https://doi.org/10.26525/jtfs2021.33.3.356

FOREST DIVERSITY AND VITALITY OF THE IMPORTANT RELICT AND ENDANGERED FERN SPECIES, *BRAINEA INSIGNIS* IN CHINA: IMPLICATIONS FOR CONSERVATION

Sun L[1, 2], Zhang Y[3], Lee SY[2], Cui Y[2], Liang P[3] & Liao W[2, *]

[1]*Guangdong Polytechnic of Science and Trade, Guangzhou, 510430, Guangdong, China*
[2]*State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, Guangdong, China*
[3]*Shenzhen Wildlife Conservation Division, Shenzhen, 518048, Guangdong, China*

**lsslwb@mail.sysu.edu.cn*

Submitted September 2020; accepted November 2020

Brainea insignis is the only species in the genus *Brainea*. It is widely distributed in the Asia and Australia continents, but usually in small, fragmented populations. Owing to its sensitivity towards habitat changes and human disturbance, the population of this fern is deteriorating. At present, it is classified as an endangered species in China. In this study, we assessed the forest diversity and vitality status of *B. insignis* in its natural habitat. A total of 63 vascular plant species belonging to 55 genera of 40 families were recorded in the 1200-m^2 study plot. The Raunkiaer's frequency index indicated that *B. insignis* was experiencing a decline in stand size and structure although the overall level of vitality was 18.58. One-way ANOVA revealed that significant differences were detected between the basal diameter, tree height, crown size, and number of leaves of *B. insignis* in the study plot, but not between tree height and number of leaves. Proper conservation management is requisite to ensure continuous survival of this relict species in the wild despite eqinterference from evolution and urbanisation from mankind.

Keywords: Diversity index, dominant species, medicinal herb, Mount Tiantou, monotypic genus

INTRODUCTION

Brainea insignis, also known as the cycad fern, is a tropical woodwardioid fern species belonging to the family of Blechnaceae. Although fossil discovery on woodwardioid ferns are scarce (Cranfill & Kato 2003), members of the genus *Brainea* are speculated to be present during the Devonian of Paleozoic and flourished during the Jurassic of Mesozoic (Wang et al. 2003). However, according to the Pteridophyte Phylogeny Group (PPG I 2016), the genus has been reduced to only one species, and the sole surviving species, *B. insignis*, was reported to thrive in the Tertiary period (Gasper et al. 2016). Today, *B. insignis* can be found growing on damp and exposed hillsides with high light availability and low soil water content, at altitudes ranging from 300 to 1700 m (Wang et al. 2013), in the southern region of China as well as widely distributed in the Asia and Australia continents. The species highly resembles *Cycas* species of the Cycadaceae in general appearance and habitat, and can be distinguished by major characteristics of its reproductive parts (Liu et al. 2017). Needless to say, studies on *B. insignis* are scarce but emerging over time. Recently, development of expressed sequence tag-simple sequence repeat (EST-SSR) markers has aided genetic structuring diversity of natural *B. insignis* populations (Liu et al. 2017) and the characterisation of the complete *B. insignis* chloroplast genome has provided insights to the phylogenetic relationship of this relict species at the genome-scale level (Yu et al. 2020). Beyond that, information such as its population structure and regeneration of this species is scarce. Being a species that was listed as a protected species of Category II in China (Order of the Forestry Bureau and Ministry of Agriculture of China 1991) as well as a near-threatened species in India (Fraser-Jenkins 2012), indicates that this species requires much attention in terms of its survival and conservation status in the wild.

In order to develop effective conservation strategies, basic information such as the forest community and species diversity could provide insights to evaluating the local threatened status of a fern species in its habitat (Balkrishna et al. 2020). Stability in the ecosystem is considered a key factor to a healthy forest stand, whereas for an endangered species, the regeneration behaviour of the species in a forest can be indicated by the presence of sufficient number of individuals from different age structure in a given population (Balkrishna et al. 2020). Considering that the stand structure of populations provides an indication of their regeneration process, we assessed the forest community characteristics and population structure of natural *B. insignis* in Mount Tiantou Nature Reserve (MTNR). MTNR is home to 1289 vascular plant species belonging to 699 genera and 191 families (Zhao et al. 2016). The nature reserve is also home to 44 protected species listed in the International Union for Conservation of Nature (IUCN) Red list and when compared with the forest area adjacent to MTNR, the preservation of biodiversity in MTNR is relatively better due to strict forest monitoring by local enforcement agencies. In this study, we aimed to obtain the vitality level of *B. insignis* in our study area and compared our data with a preliminary report conducted on the *B. insignis* forest community in MTNR in 2010 (Xu et al. 2010), as well as other similar assessments conducted previously in other forest stands in China. To provide a basic understanding of *B. insignis*, we reviewed local knowledge and conservation status of *B. insignis* in China. Recommendations for conservation management of *B. insignis* were also discussed.

MATERIALS AND METHODS

The study site is located in Jinguicun (22° 38′–22° 43′ N, 114° 22′–114° 28′ E), a low-populated village located at the eastern border of the MTNR in the Pingshan County, Shenzhen. The 20-km^2 nature reserve consists of several hilly peaks with a maximum elevation of 683 m, while the elevation range of the *B. insignis* populations extends 200–300 m above sea level. The area is covered with acidic red and yellow soil content as well as volcanic and granite rocks, while on the surface, it is dominated by secondary evergreen broad-leaved forests. The study area is characterised as subtropical to tropical humid climate which recorded an annual average temperature of 22.4 °C, and could reach the highest recorded temperature of 36.6 °C during warmer months and the lowest recorded temperature of 1.4 °C during the cold months. The annual relative humidity is 80% and the annual precipitation is 1933 mm over 140 days. Jinguicun has a hiking trail access into MTNR. As a natural habitat to *B. insignis* that is affected by human disturbance, the study site is suitable in providing an understanding to the survival of *B. insignis* in the wild.

To assess *B. insignis* at the study site, we established a plot of 1600 m^2 in MTNR. The plot was divided into 16 smaller quadrats (10 m × 10 m), randomly selected and were widely separated from each other at least 100 m apart. Species names, diameter breast height (DBH), height, and crown width were recorded for all vascular plants with DBH ≥ 1.5 cm and height ≥ 1.5 m. The number of individuals and the area coverage of undergrowth shrubs, herbs, and interlaminar lianas in a 2 m × 2 m quadrat within each 10 m × 10 m quadrat were also recorded.

Individuals with DBH of ≥ 2.5 cm were considered adults, while those that had DBH of < 2.5 cm and height of ≥ 33 cm were considered saplings. However, saplings that were < 33 cm were regarded as seedlings. Five different frequency ranks, namely, A, B, C, D and E were specified based on the range of percentage of 0–19.9, 20–39.9, 40–59.9, 60–79.9 and 80–100 respectively (Wang et al. 1996). The importance value index (IVI) (Curtis & McIntosh 1951) for each species in the plot was calculated and the dominant species were selected according to their value of importance. The formula is as follows:

$$\text{IVI} = (\text{Relative frequency} + \text{relative abundance} + \text{relative prominence})/3 \quad (1)$$

The relative prominence for tree species was based on the basal area at breast height, while for shrubs and herbs, on their percentage of coverage. Based on the Raunkiaer's frequency index, the stand classification of the dominant species was analysed for tree and shrub layers across five tree classes, i.e. class I = seedlings, class II = saplings, class III = trees that had DBH < 7.5 cm, class IV = trees that had DBH ranging from ≥ 7.5 cm, but were < 22.5 cm, and class V = trees that had DBH ≥ 22.5 cm (Wang et al. 1996).

In order to compare the results of this study with those obtained by Xu et al. (2010), species diversity within the study plots was calculated using Shannon-Wiener diversity index (H′) (Shannon & Weaver 1969), Simpson's index of dominance (C_D) (Simpson 1949), and evenness index (e) (Pielou 1966). The formula for each index is as follows:

Shannon-Wiener diversity index:

$$H' = \sum_{i=1}^{S} P_i \ln P_i \quad (2)$$

Simpson's index:

$$C_D = \sum_{i=1}^{S} (P_i)^2 \quad (3)$$

Evenness index:

$$e = H'/\log S \quad (4)$$

where, P_i represents the proportional abundance of the i^{th} species in the community and S is the total number of species.

In each study plot, we counted the number of *B. insignis* individuals and recorded the basal diameter of stem at 1.3 m above ground surface, tree height, crown size, and the number of normal healthy leaves. The level of vitality (V) of *B. insignis* in the study plot was calculated based on the formula given below:

$$V = 0.2a + 0.2b + 0.2c + 0.2d + 0.1e + 0.1f \quad (5)$$

where, a is the average leaf number for *B. insignis*, b is the average crown size, c is the number of individuals, d is the average basal diameter, e is the average tree height, and f is the number of quadrats recorded in the study plot. The level of vitality from this study was compared with those of other populations in Guangdong province (Cai et al. 2014, Liu et al. 2016, Zeng et al. 2016). One-way ANOVA was performed to determine whether or not significant differences were present between the measurements on the four morphological features (basal diameter, height, crown size and number of leaves). The measurements were assigned into several classes for calculation purposes, whereby basal diameter was assigned into five classes (≤ 0.50, 0.51–10.00, 10.01–15.00, 15.01–20.00, > 20.00 cm), tree height five classes (≤ 0.5, 0.6–1.0, 1.1–1.5, 1.6–2.0, >2.0 m), crown size four classes (≤ 2.0, 2.1–4.0, 4.1–6.0, > 6.0 m^2), and the number of leaves five classes (≤ 6, 7–12, 13–18, 19–24, > 24). Significant difference (p-value) was set at ≤ 0.05.

RESULTS

Floristic composition of MTNR

A total of 63 species of vascular plant species, belonging to 55 genera and 40 families were recorded in the study area. Rubiaceae had six species and was classified as the dominant family in the study area, followed by Euphorbiaceae, Lauraceae, and Rutaceae which had three species each (Appendix). The study area represented an evergreen community appearance and unobvious seasonal change, belonging to a typical evergreen broad-leaved forest in the subtropical monsoon zone. The forest can be categorised into three different layers on the basis of vertical structure layers, namely, tree, shrub and herb. The tree layer was further divided into another two sublayers which were upper and bottom. The upper sublayer (10–18 m in height) comprised tree species such as *Aporusa dioica*, *Diospyros morrisiana*, *Elaeocarpus sylvestris*, *Lithocarpus glaber*, *Machilus pauhoi*, *Sapium discolor*, *Schefflera heptaphylla* and *Syzygium hancei*. The bottom sublayer (6–10 m in height) had 29 other tree species such as *Cratoxylum cochinchinense*, *Gordonia axillaris* and *Itea chinensis*. The shrub layer (1–5 m in height) consisted of more than 30 plant species, including *B. insignis*, *Diplospora dubia*, *Glochidion wrightii*, *Ilex pubescens*, *Litsea rotundifolia* and *Psychotria rubra*. The herb layer was predominantly monocotyledons and ferns such as *Adiantum flabellulatum*, *Blechnum orientale*, *Dicranopteris dichotoma*, *Lindsaea orbiculata* and *Lophatherum gracile*. On the other hand, four interlayer plant species, namely, *Dendrotrophe frutescens*, *Lygodium flexuosum*, *Lygodium japonicum*, and *Tetracera asiatica* were also recorded.

Species dominance and diversity

In this study, the number (and percentage) of plant species that were grouped into ranks A, B, C, D, and E were 38 (57.58%), 15 (22.73%), 8 (12.12%), 2 (3.03%); and 3 (4.55%) respectively. The frequency distribution index was A > B > C > D < E. The first three species which had the highest IVI were treated as dominant species in each layer. In the tree layer, *L. glaber* (IVI = 61.17%), *S. heptaphylla* (9.26%), and

S. hancei (5.00%) were the dominant species (Table 1). For shrub layer, *P. rubra* (37.52%), *B. insignis* (13.69%) and *L. rotundifolia* var. *oblongifolia* (5.20%) were dominant species. The dominant herb species were *L. orbiculata* (6.95%), *D. pedata* (3.49%) and *L. gracile* (2.94%).

Lithocarpus glaber and *S. heptaphylla* had the highest percentage of standing trees in class IV (43.11 and 67.86% respectively) and lowest in class I (0.25 and 0.00%) (Figure 1). However, *S. hancei* had most standing trees in classes II and III (43.48% each), but did not have any stands recorded for class V. *Psychotria asiatica* was the highest number of standing trees in class II (62.12%), while *B. insignis* and *L. rotundifolia* var. *oblongifolia* were abundant in classes IV (76.32%) and III (77.78%) respectively. No stands were recorded for *P. asiatica* in classes I and V, *B. insignis* in class V, and *L. rotundifolia* var. *oblongifolia* in classes I, IV and V. The overall species diversity in MTNR, based on the Shannon-Wiener, Simpson and evenness indices, were 3.18, 5.12, and 0.73 respectively (Table 2).

Population structure of *B. insignis*

There was a total of 74 *B. insignis* individuals recorded in the study plot. Of these, 44 of them had a basal diameter of stem at 1.3 m above groud surface. The average basal diameter for the recorded individuals was 8.53 cm, with an average height of 41.70 cm. The average crown size was 1.54 m × 1.45 m, which had an average of 16.84 leaves per individual. The natural population of *B. insignis* in MTNR had an overall vitality level of 18.58 (Table 2). One-way ANOVA revealed that there were significant differences between the basal diameter and the tree height, crown size and number of leaves as well as between crown size and tree height and number leaves (Table 3). However, there was no significant difference between tree height and number of leaves.

DISCUSSION

Community characteristics of MTNR

MTNR has an evergreen forest stand and unnoticeable seasonal change that fits the criteria of a typical evergreen broad-leaved forest in the subtropical monsoon zone. The frequency distribution of the five ranks in MTNR is consistent with Raunkiaer's law of distribution of frequencies (Raunkiaer 1934), in which the presence of high proportion of rank E species representing the constructive and dominant species in the forest stand indicated a stable state of the present forest stand structure in MTNR. On the contrary, presence of higher proportions in rank B and rank C species would be an indication of an uneven species distribution in the forest stand. The Raunkiaer's standard frequency index suggested that ranks A, B, C, D, and E should have a standard frequency of 53, 14, 9, 8 and 16% respectively (Raunkiaer 1934). In MTNR, higher frequencies were recorded for ranks A, B and C but lower for ranks D and E. This suggested that the forest stand in MTNR was at the stage of a subclimax community, and the process to achieve the final stage of succession was ongoing.

Based on the value for importance of dominant species recorded in this study, MTNR could be regarded as a *L. glaber*–*P. rubra* community. The stand classification on dominant species within a community not only reflects its role and position, but also the stand succession as well as development trends (Kangur et al. 2005). The high proportion in class IV but low proportion in class V for *L. glaber* and *S. heptaphylla* indicated that the two species were going through a pre-development stage and the most dominant species, *L. glaber*, was experiencing a second round of secondary succession stage. Judging by the current stand classification of *L. glaber*, the species was likely to propagate growth pattern in terms of its stand size and structure. On the contrary, based on the stand classification of *S. heptaphylla*, the high proportion of stands in class IV but low proportions of stands in classes II and III indicated that although the species was growing vigorously in the study area, it was experiencing a declining trend in stand size and structure. On the other hand, stand size and structure of *S. hancei* will likely increase in future as it had larger proportions of stands in classes II and III. In the shrub layer, although *P. rubra* stands showed substantial growth in stand size, in which stand proportion in class II was greater than III and stand proportion in class III was greater than IV, the species was unlikely to achieve absolute dominancy in future succession. This was because the species was restricted to its vegetative characteristics, in which the mature

Table 1 Quantity and importance value index (IVI) of vascular plant species in Mount Tiantou Nature Reserve based on three different forest layers

Type of layer	Family	Species	Number of individuals	Relative frequency (%)	Relative abundance (%)	Relative prominence (%)	IVI (%)
Tree	Fagaceae	*Lithocarpus glaber*	320	8.21	25.70	27.26	61.17
	Araliaceae	*Schefflera heptaphylla*	20	5.13	1.61	2.52	9.26
	Myrtaceae	*Syzygium hancei*	15	3.59	1.20	0.21	5.00
	Phyllanthaceae	*Aporosa dioica*	17	3.08	1.37	0.44	4.89
	Ebenaceae	*Diospyros morrisiana*	11	3.08	0.88	0.29	4.26
	Pentaphylacaceae	*Adinandra millettii*	12	3.08	0.96	0.11	4.15
	Theaceae	*Schima superba*	5	1.03	0.40	1.69	3.12
	Primulaceae	*Ardisia hanceana*	4	1.54	0.32	0.00	1.86
	Rubiaceae	*Diplospora dubia*	5	1.03	0.24	0.02	1.28
	Iteaceae	*Itea chinensis*	5	1.03	0.24	0.01	1.27
	Phyllanthaceae	*Glochidion wrightii*	2	1.03	0.16	0.05	1.24
	Fabaceae	*Archidendron lucida*	2	1.03	0.16	0.00	1.19
Shrub	Rubiaceae	*Psychotria rubra*	206	5.44	17.98	14.11	37.52
	Blechnaceae	*Brainea insignis*	44	4.42	3.84	5.43	13.69
	Moraceae	*Litsea rotundifolia* var. *oblongifolia*	27	2.04	2.36	0.80	5.20
	Rosaceae	*Rhaphiolepis indica*	12	2.38	1.05	0.27	3.70
	Aquifoliaceae	*Ilex pubescens*	13	2.04	1.13	0.26	3.43
	Primulaceae	*Ardisia hanceana*	8	2.04	0.70	0.51	3.25
	Myrtaceae	*Syzygium hancei*	8	1.36	0.70	0.76	2.82
	Rubiaceae	*Mussaenda pubescens*	7	1.70	0.61	0.29	2.60
	Araliaceae	*Schefflerea heptaphylla*	5	1.36	0.44	0.65	2.44
	Thymelaeaceae	*Aquilaria sinensis*	5	1.02	0.44	0.46	1.91
	Dilleniaceae	*Tetracera sarmentosa*	3	1.02	0.26	0.38	1.66
	Fagaceae	*Lithocarpus glaber*	3	0.68	0.26	0.29	1.23
Herb	Lindsaeaceae	*Lindsaea orbiculata*	9	3.06	0.79	3.11	6.95
	Gleicheniaceae	*Dicranopteris dichotoma*	5	1.36	0.44	1.70	3.49
	Poaceae	*Lophatherum gracile*	5	1.70	0.44	0.80	2.94
	Adiantaceae	*Adiantum flabellulatum*	13	0.68	1.13	0.67	2.48
	Blechnaceae	*Blechnum orientale*	9	1.02	0.79	0.36	2.17

Species with IVI < 1.00 were omitted

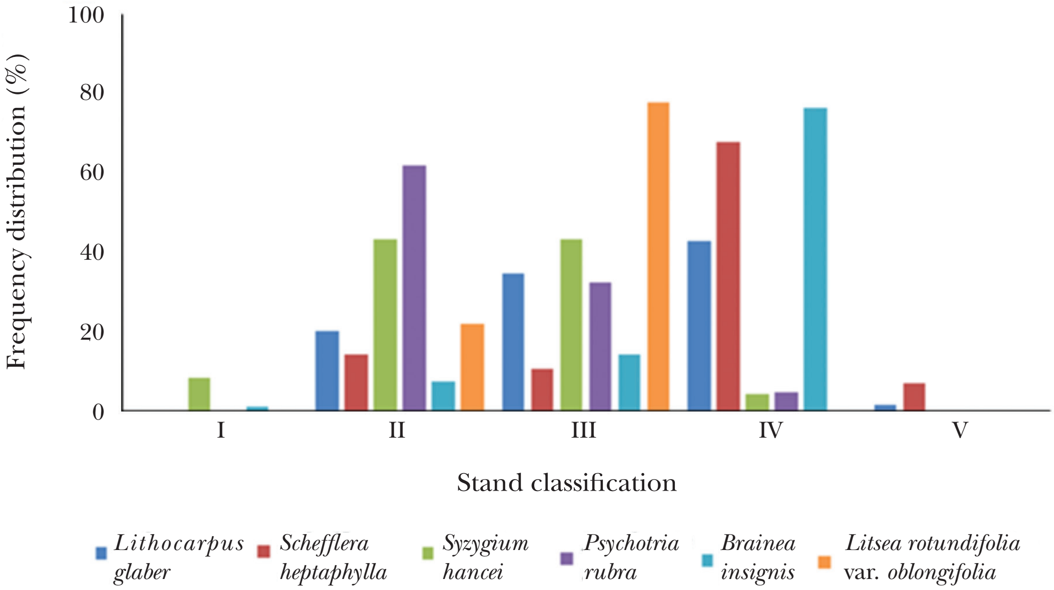

Figure 1 Stand classification and frequency distribution of six dominant species (three species each for tree and shrub layer) identified based on their importance value in Table 1; stand classification of the dominant species was based on five tree classes, i.e class I = seedlings, class II = saplings, class III = trees that had DBH < 7.5 cm, class IV = trees that had DBH ranging from ≥ 7.5 cm, but were < 22.5 cm, class V = trees that had DBH ≥ 22.5 cm

form could only reach a maximum height of 1.5 m due to its heavy branching features. By having a similar stand structure as *S. heptaphylla*, *B. insignis* stands in MTNR was also experiencing a declining trend in stand size and structure. As for *L. rotundifolia* var. *oblongifolia*, the species showed promising growth pattern for the future. Another endangered tree species, *Aquilaria sinensis*, was only represented by a handful number at the shrub layer. Considering that the *A. sinensis* tree could reach a maximum height of 15 m during maturity stage (Wang et al. 2007), the limited number of seedlings recorded in the study plot suggested that the survival of *A. sinensis* in this area could be challenging. *Aquilaria sinensis* populations are threatened by human activities, such as illegal harvesting or discriminate felling in search of agarwood (Jim 2015), thus it is listed in the IUCN Red List as vulnerable (Harvey-Brown 2018) and included in Appendix II of the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES) (UNEP-WCMC 2020). Although it may consume a long period of time, forest recovery after human disturbance is likely to be challenging but possible (Sann et al. 2016).

Population status of *B. insignis*

The population of *B. insignis* in MTNR was suffering a weak self-renewal ability which may be caused by the vegetative characteristics of its habitat such as dense canopy, insufficient growing space and other external environmental factors. Xu et al. (2010) reported that *B. insignis* population in MTNR in 2010 had slightly higher species diversity, indicating that MTNR had encountered a decrease in species diversity over the last decade (Table 2). The decreasing growth trend in *B. insignis* stand size and structure was not only observed in this study, but the same trend was also being reported elsewhere (Cai et al. 2014, Liu et al. 2016).

Natural *B. insignis* populations in habitat which receives less sunlight than usual, have low vitality level. For example, *B. insignis* stands in Jingxin Reservoir that were recorded with low vitality levels, were generally distributed in gully areas which were high in moisture content and well-shaded. Dominant species in the upper layer of the study area had high canopy density, blocking the light from reaching the forest floor. This was one of the affecting factors that contributed to the low regeneration rate of

Table 2 Diversity of study area and vegetative characteristics of natural *Brainea insignis* in Guangdong Province, China

Study area	Size (m^2) and number of quadrats	Diversity			Number of *B. insignis* individuals	Average basal diameter (cm)	Average tree height (m)	Average crown size (m)	Average number of leaves	Vitality		Reference
		H′	C_D	e						Per sample plot (SP)	Whole study plot	
Maluanshan Country Park	1200 (12)	3.45	4.42	0.65	40	NA	NA	NA	NA	NA	NA	Xu et al. (2010)
Paiyashan Nature Reserve	1200 (12)	3.76	6.03	0.69	44	NA	NA	NA	NA	NA	NA	Xu et al. (2010)
Mount Tiantou Nature Reserve	1200 (12)	3.83	8.52	0.75	34	NA	NA	NA	NA	NA	NA	Xu et al. (2010)
Guzhai Nature Reserve	1200 (24)	3.87	8.09	0.77	47	6.33	41.48	1.42 × 1.54	9.66	SP1: 10.92 SP2: 13.56 SP3: 8.63 SP4: 11.87 SP5: 9.68	11.96	Cai et al. (2014)
Jingxin Reservoir area	1600 (16)	3.19	5.38	0.75	34	7.35	36.35	1.54 × 1.45	11.33	NA	12.74	Liu et al. (2016)
Baipenzhu Nature Reserve	1200 (12)	3.79	6.36	0.81	42	12.79	46.35	1.54 × 1.54	15.58	SP1: 10.79 SP2: 18.31 SP3: 13.75	13.95	Zeng et al. (2016)
Mount Tiantou Nature Reserve	1600 (16)	3.18	5.12	0.53	44	8.53	41.70	1.54 × 1.45	16.84	SP1: 11.02 SP2: 9.41 SP3: 10.80 SP4: 11.20	18.58	This study

H′, C_D and e = Shannon-Weiner, Simpson's and evenness index respectively

B. insignis in the area. However, when compared with three natural populations in Guangdong Province, i.e. Baipenzhu Nature Reserve, Guzhai Nature Reserve, and Jingxin Reservoir area, the overall vitality level of *B. insignis* from MTNR was the highest (Table 2). We observed a similar situation for *B. insignis* stands in sample plot 1 (SP1) of our study site, where the forest canopy was slightly denser when compared with that of sample plots 2, 3 and 4 (SP2, SP3, and SP4). Although *B. insignis* are naturally distributed widely in the southern region of China, the fern is known to favour habitats along hillsides and undisturbed environment. *Brainea insignis* can only be spotted in nature reserves, where the habitat is less likely to be destroyed (Yang et al. 2011).

Conservation implications of *B. insignis*

Presently, *B. insignis* is listed under the Chinese National Key Protection plant species with a level II protection status. It was previously thought to be an endemic species in China but was later discovered scattered in small fragmented populations in neighbouring countries and throughout the South-East Asia region (Yang et al. 2011). The conservation efforts of natural *B. insignis* populations are similar to other fern species, which are mainly based on in-situ and ex-situ approaches. For in-situ conservation, the natural habitat of the fern is prioritised. Researchers have deployed various techniques to effectively identify appropriate protected areas for endangered ferns (Wang et al. 2016). One of the methods applied on natural *B. insignis* populations was through spatial model distribution using digital elevation model, orthophoto base maps and nine-date SPOT images (Wang et al. 2012). Through this technique, potential sites for the fern in the study area was predicted based on the reported habitat characteristics of *B. insignis.* However, due to limitation of low-resolution images, the output using such technique was unlikely to accurately extrapolate the spatial patterns of *B. insignis* in a highly precise manner. Therefore, conducting timely on-ground field survey and biodiversity assessments are still considered the most preferable method of choice to collect firsthand information on the habitat and population status of the fern.

As a typical mesophyte species, *B. insignis* is light-demanding and grows well in the hillside forest with moderate canopy density. However, these landscapes are often prone to fire during dry seasons (Kholia et al. 2019). In order to secure the moisture content of the surrounding, it is important that proper canopy density and forest structure is maintained. Appropriate manual forest floor cleaning may create sufficient gap to construct natural soil spore banks that allow the fern to propagate and regenerate. The potential role of spore bank in the soil has been brought up during the early 90s and was proven to be an effective method to recovering small populations, but was only practised in temperate regions (Dyer & Lindsay 1996). In Asia, India was the first to propose exploiting the efficiency of soil spore banks towards conservation of Asian ferns. Soil spore bank research is necessary and should align with the development of rural areas where deforestation for urbanisation occurs.

Based on the information compiled through the Biological Resources Programme of the Chinese Academy of Science (www.casbrc.org), ex-situ collection of living *B. insignis* is available at six botanical gardens distributed in the southern region of China. Ex-situ measures are popular and effective for fern preservation, and they complement in-situ

Table 3 Results of one-way ANOVA on the mean differences between basal diameter, tree height, crown size and number of leaves of 44 *Brainea insignis* individuals Mount Tiantou Nature Reserve

	Tree height		Crown size		Number of leaves	
	p	F	p	F	p	F
Basal diameter	1.2E6*	25.56	0.04*	4.11	2.65E6*	23.90
Tree height			5.88E11*	50.01	0.65	0.21
Crown size					4.61E10*	44.66

*significant difference (p) at ≤ 0.05

conservation programmes (Ibars & Estrelles 2012). Ex-situ fern collections in botanical gardens are important resources for fern spore banks, in which spores from endangered fern species, such as *B. insignis*, can be germinated and cultivated for replanting purposes. The concept of Fernarium, which is an ex-situ collection dedicated to only endangered ferns, was first introduced in 2012 (Bidin 2012). The major advantage of the Fernarium is to serve as an outdoor laboratory for morphological, cytological and phytochemical investigation and, at the same time, to aid in systematic classification of the pteridophytes. Botanical gardens and Fernariums are good platforms for promoting the biodiversity and delivering public awareness (Chen & Sun 2018). Therefore, it is important to strengthen the capacity and scientific research in botanical gardens in order to become an effective tool for conservation and utilisation.

Efforts to promote *B. insignis* as ornamental plant are widely carried out but not properly regulated in China. Eventually, the demand for *B. insignis* as ornamental landscaping plant has influenced the increase of illegal harvesting of wild *B. insignis* saplings. To cater for the increasing demand for this endangered fern species, regulation to monitor and to promote sustainable utilisation with minimum disturbance to natural populations is essential. For this purpose, research into cultivation and domestication of *B. insignis* has led to the development of human-aided reproduction techniques. As a result, Chinese researchers have studied the germination rate of *B. insignis* spores and the success rate of clonal stands through stem plugs and the domestication of *B. insignis* seems to be promising (Lin 2008, Gao 2011). However, in order to reduce further exploitation of *B. insignis* in the wild, constant delivery of public awareness messages is necessary to educate the locals in preserving the nature (Ghosh et al. 2017).

CONCLUSIONS

Conservation of a relict species is always in line with the precise assessment and understanding of its survival dynamics. It is unsure that the *B. insignis* will be able to survive into the future without conservation and restoration efforts. Habitat alterations and reduction of species richness and diversity in the community where *B. insignis* is distributed have imposed a declining trend of its population status in the wild. The findings of this study may provide baseline information for formulating conservation and management strategies for natural *B. insignis* populations.

ACKNOWLEDGEMENTS

The authors thank the staff of Mount Tiantou Nature Reserve for the services rendered during fieldwork. This research was funded by the Shenzhen Assessment of Rare, Endangered and Important Wild Plant Resources Project (SZCG2014044440) and the Shenzhen Dapeng Peninsula National Geopark Science and Technology Innovation Project (HT-99982020-0258; 2020–2021).

REFERENCES

Balkrishna A, Arya V & Kushwaha AK. 2020. Population structure, regeneration status and conservation measures of threatened *Cyathea* spp. *Journal of Tropical Forest Science* 32: 414–421. https://doi.org/10.26525/jtfs2020.32.4.414

Bidin A. 2012. The role of the Fernarium as a sanctuary for the conservation of threatened and rare ferns, with particular reference to Malaysia. Pp 223–240 in Heywood VH & Jackson PW (eds) *Tropical Botanic Gardens: Their Role in Conservation and Development.* Academic Press, Cambridge.

Cai Z, Wu L, Cheng F, Ma L, Liu S & Cao H. 2014. Study of *Brainea insignis* communities characteristics at Guzhai Nature Reserve, Huicheng District. *Guangdong Forestry Science and Technology* 30: 7–12.

Chen G & Sun W. 2018. The role of botanical gardens in scientific research, conservation, and citizen science. *Plant Diversity* 40: 181–188. https://doi.org/10.1016/j.pld.2018.07.006

Cranfill R & Kato M. 2003. Phylogenetics, biogeography, and classification of the woodwardioid ferns (Blechnaceae). Pp 25–48 in Chandra S & Srivastava M (eds) *Pteridology in the New Millennium.* Springer, Dordrecht. https://doi.org.10.1007/978-94-017-2811-9_4

Curtis JT & McIntosh RP. 1951. An upland forest continuum in the prairie-forest border region of Wisconsin. *Ecology* 32: 476–496. https://doi.org/10.2307/1931725

Dyer AF & Lindsay S. 1996. Soil spore banks—a new resource for conservation. Pp 153–160 in Camus JM et al. (eds) *Pteridology in Perspective.* Royal Botanic Gardens, Kew.

Fraser-Jenkins CR. 2012. Rare and threatened Pteridophytes of Asia 2. Endangered species of India—the higher IUCN categories. *Bulletin of the National Museum of Nature and Sciences, Series B, Botany* 38: 153–181.

Gao P-S. 2011. *Plug Production of Brainea insignis (Hook.) J.Sm.* National Taiwan University, Taipei City.

Gasper AL, Dittrich Vado, Smith AR & Salino A. 2016. A classification for Blechnaceae (Polypodiales: Polypodiopsida): new genera, resurrected names, and combinations. *Phytotaxa* 275: 191–227. https://doi.org/10.11646/phytotaxa.275.3.1

Ghosh S, Ganga M, Priyanka RR & Manimaran P. 2017. Endangered ornamental plant species in India and strategy for their conservation—a review. *Chemical Science Review and Letters* 6: 1457–1464.

Harvey-Brown Y. 2018. *Aquilaria sinensis. The IUCN Red List of Threatened Species*. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T32382A2817115.en

Ibars AM & Estrelles E. 2012. Recent developments in ex situ and in situ conservation of ferns. *Fern Gazette* 19: 67.

Jim CY. 2015. Cross-border itinerant poaching of agarwood in Hong Kong's peri-urban forests. *Urban Forestry & Urban Greening* 14: 420–431. https://doi.org/10.1016/j.ufug.2015.04.007

Kangur A, Korjus H, Jõgiste K & Kiviste A. 2005. A conceptual model of forest stand development based on permanent sample-plot data in Estonia. *Scandinavian Journal of Forest Research* 20: 94–101. https://doi.org/10.1080/14004080510041039

Kholia BS, Sharma S & Sinha BK. 2019. *Brainea insignis* (Hook.) J.Sm.—a conservation priority fern of North East India. *Current Science* 116: 32–35. https://doi.org/10.18520/cs/v116/i1/32-35

Lin X-X. 2008. *Studies of Phenology, Spore Propagation, and Effects of Temperature and Nutrition Concentration on Growth of Brainea insignis (Hook.) J.Sm.* National Taiwan University, Taiwan.

Liu H, Xu K, Sun H, He Q, Fan Q & Liao W. 2016. Structure characteristics and succession analysis of *Brainea insignis* community in Jingxin Reservoir Area. *Acta Botanica Boreali-Occidentalia Sinica* 36: 2094–2102.

Liu H, Yan Z, Xu H, Li C, Fan Q, Liao W & Liao B. 2017. Development and characterization of EST-SSR markers via transcriptome sequencing in *Brainea insignis* (Aspleniaceae s.l.). *Applications in Plant Science* 5: 1700067. https://doi.org/10.3732/apps.1700067

Order of the Forestry Bureau and Ministry of Agriculture of China. 1991. *The Protected Native Plant List in China (1st List)*. Ministry of Agriculture, Beijing.

Pielou EC. 1966. Shannon's formula as a measure of specific diversity: its use misuse. *The American Naturalist* 100: 463–465. https://doi.org/10.1086/282439

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54: 563-603. https://doi.org/10.1111/jse.12229

Raunkiaer C. 1934. *The Life Forms of Plants and Statistical Plant Geography*. Clarendon Press, Oxford.

Sann B, Kanzaki M, Aung M & Htay KM. 2016. Assessment of the recovery of a secondary tropical dry forest after human disturbance in central Myanmar. *Journal of Tropical Forest Science* 28: 479–489.

Shannon CE & Weaver W. 1969. *The Mathematical Theory of Communities*. University of Illinois Press, Urbana.

Simpson EM. 1949. Measurement of diversity. *Nature* 163: 688. https://doi.org/10.1038/163688a0

UNEP-WCMC. 2020. The checklist of CITES species website. http://checklist.cites.org. (accessed on 1 June 2020)

Wang B, Yu S & Peng S. 1996. *Experiment Handbook of Phytocoenology*. Guangdong Higher Education Press, Guangzhou.

Wang C, Wan J, Zhang Z & Zhang G. 2016. Identifying appropriate protected areas for endangered fern species under climate change. *Springerplus* 5: 904. https://doi.org/10.1186/s40064-016-2588-4

Wang FG, Xing FW, Dong SY & Kato M. 2013. Blechnaceae. Pp 411–417 in Wu ZY et al. (eds) *Flora of China*. Volumes 2–3. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

Wang W-C, Lo N-J, Chang W-I & Huang K-Y. 2012. Modeling spatial distribution of a rare and endangered plant species (*Brainea insignis*) in central Taiwan. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* 39: 241–246. https://doi.org/10.5194/isprsarchives-xxxix-b7-241-2012

Wang Y, Nevling LI & Gilbert MG. 2007. *Aquilaria* Lamarck. Pp 214–215 in Wu ZY et al. (eds) *Flora of China*. Volume 13. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

Wang Y, Zhang S, Li Y, Cheng J & Xing F. 2003. The floristic characteristics and distribution of National Key Protected Wild Plants in Shenzhen. *Journal of South China Agricultural University* 24: 63–66.

Xu X, Wang X, Sun Y, Lai Y, Zan Q & Liao W. 2010. Analysis on community characteristics of *Brainea insignis* in Maluanshan and its adjacent mountains of Shenzhen. *Journal of Plant Resources and Environment* 19: 63–69.

Yang FC, Zhang CL, Wu G, Liang SY & Zhang XC. 2011. Endangered pteridophytes and their distribution in Hainan Island, China. *American Fern Journal* 101: 105–116. https://doi.org/10.1640/0002-8444-101.2.105

Yu J, Wang H, Dong H, Fang Y & Xiang J. 2020. The complete chloroplast genome sequence of *Brainea insignis* (Blechnaceae). *Mitochondrial DNA Part B: Resources* 5: 2034–2035. https://doi.org/10.1080/23802359.2020.1756959

Zeng S, Ma D, Liao Y et al. 2016. Communities characteristics with the populations of *Brainea insignis* in Lianhuashan-Baipenzhu Nature Reserve, Guangdong. *Journal of Fujian Forestry Science and Technology* 43: 8–13.

Zhao Q, Liu L, Chen D & Sun H. 2016. Investigation of plant resources in Shenzhen Tiantoushan Municipal Nature Reserve. *Journal of Green Science and Technology* 14: 4–7.

Appendix Species and number of vascular plant individuals recorded in *Brainea insignis* community in Mount Tiantou Nature Reserve, Shenzhen, China

No.	Family	Species	Number of individuals				
			SP1	SP2	SP3	SP4	Total
1	Adiantaceae	*Adiantum flabellulatum* L.	0	1	0	12	13
2	Anacardiaceae	*Toxicodendron succedaneum* (L.) O. Kuntze	0	0	2	3	5
3	Annonaceae	*Desmos chinensis* Lour.	0	0	2	0	2
4	Aquifoliaceae	*Ilex chinensis* Sims	0	0	0	1	1
5		*Ilex pubescens* Hook. et Arn.	0	4	3	2	9
6	Araliaceae	*Schefflera heptaphylla* (Linnaeus) Frodin	14	4	8	9	35
7	Blechnaceae	*Blechnum orientale* L.	0	6	0	3	9
8		*Brainea insignis* (Hook.) J. Sm.	12	22	17	23	74
9	Chloranthaceae	*Sarcandra glabra* (Thunb.) Nakai	5	1	0	0	6
10	Connaraceae	*Rourea microphylla* (Hook. et Arn.) Planch.	0	1	1	0	2
11	Daphniphyllaceae	*Daphniphyllum calycinum* Benth.	0	0	2	0	2
12	Dilleniaceae	*Tetracera sarmentosa* Vahl.	0	0	3	0	3
13	Dryopteridaceae	*Cyrtomium fortunei* J. Sm.	2	0	0	0	2
14	Ebenaceae	*Diospyros morrisiana* Hance	1	1	3	8	13
15	Elaeocarpaceae	*Elaeocarpus japonicus* Sieb. et Zucc.	0	1	0	0	1
16		*Elaeocarpus sylvestris* (Lour.) Poir.	1	0	0	0	1
17	Ericaceae	*Rhododendron moulmainense* Hook. f.	0	0	0	3	3
18	Euphorbiaceae	*Triadica cochinchinensis* Loureiro	0	0	1	1	2
19	Fabaceae	*Archidendron lucidum* (Benth) I. C. Nielsen	0	0	2	0	2
20		*Dalbergia hupeana* Hance	1	0	0	0	1
21		*Dalbergia millettii* Benth.	0	0	0	1	1
22	Fagaceae	*Lithocarpus glaber* (Thunb.) Nakai	109	55	166	123	453
23	Gleicheniaceae	*Dicranopteris pedata* (Houttuyn) Nakaike	10	6	6	42	64
24	Guttiferae	*Cratoxylum cochinchinense* (Lour.) Bl.	0	0	0	6	6
25	Iteaceae	*Itea chinensis* Hook. et Arn.	3	0	1	0	4
26	Lauraceae	*Cinnamomum parthenoxylon* (Jack) Meisner	0	0	1	0	1
27		*Lindera nacusua* (D. Don) Merr.	0	0	1	0	1
28		*Machilus pauhoi* Kanehira	0	0	1	0	1
29		*Machilus velutina* Champ. ex Benth.	0	0	0	4	4
30	Leguminosae	*Astragalus camptodontus* Franch.	0	0	0	1	1
31	Lindsaeaceae	*Lindsaea orbiculata* (Lam.) Mett. ex Kuhn	56	36	7	17	116
32	Lygodiaceae	*Lygodium japonicum* (Thunb.) Sw.	1	0	0	5	6
33		*Lygodium flexuosum* (L.) Sw.	0	0	2	0	2
34	Melastomataceae	*Melastoma sanguineum* Sims.	0	0	1	0	1
35	Moraceae	*Ficus hirta* Vahl	0	1	1	0	2
36		*Ficus variolosa* Lindl. ex Benth.	0	1	1	0	2
37		*Litsea rotundifolia* var. oblongifolia	3	12	12	20	47
38	Myrtaceae	*Syzygium hancei* Merr. et Perry	31	6	11	11	59
39	Pentaphylacaceae	*Adinandra millettii* (Hook. et Arn.) Benth. et Hook. f. ex Hance	0	1	7	4	12

continued

Appendix (*continue*)

No.	Family	Species	Number of individuals				
			SP1	SP2	SP3	SP4	Total
40	Phyllanthaceae	*Aporosa dioica* (Roxburgh) Muller Argoviensis	1	7	5	1	14
41		*Glochidion wrightii* Benth.	0	0	1	1	2
42		*Glochidion zeylanicum* (Gaerthn.) A. Juss.	0	0	2	1	3
43	Poaceae	*Lophatherum gracile* Brongn.	27	0	13	7	47
44	Primulaceae	*Ardisia hanceana* Mez	1	4	3	3	11
45		*Ardisia brevicaulis* Diels	1	0	0	0	1
46		*Embelia laeta* (L.) Mez	0	1	0	0	1
47	Pteridaceae	*Pteris semipinnata*	0	1	0	0	1
48	Rosaceae	*Photinia serratifolia* (Desfontaines) Kalkman	4	0	0	0	4
49		*Rhaphiolepis indica* (Linnaeus) Lindley	0	5	1	8	14
50	Rubiaceae	*Adina pilulifera* (Lam.) Franch. ex Drake	0	0	0	1	1
51		*Diplospora dubia* (Lindl.) Masam.	0	0	3	0	3
52		*Gardenia jasminoides* Ellis	0	0	1	1	2
53		*Mussaenda pubescens* Ait. F. Hort. Kew. Ed.	0	1	1	7	9
54		*Psychotria asiatica* Wall.	51	142	177	105	475
55		*Pavetta arenosa* Lour.	0	0	0	1	1
56	Rutaceae	*Melicope pteleifolia* (Champion ex Bentham) T. G. Hartley	0	0	1	2	3
57	Santalaceae	*Dendrotrophe frutescens* (Blume) Miquel	0	0	1	1	2
58	Smilacaceae	*Smilax china* L.	0	0	0	8	8
59	Theaceae	*Polyspora axillaris* (Roxburgh ex Ker Gawler) Sweet	0	0	0	1	1
60		*Schima superba* Gardn. et Champ.	0	0	5	0	5
61	Thymelaeaceae	*Aquilaria sinensis* (Lour.) Spreng.	1	2	0	3	6
62		*Wikstroemia nutans* Champ. ex Benth.	0	0	1	0	1
63	Zingiberaceae	*Alpinia oblongifolia* Hayata	0	1	0	0	1

SP = sample plot