photoperiod); fluorescent lights providing 25 mmol m² s⁻¹). Moist chilling at 4 °C for 3 weeks is also effective at breaking seed dormancy, resulting in 56% seed germination after 30 days at 25 °C. A seed storage assessment is needed on this species.

16) *Metasequoia glyptostroboides* Hu & W.C.Cheng (Cupressaceae)

The storage behaviour of this species is suggested to be intermediate (<http://data.kew.org/sid/>), with Gordon (1992) advising maximum storage for c. 3 years at c.10% moisture content and –10 °C. Xin et al. (2004) stored seeds at –20 °C before some germination experiments. The seeds seem quite robust in storage, with viability maintained for 2 years in hermetic storage at room temperature (Campbell, 1980) and for >2 years in hermetic air-dry storage at 3 °C (Gordon, 1992). Dirr and Heuser (1987) recommend hermetic air-dry storage at cool temperatures (c. 5 °C).

Seeds of this species have a thin and fragile coat, and seeds can be of poor quality with over 90% empty seed fraction (Xin et al., 2004). For germination, pre-treatment at c. 4 °C for 6 weeks is recommended (Gosling, 2007). Based on a temperature gradient study, the optimum temperature for germination is c. 24 °C, with good germination between 19 °C and 28 °C and no germination below 10 °C (Xin et al., 2004). Seed germination can be greatly inhibited by light [65 μmol m² s⁻¹ (12 h photoperiod)], suggesting that the seeds prefer dark conditions (Xin et al., 2004). Also seed mass and germination rate of restored populations can be significantly lower than those in natural populations, indicating decreased regeneration ability in the restored populations, possibly due to inbreeding depression (Li et al., 2012a).

17) *Nyssa yunnanensis* W.Q.Yin ex H.N.Qin & Phengklai (Nyssaceae)

The optimum seed germination temperature is 25 °C, yielding 68% germination (Yuan et al., 2013). When the light conditions are changed from continuous to dark, germination is reduced to 20%. Before sowing seeds are disinfected with 30% H₂O₂ for c. 6 min and rinsed in distilled water four times. This is potentially a seed dormancy breaking treatment and soaking seeds in 200 mg l⁻¹ GA₃ is known to improve germination performance (Yuan et al., 2013). But a full characterisation of seed dormancy in this species is needed, as is an assessment of seed storage capability.

¹ The assessment is at the species level, rather than at the variety level for 'velutina' that is specified in the PSESP list.

18) *Ostrya rehderiana* Chun (Betulaceae)

The storage behaviour of this species is uncertain. This is based on the evidence that the seed lifespan is short, with germination falling from 16 to 5% after open storage at room temperature from collection through to the following spring (Zhang et al., 1988). As open storage would have likely resulted in considerable drying of the seeds and some germination was still possible after many months, the seeds are not likely to be recalcitrant (desiccation sensitive). Some seeds of *Ostrya carpinifolia* germinated after 13 years air-dry storage at 40% r. h. and 4 °C (Wildrechner, 1990), and appear to be orthodox in storage behaviour. It is recommended that *Ostrya virginiana* seeds are dried before storage (Schopmeyer and Leak, 1974). The prospects are, therefore, that seeds of *O. rehderiana* may be storable.

Fruits of this species ripen in September–October after a development period about 160–180 days (Zhang et al., 1990), but the seed produced is of inferior viability with only about 50% sound seed (Zhang et al., 1988). Seeds imbibe efficiently over 4 h and appear not to have physical dormancy (Le et al., 2013). However, in the wild the seeds germinate in April (Zhang et al., 1990), suggesting that the seed might benefit from cold stratification over winter. However, longer-term (16 weeks) low temperature treatment reduces cumulative germination, indicating the potential for dormancy re-enforcement (Le et al., 2013). Relatively long times (c. 50 days) at 15 °C are needed to break dormancy, and seeds can be treated with 400 or 600 mg l⁻¹ GA before transfer to an alternating temperature regime of 15/25 °C (Le et al., 2013). Seeds have also been germinated in common gardens and successfully transplanted to the field (Li et al., 2012b).

19) *Paphiopedilum armeniacum* S.C.Chen & F.Y.Liu (Orchidaceae)

Seed extracted from capsules harvested 120 days after pollination can be germinated *in vitro* on R medium (see Ardtiii, 1982) reaching 25% (Chen et al., 2004). The preferred conditions are 3 weeks in the dark at 25 °C, followed by transfer to the light (12 h/d). Coconut milk stimulates germination and activated charcoal (2 g l⁻¹) and banana homogenate (100 g l⁻¹) benefit the growth and development of seedlings (Chen et al., 2004). Black seeds removed from mature capsules at 180 days after pollination do not germinate. This suggests the late onset of seed dormancy, which is known in other species in this genus. Nothing is known about seed storage in this species.

20) *Paphiopedilum gratixianum* Rolfe (Orchidaceae)

Seed extracted from capsules developing for 210 days have the highest *in vitro* germination level (93%) at 25 °C in the light (16 h/d) on ½ Robert Ernst (RE) medium + NAA 0.5 g l⁻¹ + BA 0.2 mg l⁻¹ + coconut milk 50 ml l⁻¹ + casein hydrolysate 1 g l⁻¹ (Zhou et al., 2012). In contrast, seeds extracted from capsules after 300 days of development only germinate to 26%, indicating the possible onset of seed dormancy. There appears to be no information about seed storage in this species.

21) *Paraisometrum mileense* W.T. Wang (Gesneriaceae)

Freshly matured seeds germinate readily in the light (12 h/d) on 1% agar-water at constant temperatures of 20 °C and 25 °C (100%) and at alternating temperatures (night/day) of 15/25 °C (97%) and 20/30 °C (100%) (Liu et al., 2015). The fastest germination is at 25 °C, with a mean germination time of c. 8 days. As the seeds benefitted from cold stratification, enabling subsequent germination over a

wider range of temperatures, the seeds are classified as having non-deep physiological dormancy (Liu et al., 2015). A seed storage assessment is yet to be made.

22) *Parakmeria omeiensis* W.C.Cheng [is a synonym of *Magnolia omeiensis* (W.C.Cheng) Dandy] (Magnoliaceae).

Seed are contained in a fleshy, red aril which is removed by soaking in water for 1 day, followed by scrubbing off. The slightly flattened, oval seeds germinate to a low level *in vitro*. Seeds germinate when sown on Murashige and Skoog medium at 25 °C, and kept in the dark first followed by transfer to light (14/d; 2000–2500 lx) (Yu et al., 2011). An assessment of seed storage capability is required.

23) *Parrotia subaequalis* (Hung T. Chang) R.M. Hao & H.T. Wei (Hamamelidaceae)

The long spindle-shaped seeds weighing 46 mg each can germinate to 74% at 20–25 °C on sand after pre-treatment with 600 mg l⁻¹ GA₃ (Hu et al., 2012). Germination reaches 48–60% under a range of lighting conditions (total light, semi-light, and dark). The seeds for germination testing can be air-dried and held at either 3–5 °C or room temperature. After 2 years storage at room temperature germination is c. 12%. A full assessment of seed storage capability is now needed.

24) *Pinus dabeshanensis* W.C.Cheng & Y.W.Law [is a synonym of *Pinus armandii* var. *dabeshanensis* (W.C.Cheng & Y.W.Law) Silba] (Pinaceae).

Seeds of this species are large, weighing about 313 mg each. Removing the thick epispem benefits germination, as does immersing seeds in 0.2 mg l⁻¹ GA₃ for 12 h (Han et al., 2014). There appears to be no published information on the seed storage of this species.

25) *Pterocarpus indicus* Willd. (Fabaceae)

Seeds of *P. indicus* are 6–8 mm long and weigh 422 mg on average (Jøker, 2000). The seed storage behaviour is thought to be orthodox, as seeds are known to tolerate desiccation to 4% MC. Also long-term storage in liquid nitrogen seems feasible as seeds dried to 4–6% MC, or with excised embryos at 5% MC, had good recovery after cryopreservation (Krishnapillay et al., 1994). The bean-shaped seeds have a brown papery testa and do not appear to be physiologically dormant. However, the pericarp can pose a physical restraint to germination and so the seeds are extracted and sown directly in pots or in germination beds. Germination can start within about a week and seedling establishment might take up to about 3 months (Jøker, 2000). As many fruits are empty, the germination count for non-extracted seeds can be low. Therefore, it is recommended that a cut test is applied to a representative sample of fruits to reveal how many seeds there are per fruit (possibly 0, but usually 1–2).

26) *Rhododendron protistum* var. *giganteum* (Forrest ex Tagg) D.F. Chamb. (Ericaceae)

When seeds are air-dried to c. 40% RH and stored in sealed polyethylene bags at 4 °C for 360 days, germinability is still 70%. Comparable data for storage at room temperature is 59%. As for the seeds of other *Rhododendron* species, the seed is desiccation

tolerant and seed of *R. protistum* var. *giganteum* appears to be relatively long-lived (Shen et al., 2015).

Seed germination is highest (c. 80%; mean time to germinate around 10 days) at 15 °C and 20 °C in the light (14 h/d), a response that can be replicated in the nursery by sowing the seeds on the soil surface. There is a requirement for light, as sowing in the dark (24 h) results in only 2% germination (Shen et al., 2015).

27) *Sonneratia* × *hainanensis* W.C. Ko, E.Y. Chen & W.Y. Chen (Lythraceae)

Seeds of this species do not germinate in the dark. When incubated at 28/23 °C alternating temperature, seeds germinate to 69% when the photoperiod is 12 h/d, but longer photoperiods reduce germination, indicating the possibility of a high irradiance reaction (HIR). Germination reaches about 90% at temperatures from 20 °C to 40 °C, with the optimum being 35 °C (Zhang et al., 2012). Nothing appears to have been published on seed storage for this species.

28) *Taxus cuspidata* Sieb. et Zucc. (Taxaceae)

The embryo of *T. cuspidata* seed collected in autumn is very small (Qin et al., 2014). Nonetheless, the seed storage behaviour of this species is highly likely to be orthodox, as seed viability has been maintained for 5–6 years in hermetic air-dry storage at c. 3 °C (Schopmeyer, 1974).

The seed has a deep dormancy that can last for 2–3 years (Qin et al., 2014) and needs post-maturation (Cheng et al., 2004). Air-dry seeds contain ABA and have a hard coat, preventing water and gas exchange and imposing dormancy on the young embryos. Dormancy relief can be achieved by stratifying seeds at different temperatures: 15–20 °C for 4–6 months, to facilitate embryo development, followed by 3–5 °C for 3–4 months, to shorten the dormancy period. For example, a two-step treatment of 20 °C/10 °C (day/night) followed by 4 °C cold (Liao et al., 2010) enables 15% germination and is better than following warm (20 °C) – cold (4 °C) [WC] and cold (10 °C) – cold (4 °C) [CC] treatments (Liao et al., 2010). Other methods to stimulate germination include the soaking of seeds in a saturated solution of sodium carbonate for 20–30 min, grinding part of the seed coat with coarse river sand, and using a solution of 1.80 mg l⁻¹ 6-BA, 2.20 mg l⁻¹ GA₃ or 0.30 mg l⁻¹ 2,4-D for 36 h. The seeds can be incubated for 155 d at an alternating temperature of 23/15 °C (10 h day/14 h night), to enable the full differentiation of the embryo and germination to 95% (Gu et al., 2015).

4. Conclusion

A strong case has been made for the conservation status of the remaining 94% of the world's plant species to be evaluated (Corlett, 2016). Yet, any delay in assessing how to handle the seeds or spores of vascular plants increases the risk of species loss. Based on our analysis for China's PSESP (the first identified group) moving beyond species listing to improve both conservation and sustainable use options will require a significant effort to characterise the seed and spore biology of wild species. There has been a concerted effort recently to enhance the populations of six PSESP through several successful reintroduction programmes, including on *M. sinicum*, *Acer yangbiense*, *Quercus* (Cyclobalanopsis) *sichourensis*, *P. armeniacum*, *P. mileense*, *D. pulcherrima*, *C. debaoensis*, *Euryodendron excelsum* and *N. yunnanensis* (Ren et al., 2012; Sun, 2013; Sun et al., 2016; Wang et al., 2016a,b). Similar, and other, species-based studies in the future should ensure that a full assessment of the

seed and spore biology of the species is carried out as a matter of urgency.

It is highly likely that the gaps in knowledge for China's PSESP is replicated for other groups of challenging species, for example, the so-called 'exceptional' species that either produce recalcitrant seeds or few or no seeds (Pence, 2013a). These require the combined application of *in vitro* conservation and propagation, for reintroduction and research when traditional propagation methods are not adequate, and tissue cryopreservation (Pence, 2013b). Such methods in cryobiotechnology are known for a large range of species, e.g. orchids (Popova et al., 2016), and there have been recent innovations in the delivery of cryoprotectants into seed tissues (Nadarajan and Pritchard, 2014). Currently, research and training in this area of science are decentralised and a more co-ordinated effort (human and financial resources) to conserve *ex situ* threatened species of the tropics is recommended.

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