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The comparative analyses of selected aspects of conservation and management of Vietnam's national parks

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Abstract

The national parks in Vietnam are protected areas in the national system of special-use forests created to protect natural resources and biodiversity. In order to improve the effectiveness of management of national parks, the study assesses some current aspects of conservation and management of natural resources with respect to management plans, financial sources, staff, cooperative activities, causes of limited management capacity and threats to natural resources. Out of the total of 30 national parks, six are under the responsibility of the Vietnam Administration of Forestry (VNFOREST) and 24 national parks are managed by provincial authorities. It was found that most of the national parks have updated their management plans. Financial sources of funding for national parks mainly originated from the central and provincial budgets, with an average of 51% and 76% respectively. Fifty percent of national parks spent 40–60% of their total funding on conservation activities. About 85% of national parks' staff had academic degrees, typically in the fields of forestry, agriculture and fisheries. Biodiversity conservation was considered a priority cooperative action in national parks with scientific institutes. Major causes of a limited management capacity of national parks included human population growth and pressure associated with resources use, lack of funding, limited human and institutional capacity and land use conflict/land grab. Illegal hunting, trapping, poaching and fishing, the illegal wildlife trade, illegal logging and firewood collecting appeared to be the most serious threats to the conservation and management of natural resources. In addition to these results, significant differences were found between the VNFOREST and provincial parks in terms of financial sources, staff and the threat of illegal logging and firewood collecting. The authors' findings offer useful information for national park planners and managers, as well as policy makers and researchers in seeking solutions for the sustainable management of natural resources in national parks.

Keywords
Conservation, financial sources, management, management plan, national parks, staff, Vietnam

Introduction

National parks (NPs) are the areas established to protect natural biodiversity and the underlying ecological structure, support environmental processes and promote education and recreation (Dudley 2008). The importance of NPs can be illustrated by their rapid growth around the world (e.g. over the last 14 years, their number has increased by over 30%, reaching 5,436 sites in 2017) (Chape et al. 2003, IUCN and UNEP-WCMC 2017). As a symbol of national pride, NPs contribute to preventing the loss of biodiversity, maintaining the natural conditions and beauty of the landscape and ensuring the supply of ecosystem services (Schägner et al. 2016).

In Vietnam, NPs are protected areas in the system of special-use forests (SUFs) which are intended to protect nature, including rare species, forest ecosystems and genetic resources. Other objectives include the protection of landscapes, cultural and historical sites and the provision of recreation and tourism (GoV 2010). The first Vietnamese NP (Cuc Phuong NP) was established in 1962 (Vo Quy et al. 1996). Since then, the number of NPs has increased from 16 (476,621 ha) in 2002 (cited by Rambaldi et al. 2001) to 30 (1,077,236 ha, reaching ca. 3% of the total land area) in 2012 (Figure 1, MONRE 2014). According to the approved national planning system of SUFs up to 2020, Vietnam will have 34 NPs (1,166,462 ha, reaching ca. 49% of SUFs' land area and 4% of the total land area) (GoV 2014c). In 2015, the Government of Vietnam merged the two protected areas of the Du Gia Nature Reserve and Khau Ca Species and Habitat Conservation Area into a national park with a total area of 15,006 ha (GoV 2015). The increasing number of NPs supports the conservation and sustainable development of significant natural ecosystems, landscapes, historical and cultural relics and endangered and rare species in the SUFs (GoV 2014c).

Concerning the governance of Vietnam's NPs, several ministries and agencies are involved in the protection process. The Ministry of Agricultural and Rural Development (MARD) and their provincial departments have overall responsibility for managing protected areas. MARD directly administers NPs with special nature conservation status or those with areas extending across more than one province (GoV 2010, 2014b). Other NPs are managed by the Provincial People's Committees (PPCs) and their departments. In addition, each PPC is responsible for establishing and staffing the NPs' management boards, as well as allocating the capital and budget for them. The Ministry of Natural Resource and Environment (MONRE) is responsible for undertaking the state management for the biodiversity of protected areas (GoV 2008,

Figure 1. Distribution of the thirty national parks, Vietnam. Source: Adapted to IUCN and UNEP-WCMC (2017)

2014a). Together with MARD, the Ministry of Culture, Sport and Tourism guides and examines PPCs in the management of eco-tourist activities and promotes NPs as tourism destinations in the development of the tourism sector in Vietnam. However, in the context of the special-use forest system, the management and administration of protected areas, including NPs, is fragmented and the division of responsibility between administrative levels is unclear (Nguyen KimDung et al. 2012, VNFOREST 2014).

For the conservation and management of protected areas, NPs suffer from lack of funds, as well as growing investments in infrastructure development (USAID 2013). The funding for protected areas is unstable; it is derived originally from the state budget, but is channelled through central and provincial budgets, international donors and other funding bodies (e.g. ecosystem services) (ICEM 2003, VNFOREST 2014). In some NPs, the central and provincial budgets are just sufficient to cover operations and maintenance costs for protected areas (VNFOREST 2014). The numbers and ability of staff available to manage protected areas are limited, as are those who can engage in biodiversity conservation (MONRE 2014). Most of the leaders and staff in SUFs do not have any specialised knowledge about forests and biodiversity and have not undergone training in conservation skills (MONRE 2014). As a result, building the capacity of the staff plays a crucial role in the effective and efficient management of the protected areas and in achieving conservation objectives.

Despite being ranked as the sixteenth most biodiverse country in the world (Butler 2016), Vietnam faces threats to its biodiversity conservation activities, as well as its protection of natural resources. These issues focus on land conversion, population growth pressure and over-exploitation of natural resources, environmental pollution, climate change and limited human resources (MONRE 2014). In the protected areas of SUFs, VNFOREST (2014) has identified a range of pressures on natural resources, including illegal timber and non-timber forest products, illegal hunting and wildlife trade, grazing in protected areas, land grabbing and tourism.

The aim of this paper is to assess the management and conservation of natural resources in NPs of Vietnam. More specifically, various aspects of NPs were assessed with regard to their management plan, financial sources, staff, cooperative activities, limited management capacity and threats to natural resources. Different groups of NPs were also compared.

Methods

Surveys and interviews were used for the collection of data related to development and management of national parks in Vietnam. A survey method with a structured questionnaire was sent to management boards directly responsible for national park management in 30 NPs in Vietnam (Figure 1). In order to construct the survey questionnaire properly, a mixed-methods' approach was undertaken: a review of literature determining the context of management of natural resources in Vietnam's protected areas was performed (e.g. ICEM 2003, USAID 2013, MONRE 2014, VNFOREST 2014), followed by discussions with staff and management boards of NPs and then a survey questionnaire was pre-tested with six randomly-selected members of NP management boards.

After pre-testing the questionnaire and submitting comments, a structured questionnaire was developed with a total of 26 questions. This study was part of a wider tourism study in NPs of Vietnam and the survey questionnaire included questions focusing on conservation and management of NPs according to the following main subjects of interest: (1) the management plan, (2) financial sources, (3) NP staff (i.e. the number of staff, level of education and education background), (4) cooperative activities in conservation and management of natural resources, (5) causes of limited management capacity of NPs and (6) threats to natural resources (see Appendix 1). In this study, the NP management plan was formulated as a strategic overarching document regarding the management and development of the park in its current and envisaged future forms, in accordance with Thomas and Middleton (2003). The updated status of NPs' management plans was then determined. The perceptions of the respondents concerning limited management capacity and threats to natural resources were rated on a five-point Likert scale ranging from one (strongly disagree) to five (strongly agree) with a score of three being intermediate (neutral) (Likert 1932, Clason and Dormody 1994).

After contacting the heads of NP management boards in 30 NPs to explain the purpose of the survey, the questionnaire survey was sent by an e-mail; the survey itself was conducted between May and December 2016. This survey was also supplemented with phone calls to the respondents to ensure a high rate of response and to gain an insight into the questionnaire. During survey data collection, further data and information was collected through direct contact with NP units (e.g. Unit of Personnel and Administration, Unit of Planning and Finance, Unit of Science and International Co-operation), as well as provincial departments (e.g. Department of Agriculture and Rural Development) to collect data and information in order to support questions from questionnaire surveys. The final response rate to the survey questionnaire was 30/30.

Moreover, 21 interviews were conducted with randomly chosen members of NP management boards to validate questions of the survey and to gather more detailed information on conservation activities and management of NPs (see Appendix 2). Most of interviews were conducted via phone calls, while others were conducted face-to-face.

In this paper, all statistical analyses were performed using STATISTICA 12. Quantitative data were analysed using descriptive statistics. The independent samples t-test and Mann-Whitney U test were employed to compare differences between the two groups (McCrum-Gardner 2008). These tests were applicable to the data as they allowed for comparison between two independent groups with different samples sizes. The Mann-Whitney U test was carried out for attitude scores, while the independent samples t-test was used for an interval-scale variable. In addition, results were considered significant at p-value ≤ 0.05.

Results

National parks' profile

The general characteristics of the 30 surveyed NPs are presented in Table 1. The average size of a NP was 37,073 ha. Phong Nha – Ke Bang NP was the largest park with 123,326 ha (ca. 11% of the total area of 1,111,113 ha) and Xuan Thuy NP was the

Table 1. Characteristics of national parks.

National parks	Year of establishment	Area (ha)	Governance	Other designation
Ba Be	1992	10,048	PPC	RS, AHP
Ba Vi	1991	10,815	VNFOREST	–
Bach Ma	1991	37,487	VNFOREST	–
Bai Tu Long	2001	15,783	PPC	MPA
Ben En	1992	14,735	PPC	–
Bidoup-Nui Ba	2004	70,038	PPC	UBR
Bu Gia Map	2002	25,779	PPC	–
Cat Ba	1986	17,363	PPC	UBR, MPA
Cat Tien	1992	72,634	VNFOREST	UBR, RS
Chu Mom Ray	2002	56,621	PPC	AHP
Chu Yang Sin	2002	58,971	PPC	–
Con Dao	1993	20,000	PPC	RS, MPA
Cuc Phuong	1962	22,200	VNFOREST	–
Hoang Lien	2002	28,059	PPC	AHP
Kon Ka Kinh	2002	42,143	PPC	AHP
Lo Go - Xa Mat	2002	19,156	PPC	–
Mui Ca Mau	2003	41,862	PPC	UBR, RS
Nui Chua	2003	29,865	PPC	MPA
Phong Nha - Ke Bang	2001	123,326	PPC	UWHS
Phu Quoc	2001	29,421	PPC	UBR, MPA
Phuoc Binh	2006	19,814	PPC	–
Pu Mat	1997	91,113	PPC	UBR
Tam Dao	1996	34,995	VNFOREST	–
Tram Chim	1998	7,588	PPC	RS
U Minh Ha	2006	8,528	PPC	UBR
U Minh Thuong	2002	8,038	PPC	UBR, RS, AHP
Vu Quang	2002	57,038	PPC	UBR
Xuan Son	2002	15,048	PPC	–
Xuan Thuy	2003	7,100	PPC	RS
Yok Don	1992	115,545	VNFOREST	–
Total		1,111,113		

List of abbreviations

PPC: Provincial People's Committee; VNFOREST: Vietnam Administration of Forestry, Ministry of Agriculture and Rural Development; UWHS: United Nations Educational, Scientific and Cultural Organisation World Heritage Site; UBR: United Nations Educational, Scientific and Cultural Organisation Biosphere Reserve; RS: Ramsar Site; AHP: Association for Southeast Asian Nations Heritage Park; MPA: Marine Protected Area.

smallest area (7,100 ha, ca. 1% of the total area). Six NPs were found to be the responsibility of MARD, which has been decentralised from direct management by the Vietnam Administration of Forestry (VNFOREST). Twenty-four NPs were under the responsibility of provincial authorities. In addition, many NPs or NP areas were specified under international and regional agreements, including the international designations (Ramsar Site, United Nations Educational, Scientific and Cultural Organisation [UNESCO] World Natural Heritage Site and UNESCO Biosphere Reserve), as well as regional designations including the Association for Southeast Asia Nations (ASEAN) Heritage Parks (Table 1). Some NPs were also listed in the system of Marine Protected Areas.

The national park management plan

Of the 30 investigated NPs, the results showed that most of NPs (87%) had updated their management plans. In particular, 77% had management plans which had been updated by other management tools. Only four NPs (13%) indicated that their management plans had not been updated.

The other management tools (e.g. plans, actions) used management plans coordinated and integrated within 23 NPs: two VNFOREST's NPs and 21 provincial NPs (Table 2). More than 50% of the updated management plans had integrated tools for the community-based forest management and regulations for scientific research activities (91%), planning and management of sustainable tourism and ecotourism (83%) and forest monitoring programmes (74%).

In the current context of socio-economic development, 90% of the 30 NPs indicated that their management plans offered sufficient protection for their development plan(s) for local communities and region(s). Only 10% identified that their management plans were not sufficient for protection because of confusing, conflicting and overlapping institutional and legal frameworks; in addition, a lack of coordination amongst agencies and communities that had a bearing on 7% of NPs; and the non-existence of mechanisms or strategies to engage communities in the management of protected areas was identified in 3% of NPs.

Table 2. The national park management plan updated by other management tools.

Management tools	All NPs (n = 23)		VNFOREST's NPs (n = 2)		Provincial NPs (n =21)	
	n	%	n	%	n	%
Multiple-use forest management plan	9	39.13	0	0	9	42.86
Forest monitoring programme	17	73.91	2	100.00	15	71.43
Regulations for scientific research activities	21	91.30	2	100.00	19	90.48
Sustainable tourism development plan	19	82.61	2	100.00	17	80.95
Environmental impact assessment	7	30.43	0	0	7	33.33
Community-based forest management	21	91.30	2	100.00	19	90.48
Forest valuation	5	21.74	0	0	5	23.81

List of abbreviations

NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases.

Financial sources in national parks

The financial sources of the total funding for conservation and management of NPs in 2016 are presented in Table 3. The results indicated that sources of the funding were mainly derived from the state budget, i.e. a mean of 51% and 76% came from the central budget and provincial budget respectively. Support from organisations, funds from conservation programmes, revenues from forest environmental services and tourism activities contributed to the total funding for parks. In particular, in 87% of NPs, a mean of 9% of their total funding was obtained from tourism activities. The results revealed significant differences between VNFOREST's NPs and provincial NPs with respect to the central and provincial budgets and revenues from forest environmental services (Table 3).

Moreover, 50% of NPs used 40–60% of funding for their conservation activities (Table 4). The results demonstrated that 66.67% of VNFOREST's NPs spent 60–80% of their funds in conservation activities. Meanwhile, in 46% of provincial NPs, 40–60% of funds were invested in conservation activities.

Table 3. Percentages of financial sources of the total funding for national parks.

Financial sources	All NPs			VNFOREST's NPs			Provincial NPs			p-value†
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
The central budget	15	50.95	30.88	6	75.97	33.97	9	34.27	12.83	0.005*
The provincial budget	25	76.24	23.32	1	0.60	0.00	24	79.39	17.55	<0.001*
Support from domestic organisations	3	2.10	2.54	1	0.30	0.00	2	3.00	2.83	0.579
Support from international organisations	3	2.43	2.38	1	0.30	0.00	2	3.50	2.12	0.434
Funds from conservation programmes	5	5.04	4.78	0	NA	NA	5	5.04	4.78	NA
Revenues from forest environmental services	7	9.40	8.12	2	20.00	0.00	5	5.16	4.51	0.007*
Revenues from tourism activities	26	8.66	13.99	6	17.17	26.05	20	6.11	7.01	0.090

List of abbreviations

†p-values were calculated using the independent samples t-test between VNFOREST's NPs and provincial NPs; *Significant at p-value < 0.05; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation; NA: Not available.

Table 4. Percentages of national parks having investment levels of the total funding for conservation activities.

	All NPs (n = 30)	VNFOREST's NPs (n = 6)	Provincial NPs (n = 24)
Less than 20%	3.33	0	4.17
20–40%	13.33	0	16.67
40–60%	50.00	0	45.83
60–80%	26.67	66.67	25.00
80–100%	6.67	33.33	8.33
Total	100.00	100.00	100.00

List of abbreviations

NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases.

National park staff

The total number of staff within the 30 NPs was 3,127, of which 80% were forest rangers who worked in forest protection stations and 20% in other positions such as administrative and service officers. Each of the 2,501 NP forest rangers administrated an average of 444.26 ha. The mean number of staff per park was 104 persons (SD = 74.87), while 83 persons were forest rangers (SD = 68.65) for each of the 30 NPs. A significant difference in staff numbers was detected between the two groups of NPs (Table 5).

Concerning the educational level of staff, 85% working in 30 NPs had an academic education with graduates from colleges and higher education. On average, 88 NP staff had an academic education (in 30 NPs) while 37 had a non-academic education (in 13 NPs). In addition to the results, a significant difference was found between VNFOREST's NPs and provincial NPs with respect to the staff's academic qualifications (Table 6).

For academic education, Table 7 shows the education background of staff in 30 NPs. NP staff mainly specialised in the fields of forestry, agriculture and fisheries, with approximately 74% of the total number of staff.

On average, 98 members of staff in VNFOREST's NPs had an academic education in the fields of forestry, agriculture and fisheries, but only 57 persons in the provincial NPs did so (Table 8). Significant differences were found between the two groups with respect to the educational background of 'forestry, agriculture, fisheries' and 'biology, ecology, environmental protection' (Table 8).

Table 5. Staff of national parks.

	VNFOREST's NPs (n = 6)		Provincial NPs (n = 24)		p-value†
	Mean	SD	Mean	SD	
Staff	170.00	126.31	87.79	46.78	0.013*
Staff as forest rangers	141.67	125.99	68.79	37.18	0.017*

List of abbreviations

†p-values were calculated using the independent samples t-test between VNFOREST's NPs and provincial NPs; *Significant at p-value < 0.05; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation.

Table 6. National parks staff profile according to the level of education.

Level of education	VNFOREST's NPs			Provincial NPs			p-value†
	n	Mean	SD	n	Mean	SD	
Non-academic	4	63.75	93.96	9	24.56	25.97	0.251
Academic	6	127.50	52.94	24	78.58	44.23	0.027*

List of abbreviations

†p-values were calculated using the independent samples t-test between VNFOREST's NPs and provincial NPs; *Significant at p-value < 0.05; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation.

Table 7. National parks' staff by educational background.

Educational background	Staff (%)	Staff as	
		Forest rangers (%)	Others (%)
Forestry, agriculture, fisheries	74.09	83.54	36.87
Economics, business management, law	13.24	8.94	30.17
Biology, ecology, environmental protection	5.43	4.45	9.31
Tourism	2.72	1.42	7.82
Geography, geology, geographic information system	0.34	0.33	0.37
Archaeology, history, cultural studies	0.08	0.05	0.19
Other majors	4.11	1.28	15.27
Total	100.00	100.00	100.00

Table 8. Groups of national park staff by educational background.

Educational background	VNFOREST's NPs			Provincial NPs			*p*-value†
	n	Mean	SD	*n*	Mean	SD	
Biology, ecology, environmental protection	5	11.60	7.89	17	5.06	5.36	0.043*
Economics, business management, law	5	14.00	6.29	20	14.05	15.52	0.994
Forestry, agriculture, fisheries	6	97.50	38.95	24	57.46	31.22	0.012*
Tourism	3	4.33	2.52	13	4.54	5.62	0.953
Geography, geology, geographic information system	1	2.00	0.00	3	2.33	1.53	0.868
Archaeology, history, cultural studies	0	NA	NA	2	1.00	0.00	NA
Other majors	2	18.50	2.12	10	7.20	9.58	0.140

List of abbreviations

†*p*-values were calculated using the independent samples *t*-test between VNFOREST's NPs and provincial NPs; *Significant at *p*-value < 0.05; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation; NA: Not available.

Cooperation in management and conservation of NPs

It was found that 77% of 30 NPs have cooperated with scientific institutes, e.g. universities and international organisations, in the management and conservation of protected areas. Biodiversity conservation and forest management were the most common cooperative activities observed in NPs, with 77% and 57% of all NPs in them (Table 9). Similarly, 67% of VNFOREST's NPs and 79% of provincial NPs reported collaborative activities in biodiversity conservation.

Moreover, 83% of the 30 NPs indicated that their activities involved volunteers and schools in various nature conservation programmes/projects. These projects mainly concentrated on environmental education and training (70% of NPs), survey work and short work–experience placements (47%) and help with practical conservation tasks (43%).

Table 9. Percentages of national parks reporting collaborations with different activities.

Management tools	All NPs (n = 30)		VNFOREST's NPs (n = 6)		Provincial NPs (n =24)	
	n	%	n	%	n	%
Species monitoring	13	43.33	2	33.33	11	45.83
Forestry management	17	56.67	2	33.33	15	62.50
Eco-tourism management and development	10	33.33	1	16.67	9	37.50
Socio-economic development in buffer zone of the national park	12	40.00	1	16.67	11	45.83
Biodiversity conservation	23	76.67	4	66.67	19	79.17
Education and training	11	36.67	1	16.67	10	41.67
Other activities (e.g. conservation of cultural heritage, historic sites)	8	26.67	1	16.67	7	29.17

List of abbreviations

NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases.

Perspectives on the conservation and management of NPs

The management boards of the surveyed NPs were requested to use a scale from 1 (strongly disagree) to 5 (strongly agree) to score their perceptions of the major causes of limited management capacity and threats to conservation and management of natural resources.

Table 10 shows the major causes of limited management capacity for NPs: The highest average scores were obtained for 'lack of funding for the national park' (4.13) and 'population and resource use pressure within and around the national park' (4.74). The lowest average scores were found for focusing on hard infrastructure instead of conservation activities (2.53) and construction of infrastructure within the NP (2.63). The average scores for 'limited human and institutional capacity of the national park' and 'land use conflict/land grab' were equal at 3.60. NPs also dealt with 'overlapping and conflicting institutional mandates' (3.27) and 'lack of enforcement authority for national park management boards' (3.07). In these results, no significant differences were found between the VNFOREST's NPs and provincial NPs with respect to causes of limited management capacity for protected areas.

Concerning threats to natural resources in NPs, the highest average scores were found for 'illegal hunting, trapping, poaching, fishing' (4.23), 'illegal trade in wildlife' (3.93) and 'illegal logging, firewood collecting' (3.63), indicating that these were the main threats (Table 11). In particular, the highest average score for VNFOREST's NPs was 4.67 for 'illegal logging, firewood collecting', while the highest average score for provincial NPs was 4.21 for 'illegal hunting, trapping, poaching, fishing'. The only significant difference was detected between VNFOREST's NPs and provincial NPs concerned illegal logging and firewood collecting (U = 23, z = 2.51, p-value = 0.009).

Table 10. Major causes of limited management capacity of national parks, rated from 1 (strongly disagree) to 5 (strongly agree), with a score of 3 representing neutral.

	All NPs (n = 30)		VNFOREST's NPs (n = 6)		Provincial NPs (n = 24)		p-value†
	Mean	SD	Mean	SD	Mean	SD	
Lack of funding for the national park	4.13	0.86	4.00	0.89	4.17	0.87	NS
Lack of enforcement authority for national park management boards	3.07	1.23	3.17	1.47	3.04	1.20	NS
Overlapping and conflicting institutional mandates	3.27	1.17	3.17	1.17	3.29	1.20	NS
Focus on hard infrastructure instead of conservation activities	2.53	1.07	2.17	0.98	2.63	1.10	NS
Limited human and institutional capacity of the national park	3.60	0.97	3.33	1.03	3.67	0.96	NS
Population and resource use pressure within and around the national park	4.47	0.68	4.33	0.52	4.50	0.72	NS
Construction of infrastructure within the national park	2.63	0.93	2.50	0.55	2.67	1.01	NS
Land use conflict/land grab	3.60	1.43	3.50	1.05	3.63	1.53	NS

List of abbreviations

†*p*-values were calculated using the Mann-Whitney *U* test between VNFOREST's NPs and provincial NPs; NS: No statistically significant difference; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation.

Table 11. Threats to natural resources in national parks rated from 1 (strongly disagree) to 5 (strongly agree), with a score of 3 representing neutral.

	All NPs (n = 30)		VNFOREST's NPs (n = 6)		Provincial NPs (n = 24)		p-value†
	Mean	SD	Mean	SD	Mean	SD	
Illegal hunting, trapping, poaching, fishing	4.23	0.94	4.33	0.52	4.21	1.02	NS
Illegal trade in wildlife	3.93	1.08	4.17	0.75	3.88	1.15	NS
Illegal logging, firewood collecting	3.63	1.13	4.67	0.52	3.38	1.10	0.009*
Non-timber forest product collection illegally	3.40	1.25	4.17	0.75	3.21	1.29	NS
Mineral exploitation, quarrying	2.17	1.26	2.00	1.27	2.21	1.29	NS
Hydroelectric dam/projects, dams	2.57	1.31	2.83	1.47	2.50	1.29	NS
Developing dykes and canals	2.13	0.97	2.17	0.98	2.13	0.99	NS
Existing and planned routes (roads, motorways, train tracks) crossing national park or situated in its vicinity	3.07	1.29	2.33	1.51	3.25	1.19	NS
Pollution (water, soil, air, noise pollution)	3.60	1.16	3.83	0.98	3.54	1.22	NS

	All NPs (n = 30)		**VNFOREST's NPs (n = 6)**		**Provincial NPs (n = 24)**		**p-value†**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Land use change	3.07	1.26	2.67	1.37	3.17	1.24	NS
Tourism development (overlapping intensive tourism and related pressure to invest in tourist infrastructure in the national park and its vicinity)	2.77	1.07	2.83	0.75	2.75	1.15	NS

List of abbreviations
†p-values were calculated using the Mann-Whitney U test between VNFOREST's NPs and provincial NPs; *Significant at p-value < 0.05; NS: No statistically significant difference; NPs: National parks; VNFOREST's NPs: Vietnam Administration of Forestry's national parks; n: Number of cases; SD: Standard deviation.

Discussion

The results of the study identify interesting aspects regarding the conservation and management of natural resources in 30 NPs in Vietnam. It was found that some NPs were still using outdated management plans. To protect an area over a given period of time, a documented management plan needs to set out the management approach and goals, together with a framework for decision-making (Thomas and Middleton 2003). A comprehensive NP management plan is considered to be vital for the effective management of protected areas and should be accompanied by a number of other plans or related documents and tools, e.g. operational and conservation plans (Thomas and Middleton 2003, Leverington et al. 2008, Worboys and Trzyna 2015, Spoelder et al. 2015). NP managers should accept the need to establish schedules and procedures through periodic review and updating of management plans (Thomas and Middleton 2003); for example, they should revise and update their management plans within a set period (a five-year or 10-year period), during which they could forecast conditions and offer solutions to management challenges into the future. With an updated management plan, NPs could readily adapt to challenges in social and economic development at local and regional levels and protected areas would not be managed as 'islands' in isolation from their surrounding regions (McCuaig 2010).

These findings indicate that updated NP management plans lacked the potential for integrating other tools and plans (e.g. buffer zone development plan). Many NPs lacked any integration of a biodiversity monitoring programme. In this context, confusion might result when determining biodiversity conservation priorities and effective management of natural resources, e.g. the success of activities taken to conserve or recover species and their habitats. The PARC Project (2006) also found that there was no adequate process for management planning according to conservation priorities in Vietnam's protected areas. In the concept of preparing management plans, if the status of biodiversity and other such natural values had been updated, NP planners and managers would perform adaptive management of trends in biodiversity, as well as of impacts on natural resources. Revising and updating the status of management plans within a set period (e.g. 10 years) would be an effective and efficient contribution to NP management and help managers of protected areas in creating a sound investment plan for their priority activities. The evaluation and ranking of objectives and priorities in management plans would allow capacities of NPs such as finance to be allocated. According to the present findings, 10% of NPs indicated that their management plans (i.e. outdated management plans) were not flexible enough to be adapted to current local and regional development plans; for example, 7% reported a lack of coordination amongst agencies and communities in management plans which influenced the effective management and conservation of natural resources. These findings suggest that the cooperation and support of local people is needed for the successful management of NPs. The preparation and development of management plans should gain the support of key stakeholders, particularly from local communities, who would assist in delivering these plans and contribute to the effective implementation of plans in protected areas (Mishra 1994, Thomas and Middleton 2003, Spoelder et al. 2015),

Regarding financial sources, these results showed that central and provincial budgets were crucial funding sources to cover the costs of NP management and conservation. Central and provincial budgets are derived from the state budget of the Government of Vietnam. This result was consistent with Emerton et al. (2006), who stated that NPs in most countries were financed predominantly from state budgets. This was also the case with developed countries, e.g. about 69% of funding for Polish NPs in 2011 came from the public budget (Ministry of the Environment of Poland 2011) and an average of 88% of the Parks Service funding in the United States came from annual budget appropriations (Longley 2016). Emerton et al. (2006) indicated that state budget allocations for protected areas in Vietnam ranged from US$3.0 to 3.5 million per year, an average of 0.1% of Gross Domestic Product and 0.5% of total public budget allocations. Although financial sourcing from state budgets was long-term and covered basic running costs of protected areas, including NPs, it was often insufficient to finance the entire scope of activities within protected areas (Athanas et al. 2001).

In addition to the state budget, significant differences were found between the two groups of NPs with respect to central and provincial budgets (Table 3). The VNFOREST's NPs were mainly funded from the central budget, while the provincial budget supported provincial NPs. Provincial NPs received the central budget which was invested in infrastructure development projects, for example, offices and lodging facilities for staff or roads. In Vietnam, through the annual budgeting process, the Ministry of Planning and Investment was responsible for setting funding levels and negotiating budget allocations with sectoral ministries and provinces, including the state budget for NPs (GoV 2008c). As a result, the budgets for NPs were allocated annually and depended on the balance between the state and provincial budgets. Expenditure of the state budget for the VNFOREST's NPs was higher than for the provincial NPs as the state budget was estimated based on the number of staff in each NP (VNFOREST 2014). Provincial NPs generally had less access to funds than those managed by MARD (Emerton et al. 2012).

Other important sources of funding for the NPs in Vietnam included support from domestic and international organisations, such as the German Society for International Cooperation and Japan International Cooperation Agency, as well as funds from conservation programmes: for example, the Vietnam Conservation Fund (Emerton et al. 2012, USAID 2013, VNFOREST 2014). Despite being dependent upon short-term funding and projects, these sources contributed to significant amounts of financing for conservation activities, enhancing the management capacity of NPs and socio-economic development in buffer zones (VNFOREST 2014). From these survey results, some NPs had valid support from organisations (10%) and funds from conservation programmes (17%). These results suggest that NP managers should enhance their ability to attract additional funding from agencies and organisations, for example, by creating and building a team with successful funding (Athanas et al. 2001).

The Vietnamese NPs generated their own funds as revenues from forest environmental services and tourism activities. Drawing on the concept of payment for environmental services (PFES), NPs reported incomes from PFES payments from such sources as hydropower and water companies (Pham et al. 2013, Trædal et al. 2015). The development of 'innovative' financing mechanisms, such as PFES, provided increasing funding for NPs in biodiversity conservation and, at the same time, generated financial and economic incentives for local communities in buffer zones in protecting forests (Emerton et al. 2012, Pham et al. 2013, USAID 2013).

Tourism, or rather nature-based tourism, could be a particularly effective tool in the conservation and management of protected areas and can bring direct and indirect benefits; for example, tourism not only generated funds for conservation but also shaped people's attitudes to the environment and natural resources (Emerton et al. 2006, Eagles and Hillel 2008, Balmford et al. 2009). Eagle and Hillel (2008) indicated that, despite a fall in the proportion of state budgets allocated to protected areas, revenues from tourism were increasing and it was clear that NPs in Vietnam could increase their share of funding from tourism-based activities. The present study reveals a significant difference between the two groups with respect to revenues from forest environmental services: i.e. VNFOREST's NPs reported a significantly higher revenue than provincial NPs due to the implementation of PFES in NPs; however, no statistically significant difference was found between the two groups with respect to revenues from tourism activities. The results of the interviews indicated that NP managers encouraged benefits from nature-based tourism development, particularly in the economic sphere. However, nature-based tourism was a competitive market and NPs had to offer high quality as well as unique environmental characteristics to succeed in tourism development. The results of the present study suggest that both tourism and forest environmental services were crucial in increasing the funds of NPs and in creating sustainable financial sources of funding for Vietnamese NPs.

The degree of investment for conservation activities in protected areas was reflected in the percentages of financial allocations. The results showed that NPs were interested in biodiversity conservation and had prioritised investment for conservation, e.g. 40–60% of funds were invested in conservation activities in 50% of NPs and 46% of provincial NPs, (Table 4). In particular, all VNFOREST's NPs allocated more than 60% of their funds for conservation activities (e.g. conservation of species and habitat).

However, through interviews, it was found that the financial investment for conservation activities mainly depended on funding being provided to NPs and allocations of financial sources for NPs, indicating that financial allocations for biodiversity conservation in NPs could be changed over the years.

The number and quality of national park staff reflected the capacity of managerial organisation and were central to the success of the management of protected areas. Comparing data from VNFOREST (2014), the total staff size of 30 NPs grew from 2,783 in 2014 to 3,127 in 2016, an increase of 12%. However, it was found that the mean value of land being managed by each forest ranger was 444.26 ha, which was lower than Government guidelines: According to the Government of Vietnam, each forest ranger had to manage 500 ha of protected area (GoV 2010) and so the current number of rangers was deficient and NP managers were obliged to increase their number. VNFOREST's NPs were found to have significantly higher numbers of staff and forest rangers, significantly more highly qualified staff than the provincial NPs. Although 85% of NP staff reported completing an academic degree, the educational background of these staff focused on the field of forestry, agriculture and fisheries (74%), of which 84% of these fields were forest rangers. These findings demonstrated a restricted field of expertise across the group of NPs, e.g. 5% of staff reported academic education in the fields of Biology, Ecology and Environmental protection. The VNFOREST's NPs had significant higher numbers of staff than provincial NPs with qualifications in 'Biology, Ecology, Environmental protection' and 'Forestry, Agriculture, Fisheries'. In total, these results reveal that VNFOREST's NPs have a higher quantity and quality of staff than provincial NPs.

These results found biodiversity conservation in areas associated with nature protection and NP management, such as the conservation of endangered, rare species and their habitats, to be a priority area for cooperative action with scientific institutes. This demonstrates that the priority of NPs is biodiversity conservation. In addition to cooperative activities, there were organisations (specifically international and non-governmental organisations) whose interventions which aimed to promote biodiversity conservation and the management of natural resources in Vietnam (see USAID 2013). Various cooperative activities contributed to enhancing the capacity of NP staff both directly and indirectly and were able to raise financial resources for NPs. Most of the NPs (83%) also involved volunteers and schools in their nature conservation projects, particularly in environmental education and training activities. This finding was consistent with previous studies that volunteers played an increasing role in nature conservation (Wearing 2001, Halpenny and Caissie 2003).

The limited management capacity observed in NPs was attributed to the need for NP managers to deal with the human population growth and the pressure placed on protected areas by resource use (an average score of 4.47). In Vietnam, ca. 31% of population live in or near forests and depended for some part of their subsistence on forest resources (GoV 2005). More than 80% of protected areas were inhabited and the populations in their buffer zones were increasing (PARC Project 2006). The rapid population growth near protected areas was one of the main causes for the loss of biodiversity identified in the country (GoV et al. 2010). Through interviews, it was found that the livelihoods of local communities living within and around most of the NPs heavily depended on agricultural activities (e.g. cultivation and livestock rearing) and the extraction of forest products (e.g. collection of non-timber forest products). The human population growth and livelihoods of local communities were also major threats to natural resources and NP management (Nguyen Huynh Thuat and Yen Hoang Mai 2013, Le Quy Minh 2013, Duong Van Hung 2013).

Strong dependence on the state budget resulted in a lack of sufficient funds for protected areas and limited management capacity for NPs. Even in some NPs, funds only covered full operation and maintenance costs (ICEM 2003). The funds for protected areas tended to be concentrated more on infrastructure development and, to lesser degree, on operations and maintenance, than on investing in conservation activities (ICEM 2003, Ha Thi Mung and Tuyet Hoa Nie Kdam 2008, VNFOREST 2014). Facing situations with insufficient funds, it was difficult to implement long-term commitments in conservation and management of protected areas, as well as maintaining the existing condition of NPs.

Limited human and institutional capacity of NPs and the incidence of land grab, both with a mean score of 3.60, were barriers reported by NP managers. This finding was consistent with previous reports that NPs had to contend with human resources problems, such as a lack of quantity and limited quality of staff working for biodiversity conservation, as well as addressing forest environmental services and nature-based tourism, international cooperative activities and publicity and education for communities (MONRE 2014, VNFOREST 2014). This was reflected in the ratio between the educational fields of the employees being biased towards the fields of Forestry, Agriculture and Fisheries (Table 7). Land use conflicts and land grabbing occurred on NPs because of the presence of overlapping boundary phenomena (VNFOREST 2014), forest conservation policy and disjunction between the objectives of conservation and the livelihoods of communities (To Xuan Phuc 2009, Nguyen Huynh Thuat and Yen Hoang Mai 2013).

In the context of management and organisation structure, NP managers indicated some overlapping and conflicting institutional mandates (with an score of 3.27). As discussed by Nguyen KimDung et al. (2012) and VNFOREST (2014), no clear division was found between functions and tasks for managing NPs, especially provincial NPs, with the areas of responsibility being overlapping, fragmented and unclear. For example, although the provincial Department of Agriculture and Rural Development received technical instructions from their national line ministries and manages the expertise and professional work of the NPs, they were however accountable to the Provincial People's Committee. Both MARD and MONRE had a mandate to manage NPs but with different institutional objectives such as biodiversity conservation (GIZ and MARD 2012, USAID 2013). The responsibility for NPs with other designations, such as marine protected areas, Ramsar sites, ASEAN Heritage Parks and UNESCO Biosphere Reserves, was divided between MONRE, responsible for conservation and protection of wetlands, AHPs, UBRs (GoV 2003, MONRE 2004, GoV 2017) and

MARD, responsible for developing the system of MPAs (GoV 2008); in addition, MARD maintained control for protection of these protected areas. The Ministry of Culture, Sport and Tourism together with MARD had the responsibility for managing cultural, historical and environmental sites (e.g. NPs as UNESCO World Heritage Sites) and developing the country's tourism strategy and promoting tourism in the NPs (GoV 2013). The lack of enforcement authority for NP management boards influenced the capacity of NP managers (a mean score of 3.07). This finding was consistent with that of the PARC Project (2006), which noted that management boards had not been given the authority and support required to effectively carry out their duties. The authority and responsibility of management boards were unclear and there was no guidance on board structure.

The focus on hard infrastructure and the construction of infrastructure within protected areas resulted in limited management capacity for NPs. Except for office buildings, 11 out of 30 NPs had a lack of necessary infrastructure (e.g. facilities for scientific research, tourism service and environmental education centre) (VNFOREST 2014). Infrastructure in NPs should be upgraded to support managerial activities by, for example, the provision of office buildings. The PARC Project (2006) found that more than 60% of the state budget for protected areas went to infrastructure development, particularly in chronic underfunding of protected areas under the jurisdiction of Provincial People's Committees (USAID 2013). Infrastructure construction within NPs (e.g. roads, dams and dykes) led to an adverse effect on ecosystem functions, increasing human access and habitat fragmentation (USAID 2013, MONRE 2014).

Regarding threats to NP natural resources, the highest average score (4.23) was awarded to illegal hunting, trapping, poaching and fishing. This was consistent with Nguyen (2009), who noted that illegal hunting appeared to be the most serious threat to wild cattle in Cat Tien and Yok Don NPs. With demand for wildlife and wildlife products and pet trade expected to increase, pressures on illegal hunting, trapping, poaching and fishing were increasing and were threatening the existence of species and conservation of biodiversity in NPs (MARD 2003, Nuwer and Bell 2013). Species populations, particularly those of endangered species, had been continuously declining as a consequence of illegal hunting (Nguyen Manh Ha et al. 2007) and this decline in some cases was responsible for the extinction of species (Bennett et al. 2002), such as in the case of Javan rhinos in Vietnam (Baraniuk 2016). With a mean score of 3.93, the illegal trade in wildlife was the second most serious threat to many species in NPs and undermined efforts to protect natural resources. Nguyen Manh Ha et al. (2007) indicated that the number of illegal trade and violations of wildlife trade had been recorded as very high in provinces and regions of Vietnam. The illegal wildlife trade has continued unabated. Vietnam was a thriving wildlife market and an important crossroads for the illegal wildlife trade from South-East Asia to neighbouring countries (Nguyen Van Song 2008). Additionally, major sources of illegal wildlife trade are protected areas or NPs.

Illegal logging and firewood collecting were the third serious threat in NPs, even the most serious threat in VNFOREST's NPs (an average score of 4.46). The limited amount of forest cover and the high demands for timber and wood products played a large role in encouraging illegal logging in Vietnam (McElwee 2004). In particular, Vietnam was one of five principal suppliers of illegal tropical hardwood in the Asia-Pacific region to China's wood imports (Jiao 2016). Illegal logging had a negative impact on NPs, affecting the native environment and the species within them, as well as resulting in economic and social consequences. The practice resulted in biodiversity depletion, soil erosion and enhanced carbon dioxide emissions (Humphreys 2016).

Moreover, the illegal harvesting of non-timber forest products (NTFPs) (e.g. medicinal plants, honey bees and bamboo shoots) threatened the natural resources of the NPs. As commercial demand increased, harvesting rates intensified and over-exploitation of NTFPs occurs, wrecking the ecology, biodiversity and habitats of species. For example, honey was collected for both local utilisation and commercial sale and this practice had been the cause of forest fires which threatened the conservation of species in Pu Mat NP (Luu Tuong Bach and Rawson 2011).

Environmental pollution had an influence on an ecosystem, biodiversity losses and degradation of NPs. For example, water pollution could lead to the destruction of natural habitats (MONRE 2014). Environmental pollution occurred due to anthropological activities in NPs' buffer zones and overheated economic development (Haneji et al. 2014, Khai and Yabe 2014). The rapid development of industries and an increase in shrimp farming resulted in biodiversity losses in wetlands such as Tram Chim NP (Khai and Yabe 2014).

Land use change, existing and planned routes, having the same average scores of 3.07, threatened biodiversity and natural resource management. The conversion of natural forests and wetlands to other forms of land use (e.g. agriculture and aquaculture, industrial plantations, construction land) has led to fragmentation of ecosystems and natural habitats and contributed to the degradation and loss of biodiversity (MONRE 2014, Khai and Yabe 2014). Existing and planned routes increased access to protected areas and added to the fragmentation of forests, wetlands and other isolated natural habitats (USAID 2013). For example, the construction of Cam Lo – Tuy Loan highway, a section of the Ho Chi Minh Trail, crossed the core zone of Bach Ma NP (with a total of 9 km and 49 ha of forests) (Vietnam News 2016), influenced biodiversity losses and habitat fragmentation. In many cases, routes are implicated in the illegal trade of wildlife and logging (McElwee 2004, Nguyen Manh Ha et al. 2007).

Developing dykes, canals and hydroelectric/dam projects had an impact on the loss of biodiversity resources in NPs, such as habitat loss and fragmentation (Carew-Reid et al. 2010, USAID 2013). Illegal mineral exploitation appeared to be a threat to the environment and conservation of wildlife in some areas of NPs such as illegal gold mining activities in the Pu Mat and Chu Mon Ray NPs (Luu Tuong Bach and Rawson 2011, Dinh Chieu 2017). Mineral exploitation in NPs' buffer zones affected the ecosystem and habitat of species. For example, illegal sand mining, upstream of Dong Nai River, the Cat Tien NP's buffer zone, has affected the natural flow of water into the river, causing landslides and soil creep and has had an adverse influence on animals in the park with noise and air pollution (Dinh Du et al. 2017).

Despite scoring 2.77, tourism development could be a potentially important threat to natural resources in NPs. Nature-based tourism might have negative impacts on protected areas, affecting both the environment and species within them (Steven et al. 2011). Increasing numbers of tourists could threaten the fragile ecosystems of NPs with, for example, their accompanying increased accumulation of waste (Nguyen Huynh Thuat and Yen Hoang Mai 2013). The development of tourism infrastructure influenced environmental degradation and habitat fragmentation (Nguyen Huynh Thuat and Yen Hoang Mai 2013, Duong Van Hung 2013). In addition, the lack of a tourism development plan could threaten the sustainability of NPs (Le Quy Minh 2013). On the other hand, tourism might also have a considerable potential for raising funds for NPs, therefore contributing to biodiversity conservation and management of natural resources (Athanas et al. 2001, Emerton et al. 2006, Eagles and Hillel 2008, Balmford et al. 2009; Schägner et al. 2016).

Conclusion

Vietnam's NPs function within the system of special-use forests, which is considered the backbone of the national strategy for nature protection in the country (PARC Project 2006). Many NPs or areas within NPs are recognised internationally: one is a UNESCO World Heritage site, nine are included in the UNESCO World Network of Biosphere Reserves and areas within seven NPs are protected under the Ramsar Convention. Five NPs are recognised regionally as the ASEAN Heritage Parks. Five NPs are in the national system of Marine Protected Areas.

These findings identify some challenges faced by the conservation and management of natural resources in 30 NPs, including six VNFOREST's NPs and 24 provincial NPs. The results showed that about 87% of management plans of NPs had been updated. Financial sources for NPs mainly came from the state budget and most of NPs spent about half of their funds on conservation activities. Despite principally depending on the state budget, NPs had opportunities for increasing funding for their conservation and management; for example, NP managers could increase their own funds from nature-based tourism development. Also, having varied sources of funding could help NP managers in their management decisions and ensure the effective implementation of long-term commitments in conservation activities and natural resource management.

Most NPs' staff were found to have academic degrees. However, this educational background was found to offer little variety across disciplines. Hence, NP managers should continuously concentrate on the development of human resources, including the quantity and quality of staff for undertaking the assigned tasks effectively. In Vietnam's NPs, biodiversity conservation was a priority cooperative action with scientific institutes.

The present findings indicate the most common causes of limited management capacity of NPs to be (1) the pressure placed by the human population growth and resource use pressure within and around protected areas, (2) the lack of funding, (3) limited human and institutional capacity and (4) land use conflict/land grab. These results also indicated that (1) illegal hunting, trapping, poaching and fishing; (2) illegal wildlife trade; and (3) illegal logging and firewood collecting were regarded as the most serious threats to natural resources in NPs. Significant differences were also found between the VNFOREST's NPs and provincial NPs with respect to financial sources of funding (the central and provincial budgets, revenues from forest environmental services) and staff. Except for the threat of illegal logging and firewood collecting, no significant differences were detected between the two groups with respect to causes of limited management capacity and threats to natural resources.

Further research can build upon the findings of this study to seek solutions and strategies for effective management of NPs. The findings of this study partly may provide useful information for protected area planners, managers and policy makers, as well as researchers and allow them to more effectively manage and conserve the biodiversity of Vietnam's NPs. It is hoped that this study will support the effective management of NPs and the sustainable management of natural resources and biodiversity protection in Vietnam.

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Appendix I

Questionnaire for the survey on conservation and management of the national park.

I. General information about the national park	
1. Name and location of the national park	
a) Name of the national park: …………………………… b) Location: ……………..…District…….………………Province……….	
2. Who is responsible for the administration of the national park? *(Please mark (√) only one appropriate box)*	
□ Provincial People's Committee (Please specify…………………….………….) □ Vietnam Administration of Forestry, Ministry of Agriculture and Rural Development	
II. Area and designations	
3. What is the area of the national park and its buffer zones?	
a)…………….... ha of the total area of the park, including ……….ha of marine area b)………………ha of buffer zones, of which marine area covers ..………..ha	
4. Other designations of the national park,	
a) Was the national park or national park areas recognized/designated regional/international agreements? *(Please mark (√) only one appropriate box)*	
□ NO □ YES, Go to b)	
b) Please select regional/international designations? *(You may choose more than one by marking (√) appropriate boxes)*	
□ UNESCO Natural World Heritage Sites □ UNESCO Biosphere Reserves □ Wetlands of International Importance (Ramsar Sites) □ Others (Please specify……………………………………)	□ ASEAN Heritage Parks □ Marine Protected Areas
II. Management Plan	
5. Concerning the national park management plan,	
a) Which updating status of the management plan has been applied for current management of the national park? *(Please mark (√) only one appropriate box)*	
□ Outdated Management Plan □ Updated Management Plan □ Management Plan updated by more management tools. Go to *b)*	
b) Please select other management tools as flows: *(You may choose more than one by marking (√) appropriate boxes)*	
□ Community based forest management □ Multiple – use forest management plan	□ Forest valuation □ Forest monitoring programme
□ Environmental impact assessment □ Regulations for research activities in the national park □ Sustainable eco-tourism/tourism development plan □ Others (Please specify………………………………………)	
6. In the current context of socio-economic development,	
a) Does the management plan provide a sufficient protection in relation to developmental plan(s) of local communities and region(s)? *(Please mark (√) only one appropriate box)*	
□ YES □ NO. Go to *b)*	
b) If NO, it is a result of *(You may choose more than one by marking (√) appropriate boxes)*	

□ Lack of constantly updated debates
□ Lack of coordination among agencies and communities that have a bearing on the park
□ Confusing, conflicting and overlapping institutional and legal frameworks
□ Non-existence of mechanisms and strategies to engage communities in the conservation of the park
□ Others (Please specify………………….)

III. Financial sources

7. Concerning financial sources of the total funding for the national park in 2016,

a) What is the percentage of financial sources of the total funding?
(You may choose more than one by marking (√) appropriate boxes)

Financial sources	Percent	If possible, please specify in VND million
□ The state budget		
□ The provincial budget		
□ Supports from domestic organizations (Please specify………………………………………)		
□ Supports from international organizations (Please specify………………………………………)		
□ Funds of conservation programs (Please specify………………………………………)		
□ Revenues from forest environmental services		
□ Revenues from tourism activities		
□ Others (Please specify………………………………………)		
Total	100	

b) What is the percentage of the total funding for the national park invested in conservation activities in 2016?
(Please mark (√) only one appropriate box)

□ 10	□ 20	□ 30	□ 40
□ 50	□ 60	□ 70	□ 80

□ Other (Please specify ………… %)

IV. Cooperative activities

8. Have the national park cooperated with scientific institutes in conservation and national park management?
(Please mark (√) only one appropriate box)

□ NO
□ YES. Go to *b),* and *c)*

b) If YES, which scientific institutes?
(You may choose more than one by marking (√) appropriate boxes)

□ Vietnamese Academy of Forest Sciences
□ Vietnam Academy of Science and Technology
□ Domestic universities (Please specify………………………………………)
□ Foreign universities/institutes (Please specify………………………………………)
□ NGOs (Please specify………………………………………)
□ Others (Please specify………………………………………)

c) What kind of cooperative activities have been cooperated with scientific institutes in the national park?
(You may choose more than one by marking (√) appropriate boxes)

□ Species monitoring	□ Education and training
□ Biodiversity conservation	□ Forestry management
□ Eco-tourism management and development	□ Cultural heritage conservation

□ Socio-economic development in buffer zone of the national park
□ Others (Please specify………………………………………)

9. For conservation programmes/projects in the national park,

a) Has the national park involved schools, volunteers in various projects?
(Please mark (√) only one appropriate box)

□ NO
□ YES. Go to *b)*

b) If YES, please specify in which?
(You may choose more than one by marking (√) appropriate boxes)

□ Environmental education and training/Education services
□ Help with practical conservation tasks
□ Survey work and short work – experience placements
□ Others (Please specify……………………………………)

10. How many academic papers were published by park's staff in the previous 5 years, including co-authors of papers?
(Please mark (√) only one appropriate box)

Subject area of	Journals	Books	Other papers (Please specify…………)
□ Biology, ecology, nature conservation			
□ Economics, business management, law			
□ Forestry, agriculture, fishery			
□ Tourism			
□ Geography, geology, geomorphology			
□ Archeology, history, cultural heritage conservation			
□ Others (Please specify……………)			

V. Causes of limited management capacity

11. Please tell us how you feel about the following statement concerning causes of limited management capacity of the national park towards conservation and natural resource management?
(For each statement, please circle the number that is appropriate under one of the five categories which applies SD - Strongly disagree, D - Disagree, N - Neutral, A - Agree, SA - Strongly agree)

	SD	D	N	A	SA
a) The park suffers from a lack of funding for conservation activities and national park management	1	2	3	4	5
b) The park suffers from a lack of enforcement authority for national park management boards	1	2	3	4	5
c) There are overlapping and conflicting institutional mandates	1	2	3	4	5
d) The park has focused on hard infrastructure instead of conservation activities	1	2	3	4	5
e) The park suffers from a lack of limited human and institutional capacity	1	2	3	4	5
f) There is the population growth and resource use pressure within and around the park.	1	2	3	4	5
g) There is under construction of infrastructure within protected areas	1	2	3	4	5
h) There is suffering from land use conflict/land grab	1	2	3	4	5

VI. Threats to natural resources

12. Please tell us how you feel about the following statement concerning current threats to biodiversity and natural resources in the national park?
(For each statement, please circle the number that is appropriate under one of the five categories which applies SD - Strongly disagree, D - Disagree, N - Neutral, A - Agree, SA - Strongly agree)

	SD	D	N	A	SA
There is illegal hunting, trapping, poaching, fishing	1	2	3	4	5
There is illegal trade in wildlife	1	2	3	4	5
There is illegal logging, firewood collecting	1	2	3	4	5
Illegal non-timber forest product collection is present	1	2	3	4	5
Mineral exploitation or quarrying is present	1	2	3	4	5

There are hydroelectric dam/projects, dams	1	2	3	4	5
Dykes and canals are under development	1	2	3	4	5
Existing and planned routes (roads, motorways, train treks) cross the park or are situated in its vicinity	1	2	3	4	5
There is pollution (water, soil, air, noise pollution)	1	2	3	4	5
There is land use change	1	2	3	4	5
There is the tourism development (overlap intensive tourism and related pressure to invest in tourist infrastructure in the national park and its vicinity)	1	2	3	4	5

VII. The national park staff

13. How many employees work in the national park, according to level of education and educational background?
(You may choose more than one by marking (√) appropriate boxes)

	The total number of staff	Staff as forest rangers	Staff as others
a) Levels of education of national park staff			
□ Unlearned			
□ Primary school and lower			
□ Intermediate school			
□ High school			
□ College graduates and higher. Go to *b)*			
b) National park staff by educational background in the field of			
□ Biology, ecology, environmental protection			
□ Economics, business management, law			
□ Forestry, agriculture, fishery			
□ Tourism			
□ Geography, geology, geographic information system			
□ Archeology, history, cultural heritage conservation			
□ Other majors (Please specify……………)			

VIII. General information on the respondent

***All personal information will be confidential*.**

a) Position in the national park: …………………………………..
b) Email address: …………………………………..
c) Telephone number: ………………………………
d) Date of response: …………………………………

If you wish to provide feedback for improvement of this survey, please add any further comments/ suggestions below.

Appendix 2

Questions for interviews with members of national park management boards

1. What do you think about the development of ecotourism/nature-based tourism in the national park as alternative livelihoods of local residents who live within and around the park?
2. What are your opinions about trends of financial sources on funding for the park as well as conservation activities and natural resource management?
3. What do you think about the roles of the park's own fundraising (e.g. revenues from tourism activities) as well as its financial autonomy in the contribution to conservation and management of the park?

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Conservation and trade of wild edible mushrooms of Serbia – history, state of the art and perspectives

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Abstract

Wild edible mushrooms have received significant scientific and socio-economic attention in the last few decades, since they have become the subject of a booming trade business. Through last decades, Serbia, a small country positioned in the South East of Europe, has become a source country for extensive export of commercially important species of wild mushrooms. The data used for international analyses of national policy on mushroom protection and trade are cited usually from personal communications and therefore are not really reliable. Extensive investigations into diversity or ecology of macro fungi in Serbia have never been undertaken. The forestry sector, which is managing all forests in the country, has absolutely neglected its role in ecosystems while habitats of macro fungi have been permanently destroyed. There are only two legal acts that refer to mushroom protection directly and none aims to protect their habitats or diversity in practice. In this contribution, a comprehensive review of official data on research, conservation, socio-economic importance and legislation on wild edible mushrooms and truffles in Serbia was provided. Additionally, the application of existing legal acts on conservation of macro fungi and data on wild mushroom trade in the period between 1993–2016, during which time the trade control has been initiated was analysed. The currently valid system of conservation and trade control are discussed in the frame of protection of wild mushroom species and their habitats and measures for upgrading this system in order to meet the requirements of the sustainable use of natural resources in the socio-economic conditions of Serbia are proposed.

Keywords

Wild mushrooms conservation, wild mushrooms trade, mushrooms of Serbia, truffle trade in Serbia

Introduction

Mushrooms have probably been a part of the human omnivore diet ever since humans have evolved as a species. Actually, it is quite possible that many fungal species developed the highly nutritious sporocarps concurrently with the evolution of omnivores, as a very small number of animal species has been reported to be strictly mycophagous (Witte and Maschwitz 2008). This group, often called macro-fungi, includes all species that produce sporocarps larger than 1 mm. Through human evolution, fungal species that produced edible sporocarps may have served as a good source of food in the specific times of the temperate seasons (as in Europe) – they could be gathered for immediate consumption or dried and preserved for cold seasons when nutrient rich food was scarce (Peintner et al. 2013, Heilmann-Clausen et al. 2015). Once, gathering of mushrooms was a necessity, now it is a relaxing hobby or a good business, depending on the country's resources and socio-economic status. Regarded at one time as staple food, mushrooms have now become a luxury and a delicacy.

Due to the concentration of inhabitants in large cities and social evolution that defines working time, the habit of collecting mushrooms for food in Europe became unavailable for the majority of those who consume them. Even though many of the saprotrophic species could be easily cultivated, very few are part of the usual European diet – in the market, the white button mushroom (*Agaricus bisporus* (J. E. Lange) Imbach), oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm) and Asia-origin shii-take (*Lentinula edodes* (Berk.) Pegler) dominate (Valverde et al. 2015). The ectomycorhizal (ECM) species, that are far more appreciated, are still collected from their natural habitats and that is why they fetch far higher prices. For that reason, in some European countries, the wild mushroom trade has become a huge business in the last few decades (Sitta and Floriani 2008). Here it is mainly referred to species of porcini (*Boletus edulis* Bull.:Fr., *B. aereus* Bull.:Fr., *B. reticulatus* Schaeff. and *B. pinophilus* Pilát & Dermek), chanterelle (*Cantharellu scibarius* Fr.:Fr., *Craterellus lutescens* (Pers.: Fr.) Fr.), morels (*Morchella deliciosa* (Fr.: Fr.) Quélet, *M. vulgaris* (Pers.: Fr.) Boudier, *M. esculenta* (L.: Fr.) Pers.) and milky caps (*Lactarius deliciosus* (L.: Fr.) S.F. Gray, *L. deterrimus* Gröger). The most expensive of them are certainly European truffles (*Tuber magnatum* Pico, *T. melanosporum* Vitt., *T. aestivum* Vitt., *T. brumale* Vitt, *T. borchii* Vitt.).

The expansion of commercial harvesting in Europe has resulted in the introduction of national, regional and even communal regulatory and licensing systems in several countries, but with significant differences (Brainerd and Doornbos 2013). In Scandinavia, fungi gatherers have open access and can pick as long as they do not harm property (Saastamoinen 1999). Finland promotes the greater harvesting of fungi as an under-utilised resource (Härkönen and Järvinen 1993), while, in the Netherlands, gathering of fungi is strongly discouraged through codes and local acts (Arnolds 2001). In France and Italy, there are gathering permits and timing and the volume of harvest is regulated through daily limits and harvesting calendars depending on regional regulations (in France there are no national rules, Regis Courtecuisse, personal communication). In some regions in Italy, this is complemented by the requirement to pass a proficiency test (Brainerd and Doornbos 2013). The aim of this paper was to review diversity, conservation, socio-economic importance and legislation on wild edible mushrooms and truffles in Serbia from different perspectives, for the first time presenting a realistic dimension for research, harvesting and trade. In order to make available all the written data on the topic, existing publicly available recourses and also some personal testimonies where the written data were missing were listed.

Methods

The review first describes the environment that has determined the state of fungal communities in Serbia: country position, nature, climate and socio-economic state concerning the topic. All research data that could be detected have been briefly presented on macro fungi as well as the regulations concerning mushroom conservation and trade. In the second part of the paper, some analyses have been undertaken to enable direct insight into the efficiency of the country's legislation system concerning mushroom trade.

In order to visualise the realistic state of knowledge on macro fungal diversity, data were used from available literature to compile the map of areas that have been investigated so far. To compare these data to the level of usage of forests for mushroom harvesting, the points of mushrooms purchase from the gatherers were added, based on the data obtained from the Institute for Nature Conservation of Serbia (INCS). Records from historical literature were not included in this presentation due to the unreliable nomenclature and imprecision of findings. Truffle distribution was illustrated elsewhere (Marjanović et al. 2010a).

In order to analyse the efficiency of the current system of regulation for the collection and trade with wild mushrooms, all data that could be obtained from the public institutions have been used. The data provided by INCS include: the annual quota (AQ, the annual amount that can be permitted for harvesting); the amounts that were permitted for harvesting and trade for each year from the beginning of the regulation in 1993 up to the year 2016 (AGH, annual gross harvest); the data on the purchasing points (affiliated addresses where the trading companies buy mushrooms from the harvesters, which can be subject to inspection) and numbers of companies that applied for permission for the period 2006–2016. As almost all mushrooms gathered in Serbia are exported, the authors included in their analyses the data obtained from the Customs service of Serbia on annual gross export (AGE) and annual gross values (AGV) of mushroom exports in Euros, as provided in the export documents. In order to avoid misinterpretation of the presented data due to the weather influence on mushroom production, the data on annual average maximal rainfall (AMR) for the country obtained from the Institute of Hydrology and Meteorology of Serbia were included. For comparison of the data, correlation coefficients were calculated using the software Microsoft Excel 2007.

Results

The positioning and nature of Serbia

Serbia is located in the heart of the Balkan Peninsula, a region not geologically old, but with a geographic position and geologic, topographic and climatic diversity that have produced an environment conducive to very high biodiversity rates at species, community and ecosystem levels (Stevanović et al. 1995, Myers 1999, Figure 1). The northern part - province Vojvodina belongs to the flat Pannonian Basin with a typical semi-arid continental climate. Central Serbia is a hilly depression surrounded by high mountains of differing origins and ages, with a sub-mediterranean, semi-arid, mild climate. Mountains produce a rain shadow and humidity gradient from the humid climate with a strong mediterranean influence in the western part of the country (average annual precipitation 720–900 mm,1500 mm in the mountains), to the very arid and warm climate in the south-east (650–700 mm, 1000 mm in the mountains). Average yearly temperatures vary between 9.5–11.7 °C in the lowlands (0.5–5 °C in the mountains). The coldest month is January with averages between – 0.6 °C in mountains and 0°C in lowlands, while the warmest is July with averages 11–22 °C in the lowlands and 11–16 °C in the mountains. The Northeast receives only 520–590 mm of precipitation yearly and the temperature difference between annual minimum and maximum can be as much as 80 °C. More than 20 different soil types have so far been recorded in Serbia.

The natural vegetation of Serbia consists primarily of temperate forests dominated by numerous ECM species of Fagaceae and few species of Pinaceae. Native lowland forests are dominated by pedunculate oak (*Quercus robur* L.) mixed with poplars (*Populus alba* L., *P. nigra* L.) and ash (*Fraxinus angustifolia* Vahl, F.) in wet areas and linden (*Tilia cordata* Mill.) and maples (*Acer sp.*) in drier soils. Most hilly regions are dominated by various oaks (*Querscus cerris* L., *Q. frainetto* Ten., *Q. petrea* (Matt.) Liebl., *Q. pubescens* Willd.), but also hornbeams (*Carpinus betulus* L., *C. orientalis* Mill.*, Ostrya carpinifolia* Scop.), linden (*Tilia argentea* D.C.) and hazelnuts (*Corylus avellana* L.*, C. colurna* L.). Typical European forests dominated by European beech (*Fagus sylvatica* L.) or Norway spruce (*Picea abies* (L.) H.Karst.), occur at the higher elevations, while the uppermost regions can be inhabited by some endemic pines (*Pinus heldreichii* Christ.*, P. peuce* Griseb., *P. mugo* Turra). The majority of the native vegetation has been continuously destroyed since human civilisation appeared in Europe. The percentage of the preserved forests varies from there being almost no forests in the areas of intense agriculture (northern flat areas and river valleys) to the somewhat forested mountains, National parks and hunting reservations.

Brief overview on published scientific data on biodiversity of macro fungi of Serbia

What marks the written scientific work on macro fungi in Serbia is the fact that there has never been any serious mycological taxonomic literature written in Serbian or any of the languages spoken in the ex-Yugoslavian republic, in which Serbia until recently

Figure 1. Geographical position of Serbia in the Balkan Peninsula.

belonged. All investigations on diversity of epigeic macro fungi of Serbia were based on sporocarp descriptions (Listed in supplementary material 1, Figure 2). The vegetation types present in Serbia are very well characterised (Kojić et al. 1998), but the knowledge on fungal communities within those vegetation types is minimal (See supplementary material 1). Unfortunately, authors with the background in forestry in Serbia consider forests exclusively as timber production or hunting tourism areas (See supplementary material 1). The importance of macro fungal saprotrophs in forest ecosystems is not recognised in their work (wood degrading species were usually treated as pests), while the existence of ECM has only recently been documented (Katanić et al. 2015a, b). The single experimental attempt of using seedlings inoculated with ECM fungi in forestry was in remediation of polluted soils (Karličić et al. 2016). Finally, the cooperation between forestry specialists and biologists on this topic is almost non-existent (Marjanović 2000).

Unlike other macro fungi, interest and research on truffles has been flourishing in the last two decades (details in supplementary material 1). The check-list of 12 species

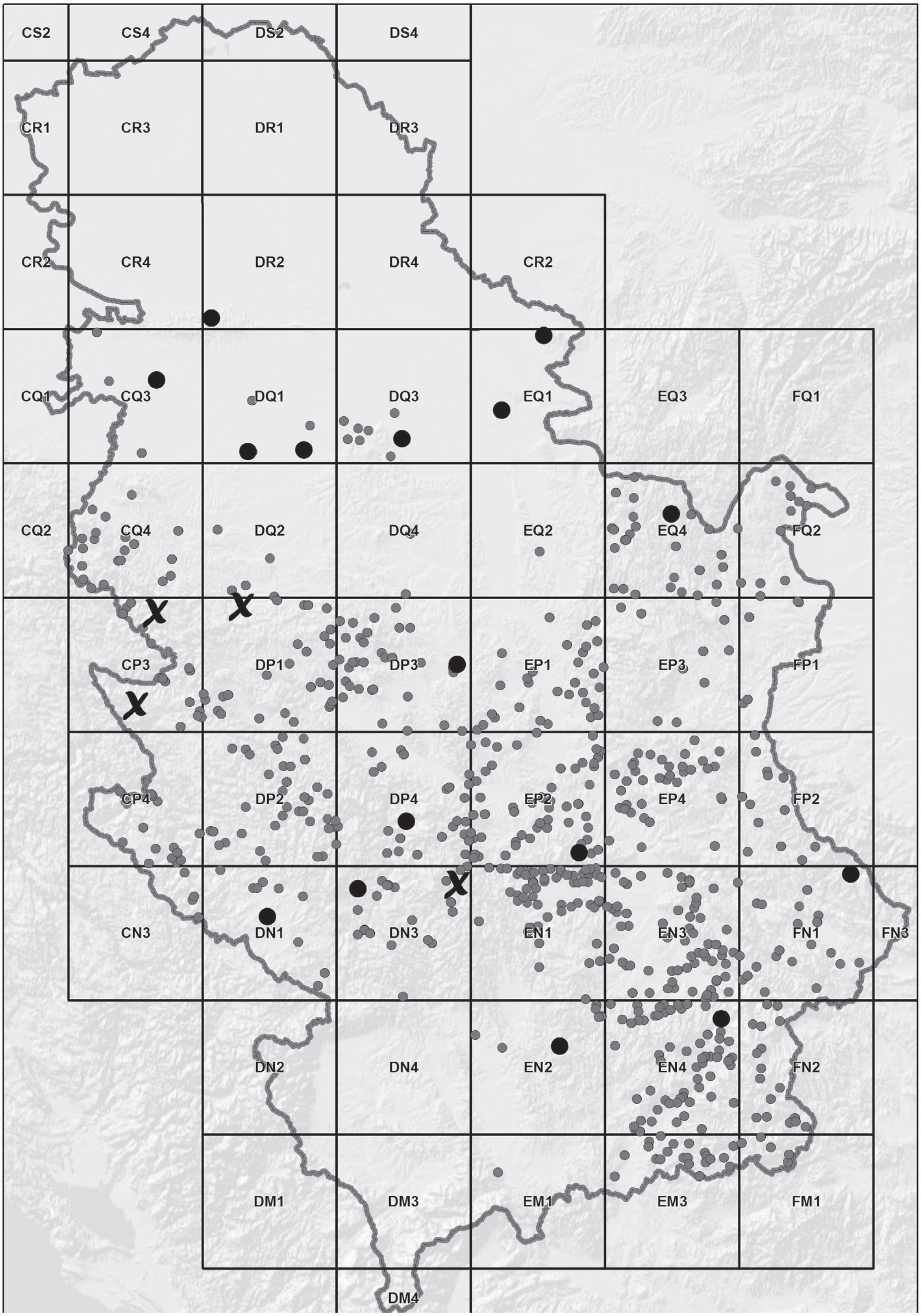

Figure 2. Mushroom research and purchase map**.** Small dots - points of epigeic mushrooms purchase from the gatherers based on the data obtained from the INCS; big dots - data on epigeic macro fungal diversity existing; X–diversity and ecology data existing.

of true truffles have been described using morphological features of ascocarps combined with the analysis of rDNA sequences (Marjanović et al. 2010a) and a new species from the mountain Tara was described (Milenković et al. 2016). Detailed descriptions of ecological features and habitats of the economically most important truffle species in Serbia (*Tuber aestivum* Vitt. and especially *Tuber magnatum* Pico) have been published recently (Marjanović et al. 2010a,b, 2013a,b, 2015, Bragato and Marjanović 2016). The single official national collection of fungal dry exsiccates is in the Natural Museum in Belgrade (See supplementary material 1), while so far, the authors are not aware of any official attempts about forming the national collection of macro fungal mycelia.

Socio – economical importance of wild macro fungi in Serbia

Within the authors' knowledge, there have been no data on using mushrooms in the local diet by inhabitants in the territory of present-day Serbia until the 19th Century, when the first written data on mushrooms appeared (See supplementary material 1). The long-lasting impoverishment of the inhabitants, caused by the previous rule of Ottomans (14–19th Century), probably induced the widespread usage of forests products, but the written data on that is scarce. Nowadays, mushrooms are normally gathered by people who enjoy eating them (hobbyists, inhabitants of the rural parts), but the major reason for harvesting mushrooms is gaining income by selling them to the trading companies. Only symbolic amounts of the wild mushrooms are consumed within the country. The species widely used in the local cuisine and sold on the local markets are: *Boletus edulis* Bull..:Fr*, B. reticulatus* Schaeff., *B. aereus* Bull.:Fr.*, Cantharellus cibarius* Fr.: Fr., *Lactarius piperatus* (L.: Fr.) Pers., *Amanita caesarea* (Scop.: Fr.) Pers., *A. rubescens* (Pers.: Fr.) Pers., *Macrolepiota procera* (Scop.: Fr.) Singer, *Laetiporus sulphureus* (Bull.: Fr.) Murrill, *Russula cyanoxantha* (Schaeff.) Fr., *R. virescens* (Schaeff.) Fr. *and Agaricus campestris* L.: Fr. (Marjanović, Ž. unpublished).

Harvesting and trade with economically important species of mushrooms started in the early seventies of the last century, when Serbia, one of the least developed countries of Europe, with its turbulent war history and political instability, became one of the source countries for edible mushrooms (Sitta and Floriani 2008). Italy began importing fresh porcini from Yugoslavia during the early 20th century and, by 1930, commercial imports of fresh porcini had reached significant levels (Bellini 1933). According to Sitta and Floriani (2008), Serbia has been an important exporter of fresh and dried mushrooms to Italy due to the high quality of products. The economical breakdown of the country and extreme poverty of inhabitants during 90s pushed many of them to search for income in collecting species of interest for trading companies. At the time, social events called "The Days of Mushrooms" started to be organised all over the country with the goal of educating and providing wide publicity on recognising edible mushrooms. Subsequently, an increasing number of people started to collect them for personal use or for sale, while wild mushrooms harvesting in Serbia became an important business and the number of mushroom trade companies has expanded (Figure 3).

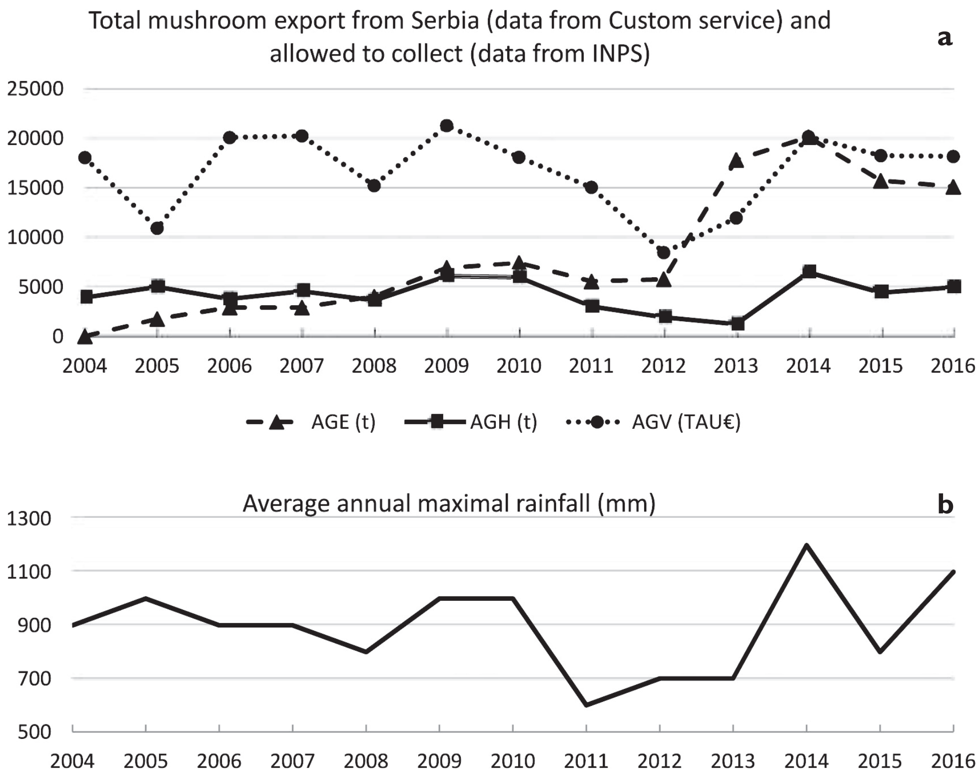

Figure 3. a Comparison between permitted and traded amounts of wild mushrooms in Serbia for the period 2004–2016. AGE of all mushroom species under control and their AGV as indicated on the export documents and provided by the Custom service of Serbia and AQ according to INCS **b** Average maximal rainfall rates for the investigated period.

Since their detection in the 70s , the traditionally illegal truffle market, mainly held by the Slovenian smugglers, has been the only route for selling truffles from Serbia (Ivan Ratoša, personal communication). In early 2000s, Milenković M. (See supplementary material 1) was organising paid courses (for 150+ people) and sold privately written instructions on truffle hunting on an even larger scale (M. Milenković, personal communication). Due to these activities, the number of truffle hunters in Serbia grew rapidly and the black market has been flourishing ever since. The majority of the truffle export is nowadays going through illegal routes (M. Milenković, personal communication), but the level of legal trade is also growing (Figure 5).

Mushroom collecting due to socio-economic drivers is not the crucial factor affecting the conservation of fungi. The far larger danger is the excessive and frequently uncontrolled harvesting of vast amounts of wood. In the last two centuries, forest cover in central Serbia was significantly decreased, from 80 % in 1801 to 21.4 % just after the Second World War (Aleksić and Vučićević 2006). Today, forests in the Republic of Serbia cover 2,252,000 ha, 29.1 % of the country's area: 37.6 % in Central Serbia and 7.1 % in Vojvodina (Banković et al. 2009). The majority of the harvested wood is used for heating, since 40.9 % of households in Serbia still use wood for heating and even cooking all year around (Glavonjić 2011). The state-owned agencies for forest management that are in charge of all forests in the country except National parks (Srbijašume and Vojvodinašume) perform very symbolic afforestation–only 7 % of forests belong to plantations, but with non-native tree species (American hybrids of *Populus sp.*, *Paulownia tomentosa* (Thunb) Steud., *Pseudotsuga menziesii* (Mirb.) Franco, invasive *Robinia pseudoaccia* L.) or non-native for the habitat (*Pinus nigra* J.F.Arnold or *Picea abies* L. H. Karst. on all habitats, Ivetić 2015).

Another major socio-economic danger for macro fungal communities in Serbia is the problem of pollution (uncontrolled air pollution, application of fertilisers and pesticides) absolutely not recognised by the forestry sector and a serious problem with waste deposition (http://www.sepa.gov.rs/). Only 60 % of the municipal waste is gathered, while all the rest ends up in the natural environment (http://www.sepa.gov.rs), with traditional places for disposing of the unwanted waste of different origin being forests.

Mushroom conservation, legislation and trade control in Serbia

The first attempt at placing macro fungi into a process of protection was the proposed preliminary red list of that time Yugoslavia (Ivančević 1995, 1998). However, these data were quite useless assuming the fact that: the checklist of detected species in the territory of Serbia (or previous Yugoslavia) has never existed; there are only three spots for which any kind of data officially exists about abundance of sporocarp production based on the few seasons' examination: (Čolić 1967, Ivančević and Marjanović 1987, 1988, 1990, Marjanović 2000); no survey on the country level has ever been conducted in order to obtain even an estimation on the diversity or abundance. Still, there are many legal acts that passively concern macro fungi. On the international level, Serbia has joined the Convention on Biological Diversity (CBD), Convention on the Conservation of European Wildlife and Natural Wildlife (Bern convention, www.zzps.rs). Eight percent of the total territory has been assigned for 61 IPA sites (Important Plant Areas, Stevanović and Šinžar-Sekulić 2009), while under the Bern convention, the EMERALD network within Serbia is expected to cover 11.54 % of the territory with 61 officially nominated sites proposed (www.zzps.rs). The State protects 6.5 % of the territory within: 5 national parks, 16 parks of nature, 20 areas of landscapes of exceptional quality, 70 reserves of nature, 314 monuments of nature and 4 habitats (www.zzps.rs). All of them, plus areas of international importance for nature conservation, constitute the Ecological net of Serbia (Official Gazette of the Republic of Serbia No 102/10) that covers 20.49 % of Serbian territory. There is only one protected fungal habitat in Serbia (www.zzps.rs), but through protecting other areas of high biodiversity, they were passively included. Also, macro fungi recently appeared in the Biodiversity Strategy of the Republic of Serbia for the period 2011–2018, issued by the Government of Serbia in compliance with international agreements and the Serbian Law on Ratification of the Convention on Biological Diversity (Radović and Kozomara 2011). On the other hand, fungi are not mentioned even once and mushroom gatherers and traders do not exist as stakeholders in the Forestry Development Strategy for the public of Serbia (2006).

On the national level, general Laws on nature protection (See supplementary material 2) passively regulate fungi by regulating protection of the environment, biodiversity, diversity of ecosystems or landscapes in the country, in a way that should meet the requirements of the EU legislation system, through processes for EU joining that are expected in the near future. The Law on Forests, issued by the Ministry of Agriculture and Forestry (MAF) directly treats mushrooms in two articles: Article 9 forbids collection of any secondary forest products, unless stated differently by that same Law; Article 62 allows collection of secondary forest products only upon approval of the forest manager. It is not stated how these two articles were correlated with other legislation acts that refer to wild mushrooms.

Legal acts that directly regulate macro fungi protection are components of the Law on Nature Conservation (See supplementary material 2) issued by the Ministry of Environmental Protection (MEP): Regulation on protection of protected and strictly protected wild species of plants, animals and fungi (in the future text "The Regulation", Official Gazette of the republic of Serbia, No 5/10, 47/11, 32/16) and Bylaw on placing the use and trade of wildlife under control (in future text "The Bylaw", Official Gazette of the republic of Serbia No. 31/05, 45/05, 22/07, 38/08, 9/10, 69/11). The Regulation lists 38 strictly protected and 26 protected macro fungal species, including 15 species that may be harvested and traded under control (supplementary material 2: Tables 1 and 2). The document provided no explanation on the criteria for compiling the lists in this legal act. By The Regulation, strictly protected species and their habitats are forbidden to be disturbed in any way and should be monitored and repopulated if possible. Species listed as "protected" can be collected under conditions regulated by other legal acts, but the regulation of their protection is not clear.

The current version of The Bylaw allows the controlled harvesting and trade with 15 species of macro-fungi (supplementary material 2: Table 2). Since the beginning of its application, the list of species has been changed few times: originally (1991), it included all species of *Lactarius sp., Morchella sp., Agaricus sp., Boletus edulis*, *Cantharellus cibrius* and *Pleurotus ostreatus*; in 1996, *Amanita caesarea* was added and removed again in 1999 together with *Morchella sp*., while two inedible species of *Bovista sp.* appeared in 1996 and were removed in 2005, when truffle species appeared on the list. *Tuber brumale* that was listed in 2005 was exchanged for *T. macrosporum* in 2011. No rationale was provided for any of these changes nor explained in any way.

The Bylaw forbids trade on the species listed outside the frameworks described and regulates the methods of sporocarp collection to avoid damage to soils and plants in the ecosystems. It declares a taxation of 10% of the nominal value per kg of sporocarps for every species, for the "permission to harvest and trade" (in further text The Permission). Only legal entities that are registered for such business are eligible for applying (natural persons undertaking mushrooms harvesting or trading are not included). The Permissions issues MEP after the opinion of INCS. INCS is obliged to perform monitoring of mushroom production and habitat state and to decide on an annual quota allowed for harvesting (AQ). The Permission holders are obliged to organise education and issue certificates on mushroom recognition for the gatherers whom they engage, as well as providing the annual reports on harvesting to the MEP. The Bylaw defines penalty payments for those who do not obey the rules defined in the text (legal entities or natural persons). It does not define who is supposed to control fungal habitats *in situ* for illegal activities or who is supposed to be informed about the illegal mushroom gatherers or to whom.

The functioning of the regulation of mushroom trade in practice

One of the aims of this paper was to investigate the actual practice of macro fungal trade control in Serbia, hence the analyzes of the data obtained from legal public sources and evaluation of the effects and level of application of Legal Acts in the period 1993–2016 since The Bylaw was established has been performed. Comparison of the data on AGE of all mushroom species under control and their AGV, together with the AGH according to INCS were presented in Figure 3a, while the values for AMR were presented in Figure 3b. The correlation coefficients for all the data presented in Figure 3a and b were calculated and only the AGH appeared strongly correlated with AMR (r = 0.85). AGH was weakly correlated with AGV (r = 0.5), which was not the case with AGE and AGV (r = 0.36). Rainfall rates did not significantly influence the values of export (r = 0.34), while no correlation was detected between AGE and AGH (r = 0.08). The number of companies that have applied for the Permissions did not influence the gross amount of trade in the examined period (data not shown).

The most ubiquitous mushrooms that have been the subject of trade on a large scale in Serbia were *Boletus sp.* (50.2 % in 2013 to 80.3 % in 2014, on average 68.5 %) and *Cantharellus cibarius* (11 % in 2014 to 41.2 % in 2013, on average 25.1 %, data from supplementary material 3). In Figure 4, AQ and AGH for these species were compared (other species that were listed in The Bylaw were collected and traded in much smaller amounts - less than 10 % of AGH and therefore not presented, see supplementary material 3). By calculating the correlation coefficients between AGH (Figure 4) and AMR for the period 2004–2016 (Figure 3b), a strong influence of rainfall on trade with *Boletus* sp. (r=0.85) was detected but not on *Cantharellus cibarius* (r = 0.35). The data on truffle species (*Tuber magnatum*, *T. aestivum* and *T. brumale* / *T. macrosporum*) were presented in the Figure 5.

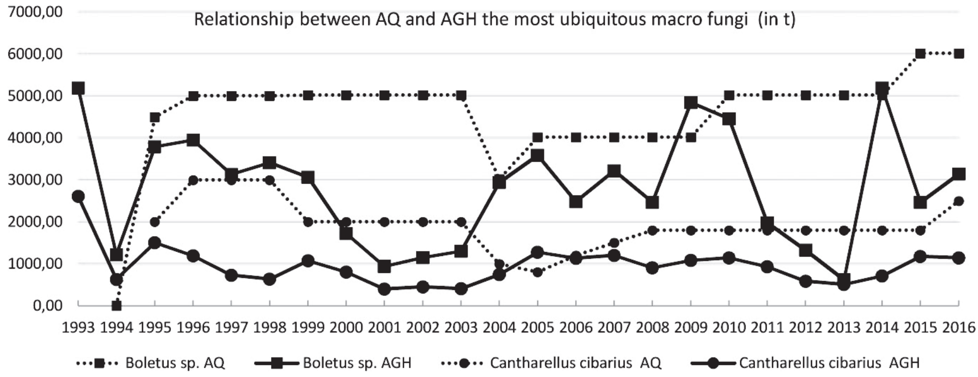

Figure 4. The estimation and demand of the most ubiquitous mushrooms trade in Serbia. AQ and AGH according to INCS for porcini *Boletus sp.* (*B. edulis*, *B. aereus*, *B. reticulates* and *B. pinophilus*) and *Cantharellu scibarius*.

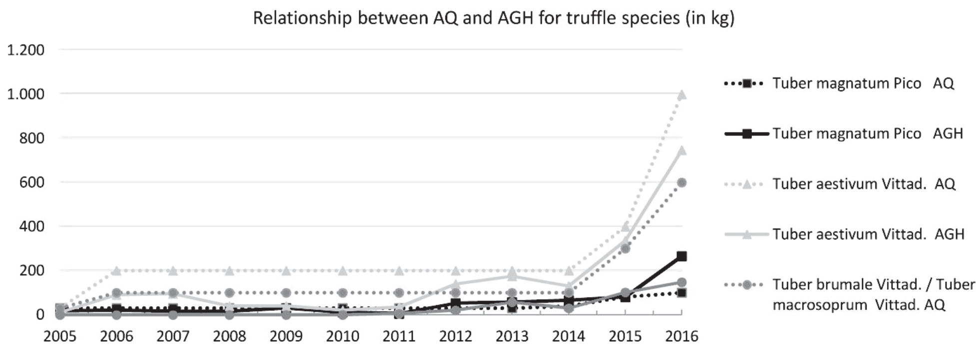

Figure 5. The estimation and demand of truffles trade in Serbia. AQ and AGH according to INCS for truffle species.

Discussion

In this contribution, the authors have provided a comprehensive overview on different types of data on macro fungi from the country positioned on the borderlines of different climate, geological and vegetation zones of the Balkan Peninsula, with consequently high rates of biodiversity. Throughout history, common fungal habitats forests, once covering probably the great majority of the country, have become endangered. At the present time, with evident planet climate and atmosphere composition equilibriums changing as a consequence of global forest decrease, the desire for their preservation and enlargement has become evident (Meyfroidt and Lambin 2011). In the background, the organisms i.e. the fungi that serve crucial roles in maintaining the equilibrium of the forest ecosystems, especially in temperate regions with seasonally changing climates are hidden. Whether they degrade wood or forest litter enabling nutrient cycling and taking direct roles in soil formation or form symbiotic relations with trees enabling them to reach unavailable nutrients and water, forest ecosystems could never have functioned without complex macro fungal communities that obligatorily accompany them (Dix and Webster 1994). Therefore, when conservation of macro fungi is referred to, it must be kept in mind that it does not just mean protecting species, but it always means protecting entire ecosystems in their habitation.

Considering macro fungi conservation, differences should be made between the so-called rare species and those harvested in large amounts. In Serbia, the former are listed as 38 strictly protected species, a significantly smaller number than bordering Croatia, which strictly protects 314 species (http://www.dzzp.hr). From the content of the list (See supplementary material 2, Table 1) to a local specialist, it appears clear that there are many rare species that can be found in Serbia, but were not listed (for example, *Cortinarius* sp. or *Inocybe* sp., the species rich genera and which are numerous in Serbia (Marjanović 2000), are missing). Within Europe, the lists of protected species have recently been recognised only as the basic line for conservation of fungi, while habitat preservation, its reclamation and *ex situ* conservation have been proposed practices (Courtecuisse 2001). In Serbia, The Regulation forbids destroying habitats of strictly protected species, but would the average person intending to destroy the habitat (either by harvesting the wood or by depositing pollutants) ever think if such a spot hosts a very rare mushroom? Apparently, without habitat investigation, mapping, clear marking on spot and monitoring, the protection on paper has absolutely no effect (Dahlberg et al. 2010, Brainerd and Doornbos 2013).

Species that have been harvested on the large scale are listed as "protected" in The Regulation (See supplementary material 2, Table 2) and some of them are regulated by The Bylaw, with odd occasional changes in the list content. The appearance of inedible *Bovista sp*. was odd enough and their extraction from the list was no surprise. On the other hand, *Amanita caesarea* and *Morchella* sp., are widespread, collected and sold in Serbia, but were extracted from the list in 1999 and have never appeared there again. The protection of habitats for listed species was regulated briefly by The Bylaw, but this applies only to the gatherers and not to other stakeholders. On the other hand, the detailed investigations of habitats defining ecological demands of economically important macro fungal species are recent and sporadic (Marjanović et al. 2010a, b; 2013a, b; 2015; Bragato and Marjanović 2016). However, such an approach revealed how crucial it was to understand inseparable ecosystem features like soil water, nutrients and organic matter seasonal dynamics, the size of the soil particles that define the size and distribution of soil pores, which further define the dynamics of soil aeration, up to the influence of seasonal leaf development and senescence on dynamics of nutrient uptake by the trees or their transport toward roots. All these elements are obligatory for appearance and fructification of the most precious ECM mushroom in the world, *Tuber magnatum* (Marjanović et al. 2015) and a small change in these vulnerable ecosystem dynamics may lead towards species disappearance from the site (Bruns 1995) .

The recent legal acts of Serbia concerning fungal conservation were certainly influenced by similar acts of other EU countries and probably shaped by the political demands of processes leading towards EU joining. Forest protection, their sustainable usage and reforestation are very highly listed goals in the Law on forests (2015), but actual research (Ivetić 2015), the reports of non-governmental organisations and the situation observed *in situ* reveal a very different reality. According to the recent report of REC (2009) about illegal logging in Serbia: 64.7 % of forests are very low quality coppice forests; officially registered volume of logging (in gross amount) ranges from 3–3.5 million m^3 annually, but estimations are that this amount could be even 9 million m^3 – equal to the estimated annual wood increment; the estimated illegal wood cut in privately owned forests (about 47 % of all forests in Serbia) is ca. 1.2 million m^3 annually. The areas abandoned from agriculture or clear cuts are permanently changed environments concerning EMC communities (Jones et al. 2003) and in Serbia usually quickly overgrown by black locust and other invasive species that do not form ECM (Radtke et al. 2013). Apart from areas where clear cuts and intense coppicing were left to naturally regenerate, the authors are not aware of any reforestation attempt with native tree species in a suitable habitat. While establishment of autochthonous ECM fungal communities in non-native plantations is not expected, in coppice forests that dominate the forest resources of Serbia, degradation of such communities is highly likely as the amount of carbohydrates that reaches ECM roots is much lower and the micro-environment is significantly changed (Bruns 1995, Jones et al. 2003).

A recent detailed report based on 30 years of investigation revealed no influence of sporocarp collection on populations of ECM fungi in undisturbed habitats (Egli et al. 2006), changing the opinion of the mycological scientific community on measures for mushroom protection (Brainerd and Doornbos 2013). However, the data which is presented here reveal the level of the legislation application in Serbia. The discrepancy between AGE and AGH is obvious (Figure 3). While AGH of sporocarps and the most important environmental factor for their production (rainfall) were strongly correlated, this was not the case with AGE. It is not clear from where these huge exported amounts of mushrooms could originate, but it might be possible that part of them were re-exported from other countries. The AGE was lower than or following the curve of AGH up to the year of 2008, when export started to be higher than permitted, especially from 2012 when 2–10 times more mushrooms were officially exported than was permitted for harvesting. The possible explanations are that Permissions were falsified, that they were issued illegally or that there was a serious violation of the Customs' procedure.

Simultaneously with the vast growth of the AGE (2012–2014), the AGV was dropping (Figure 3). While the average price of one kg of mushrooms in 2006 was 6.8 Euros, in 2013 (when AGE was more than 10 times higher than AGH), the average price per kg was 0.7 Euros (calculated from the raw data presented in Figure 3a). Palumbo and Sitta (2005) reported that the prices of dried porcini in Italy (main target country for Serbian mushrooms) for 2005 were between 40 and 70 EUR/kg. In the data that have been received from the Customs service, it was not stated if the sold mushrooms were fresh, frozen or dried, but it is hard to imagine that they could ever cost 0.7 EUR/kg on average. The probable explanation is that the values on the invoices that were provided to the Customs service were manipulated.

The AQ for the most important commercial mushrooms was obviously changing during the period of investigation (Figures 4, 5), but no explanation could be found for this, as the monitoring proposed by The Bylaw has never been undertaken. For truffles, AQ grew rapidly from 2014, while for *T. magnatum* in 2016, it was exceeded by a factor of 2.5 (Figure 5). This was not the first time for such practice (Figure 4), but the legal mechanism enabling it is not known. Obviously, the regulations on trading with protected mushroom species has been severely violated starting from 2008. On top of these official data, it must be added that significant amounts of mushrooms, especially truffles, have been regularly smuggled over the borders in the last decade. The taxation for harvesting and trading with truffles is extremely high, while the smuggling routes have been well established a long time ago, the reason why the truffle smugglers are in a much better position than the legal traders in Serbia. Additionally, such taxation for truffle trading does not exist in other truffle source country, which discourages the legal truffle trading in Serbia.

Proposals for the legal measures that would improve the effects of conservation of rare and sustainable use of commercial mushrooms as a natural resource of Serbia

After serious evaluation of the situation in Serbia, considering solutions taken in other EU countries (Moore et al. 2001, Brainerd and Doornbos 2013), as well as respecting the newest scientific achievements concerning macro fungi and their habitats, some measures are suggested that could lead towards much more effective conservation and control of trade with wild mushrooms.

Considering the current status of the existing resources, the logical starting point for establishing the background for forming the official National database on macro fungal species in Serbia would be publishing the data on collection in the Natural Museum in Belgrade. In this way, the check-list would be formed and the mapping and monitoring system would be facilitated. Only then the realistic list of strictly protected species could be compiled, their habitats properly marked and further legally protected from disturbance.

Harvesting of the commercial epigeic species regulated by The Bylaw has a large economical potential and the preservation or enlargement of their fructification, which depend strictly on preservation of stability of their habitats, should be of special interest. They inhabit soils and ecosystems that are widely present, the reason why their protection should be strongly connected to the sustainable wood harvesting organised in an appropriate way (e.g. according to the recommendations in Kraus and Krumm 2013). As the number of researchers specialised in macro fungal ecology in Serbia is minimal, the projects supported by the Government should be set to enlarge the number of people working in this area. Such projects should involve combined investigations of fungal communities (ECM morphotyping or molecular analyses) and fructification, accompanied by the investigation of seasonal dynamics and spatial distribution of mycelia for all highly exploited mushroom species in selected spots (e.g. van der Linde et al. 2012, Ovaskainen et al. 2013). These data should be correlated with microclimate parameters in order to define specific indicators of habitat functionality and fungal population dynamics (Marjanović et al 2015). Such parameters would be reliable subjects of monitoring and key factors for decision-making on conservation practices.

Reforestation of deserted bare land with autochthonous tree species inoculated with local strains of ECM fungi would be the best way to invest in renewable resources - mushroom and wood production (Schwartz et al. 2006). Under field conditions, late-stage fungi like porcini were able to compete effectively with early stage fungi for colonisation of new roots only if they were already associated with other living roots (through mycelia), while spore-based inoculations failed (Deacon et al. 1983, Fleming 1983, 1984). Also, mycelia of saprotrophs that perform final stages of wood degradation are highly threatened by wood exploitation (Stokland et al. 2012), may be interesting material for preservation. Therefore, in addition to the improvements *in situ*, simple measures that could be effectively developed from the existing resources in research institutions include the formation of the National Bank of macro-fungal mycelia, in order to compensate for disappearing habitats/substrates and provide the basis for their possible reclamation (Courtecuisse 2001).

The most endangered and the most economically potential macro fungal species in Serbia is certainly *Tuber magnatum*. The very specific ecological demands of this species and continuous destruction of its habitats at the global level, combined with the high demand on the market formed its very high prices (up to 4000 EUR per kg of the best quality in Italy). The white truffle of Piedmont or Alba has been long accepted as a strictly Italian delicacy, but since the natural habitats in Italy have been permanently destroyed (Gilberto Bragato, personal communication) and the new habitats discovered first in Istria (probably early 20th century) and, later on, in Serbia (Marjanović and Milenković 1998) and Hungary (Bratek et al. 2004), the market has been widened. As this truffle is of highest economic importance for all these countries, the regional initiative for protection of existing habitats and reintroduction on suitable lands would be the best solution for its preservation and sustainable use. This truffle competes with the wood industry (its dominating host is *Quercus robur*, highly prized for its wood quality) and agronomy, as the soils it inhabits are of the highest quality. Therefore, a well organised action at state level must be applied for strict protection of existing habitats and detecting areas where this truffle species could be reintroduced.

Following these proposals, legislation regulating macro fungi protection and trade should be substantially changed. The conflict of jurisdiction between the Law on Forests and the Law on Nature Protection indicates a need for further harmonisation on the conflict of interests. If biodiversity conservation should be a priority, then the protection of habitats of strictly protected fungi must be an official part of the Law on Forests, which should enable monitoring governed by the MEP and INCS. As it does not recognise mushroom gathering as an economic interest for rural citizenship at all, a clearly stated connection to The Bylaw should be included. The Permissions issued by MEP that the holders buy from the State for a relatively high price, should be recognised in the field by the forest managers. The current chaos in legislation may be solved only by agreement between all stakeholders of the forest ecosystems - users, specialists and MEP and MAF, at State level.

Concerning the worrying information on the multi-level violation of the regulations on the mushroom trade, it is believed that the State should try to understand the causes of such a situation and react adequately. The number of citizens (mostly from the impoverished rural regions) that are involved in the mushroom trade in Serbia is increasing; the reason why the valid attempt for limitation of this business is obviously not functioning. The only outcome is the creation of the mighty black market, which is endangering the natural resources in the country and attracting criminal characters. Reduction in the taxes for mushrooms (especially truffles) trade and introducing the issuing of licences for gatherers, as in other EU states (Brainerd and Doornbos 2013), would meet the interests of both State and traders and reduce the number of illegal gatherers. This way, they would achieve their role in the entire system and become visible to the instruments of the Law. Investing in the currently non-existent control of the mushroom gathering in nature by formation of an educated ranger net, as well as detecting and sentencing of the illegal traders and gatherers, would be much more effective than the existing system of office- and Customs- based regulation. In addition, many members of the local mushroom gathering societies could be introduced to the monitoring process. This way, jobs could be created in very impoverished regions where the mushroom gathering is a significant source of income. In other words, sustainable usage of mushrooms as natural resources that would allow the trade to develop, but with investment in habitat protection, development and control would be the only model that would satisfy the needs of all stakeholders involved.

Conclusions

The current state of knowledge, research and published data on diversity, ecology and population dynamics of macro fungi in Serbia is not satisfactory. The existing regulations on mushroom conservation and trade control in Serbia are confusing, non-efficient, hardly applicable and regularly violated. In order to fulfil the requirements of mushroom conservation and sustainable harvesting from the existing forest ecosystems, actions are proposed that would be efficient in practice: creating a fund that would enable protection and conservation of fungi and their habitats at the State level; directing the research towards fungal population and ecosystems dynamics using up-to-date techniques; establishing agreements between all stakeholders of the forest ecosystems - users, specialists, managers, MEP and MAF and then reforming the existing regulations at the State level; for endangered species, organising habitat mapping, clear on-spot marking and monitoring by specialists and INCS; for commercially important species, organising wide-range monitoring that would include mushroom gathering societies, traders, professional gatherers, specialists and INCS; introduce the wide practice of enlargement of areas afforested by autochthonous tree species, inoculated by autochthonous ECM fungi on suitable soils. Due to the highly threatened habitats and high commercial potential, strictly protect truffle habitats and organise their re-introduction on suitable land; stimulate establishment of truffle plantations and reconstruct the regulations towards better application in practice that would be obligatory for all stakeholders (including the forestry sector). The formation of an educated organisation of rangers that would inspect the activities *in situ* would be the best solution against illegal collection and trade of mushrooms, while the systematic reform of The Bylaw should enable strict definition of rules in this field.

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Supplementary material 1

Overview of mycological literature, research and herbaria collections on macro fungi of Serbia with reference list

Authors: Mandić Radomir, Adžemović Mesud, Marjanović Žaklina

Data type: Additional data PDF.

Explanation note: The document provides brief descriptions of the scientific and hobbyist published data on epigeic and hypogeic macro fungi in Serbia, including the history of their recognition with reference lists. It also provides information on official herbaria collections of macro fungi.

Link: https://doi.org/10.3897/natureconservation.25.21919.suppl1

Supplementary material 2

The lists of strictly protected and protected species of macro fungi in Serbia

Authors: Mandić Radomir, Adžemović Mesud, Marjanović Žaklina

Data type: Additional data PDF.

Explanation note: The document lists the legal acts that regulate macro fungi and their habitats in Serbia, as well as species that are regulated by The Regulation and The Bylaw.

Link: https://doi.org/10.3897/natureconservation.25.21919.suppl2

Supplementary material 3

Annual amounts of mushrooms allowed to harvest (in kg, according to INCS)
Authors: Mandić Radomir, Adžemović Mesud, Marjanović Žaklina
Data type: Data set (Excel spreadsheet).
Explanation note: The document provides table with official data on mushroom and truffle amounts that have been allowed to harvest since 1993, according to INCS.

Link: https://doi.org/10.3897/natureconservation.25.21919.suppl3

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RESEARCH ARTICLE

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Infanticide in brown bear: a case-study in the Italian Alps – Genetic identification of perpetrator and implications in small populations

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Abstract

Sexually Selected Infanticide (SSI) is thought of as a male reproductive strategy in social mammalian species, because females who lose cubs may quickly re-enter oestrus. SSI has rarely been documented in non-social mammals and, in brown bears, SSI has been studied mainly in an eco-ethological perspective. The authors examined the first genetically documented infanticide case which occurred in May 2015 in brown bears in Italy (Trentino, Central-Eastern Alps). The infanticide killed two cubs and their mother. Hair samples were collected from the corpses as well as saliva, through swabs on mother's wounds, with the aim of identifying the genotype of the perpetrator. The samples were genotyped by PCR amplification of 15 autosomal microsatellite loci, following the protocol routinely used for individual bear identifications within the Interregional Action Plan for Brown Bear Conservation in the Central-Eastern Alps (PACOBACE). Reliable genotypes were obtained from the mother, cubs and putative perpetrator. The genotypes were matched with those populating the PACOBACE database and genealogies were reconstructed. Both mother and perpetrator genotypes were already present in the database. Kinship analyses confirmed mother-cubs relationships and identified the father of the cubs. In this study, for the first time, the authors used the open-source LRmix STUDIO software, designed to analyse human forensic genetic profiles, to solve a case in wildlife. Through LRmix STUDIO, those alleles that do not belong to the victims were isolated and, finally, the perpetrator was identified. This study presents a method that allows, through the application of different models, the genetic identification of the conspecific perpetrator with the highest probability. The identification of the infanticidal male is relevant for the better management and conservation of wild populations with small effective population size (Ne) and low population growth rate, especially in the case of recently established populations in human-dominated landscapes. This procedure will have predictably wide applications, supplying important data in the monitoring of small and isolated populations.

Keywords

Conservation genetics; LRmix STUDIO; Low template DNA; Small population; *Ursus arctos*

Introduction

Infanticide, the killing of dependent offspring by conspecifics, has been thought of as a component of intersexual conflicts in social mammals (Hrdy 1979, Hrdy and Hausfater 1984). Most reports describe adult and sub-adult males killing young-of-the-year or yearling cubs (Taylor et al. 1985, Dean et al. 1986, Olson 1993, Craighead et al. 1995, Derocher and Wiig 1999). Others document the killing of cubs by unmated females (Dean et al. 1986, Hessing and Aumiller 1994). Although the victim was consumed in most instances, cannibalism could not be invoked as a general cause of aggression (Dean et al. 1986, Hessing and Aumiller 1994, Karamanlidis et al. 2015). Similar to other species, such as African lions *Panthera leo* (Packer and Pusey 1984) and red deer *Cervus elaphus* (Bartoš and Madlafousek 1994), infanticide in bears is most likely related to male reproductive success (Swenson et al. 1997). SSI can be a male reproductive strategy where the killing of unrelated offspring induces premature oestrus in mothers and increases the opportunity to breed with them (Hrdy 1979). SSI is common in size-dimorphic species with a polygamous mating system (van Schaik 2000) and occurs during the mating season in seasonal breeders that have extended maternal care and lactational anoestrous (Bellemain et al. 2006a, Zedrosser et al. 2009). SSI has been described in some North American and Scandinavian brown bear populations (Wielgus and Bunnell 1995, Swenson et al. 1997, Bellemain et al. 2006a, Libal et al. 2011). Resistance to infanticide may be costly: a female may sustain serious injuries in defending her offspring and sometimes the mother dies in an attempt to defend her cubs. In isolated populations with a small number of reproductive adults, SSI can negatively impact the long-term conservation of the species, especially in the case where the female is killed while protecting her cubs. Taking this into account, the genetic identification of the perpetrators could give concrete indications for the management of small populations (e.g. placing radio-collars on infanticidal males to track them). Nevertheless, genetic studies for identifying infanticidal males have received little attention.

In this paper, the authors report the first observation of infanticide in brown bears documented through genetic analysis in Italy. On 10 May 2015, as part of the field and genetic long-term monitoring of the brown bear population re-introduced in Trentino, central-eastern Italian Alps (De Barba et al. 2010a), in Costa Lugiangia (Tuenno), the corpses of three brown bears were found: an adult female and two cubs-of-the-year. Death was not caused by anthropogenic causes. Their injuries, in fact, led to the hypothesis that the aggressor was an adult male bear. The cubs were killed and partially consumed and the female was consumed as well and covered with plant material. The mother (the only one with substantial parts still to be consumed) was found covered with earth, branches and foliage, which is typical of the behaviour observed in bears in an attempt to defend carcasses from scavengers. Another typical ursine behaviour involves dragging carcasses to places inaccessible to scavengers: the corpses were found, indeed, in a very dense forest area. Moreover, the day before, the person who found the first cub's corpse had heard very loud noises coming from the woods and these could have been derived from an intense struggle. This is just one of the indications that the mother had defended her cubs fiercely. It was observed that trees had been broken and that there were claw marks on the trunks. The suspects are, therefore, all the adult male bears considered present in the population in 2015.

Since an infanticidal bear can affect the growth rate of a small and isolated population (Gosselin et al. 2015), the results of this study are particularly relevant for the management of other wild small populations located in structurally fragmented and human-dominated landscapes.

Methods

Study area, population and sampling

In 2015, the brown bear population, re-introduced in central-eastern Italian Alps, extends mainly in the western part of the Trento Autonomous Province (PAT), across an area of ca. 20794 km^2 (including movements of young dispersal males). The females permanently occupy a smaller area (1303 km^2) entirely located within the PAT. The Extent of Occurrence is estimated as 100% minimum convex polygon, delimited by all the validated indices of presence (Groff et al. 2016). In 2015, the bear density in the territory occupied by females was approximately 3.4 bears/100 km^2 (44 individuals in total, including the cubs-of-the-year).

This population has been continuously monitored during the last 17 years by both genetic and direct observation procedures (AA VV 2010, De Barba et al. 2010a). The main monitoring targets are: the identification of most of the individuals; the assessment of all reproductive events; the reconstruction of the population pedigree and demographic structure. Moreover, the reference genetic database allows identifying problematic bears that have a high level of interface with humans with humans and allows implementing effective actions for conservation, prevention and mitigation of conflicts. Although such bears represent only a small part of the bear population, they usually cause the majority of all human-bear conflicts, while most bears come into conflict with humans only rarely or never.

In the period between 2003 and 2015, the authors collected and analysed ~7800 biological samples, of which ~7700 (hair samples, scat, saliva samples, urine and blood samples on snow) were collected through non-invasive techniques during the monitoring programmes and ~100 were of an invasive origin (tissue, hair samples, teeth and bone sample). Tissue, teeth and bone samples were taken during necropsies of animals which had died of natural causes or had been killed by traffic or poaching, while hair samples came from animals live-captured for radio-tracking studies.

During the genetic monitoring carried out in 2015, 45 different bears (24 females and 21 males) were identified of which 25 were adults: 13 females (>3 years old, reconstructed on the basis of field data and genetic pedigrees) and eight males (>4 years old). In addition to these bears, the authors considered in the population even those bears that had been sampled during 2012–2015 (the last sample collected not before than 2012) and not known as dead in 2015. Thus, the total number of individuals present in 2015 was 57 (of which 14 were adult females and 12 were adult males).

Following the infanticide case, in 2015 two types of biological samples were collected: three samples of hairs from the corpses and four saliva swabs, by swabbing the mother's injuries, hoping to isolate the DNA of the perpetrator. The samples were preserved dry until the DNA extraction.

DNA isolation and amplification

DNA from hairs was extracted using the ZYMO Research ZR-96 Genomic DNA™ – Tissue MiniPrep Kit (CA, U.S.A.) and DNA from swabs was extracted using the QIAGEN QIAamp® DNA Investigator Kit (Hilden, Germany), following the manufacturer's protocol. The amplification and analysis of microsatellites were carried out by updating the protocol described by De Barba et al. (2010b). For the parentage analysis, the authors amplified 15 loci (Ostrander et al. 1993, Paetkau and Strobeck 1995, Taberlet et al. 1997, Paetkau et al. 1998, Bellemain and Taberlet 2004) in three multiplex-PCR: M1 (cxx20, G10M, G10P, Mu11, Mu15), M2 (G1D, G10X, Mu23, Mu50, Mu59) and M3 (G10C, G10H, G10L, Mu09, Mu10); for the individual identification, the authors used routinely only 10 loci (M1 and M2). Sex was identified using the amelogenin gene (AMG, Ennis and Gallagher, 1994) and confirmed using the SRY gene (Taberlet et al. 1993). A multitube approach was used (Taberlet et al. 1996, Adams and Waits 2007) with positive and negative controls in each step (Pompanon et al. 2005). One primer of each pair was 5'-labelled with 6-FAM, HEX, NED or PET dyes. STR fragments were detected and sized on an ABI Prism 3130XL Genetic Analyzer DNA sequencer (Thermo Fisher Scientific, Waltham, MA USA). The electropherograms were collected by the DATA COLLECTION Software v.3.0 and analysed by the GeneMapper Software v.4.0 (Applied Biosystems by Thermo Fisher Scientific).

Statistical analysis

Low genetic variability in the sampled population and small numbers of markers used in genotyping, might lead different individuals to show the same multilocus genotype. This shadow effect (Mills et al. 2000) can be minimised by increasing the number of loci genotyped. Two probability-of-identity formulations were used (Waits et al. 2001): PID_{unb} (unbiased for small sample size) and PID_{sibs} (the expected PID between sibs), which defined respectively the lower and upper bounds assuming that the sampled population included only unrelated individuals or sibs. GenAlEx v.6.502 (http://biology-assets.anu.edu.au/GenAlEx) was used to evaluate the suitability of the marker set chosen for individual (and victims') identification. The matches were calculated amongst the genotypes with the option MATCHES in the MULTILOCUS menu, which automates the detection of repeated genotypes within the dataset, by comparing the profiles of the victims directly with those present in the reference database, to find out from which one they originate.

To ensure as much as possible the proper genetic reconstruction of the cubs' pedigree, the paternity probabilities were calculated by comparing the results of two software taking into account the allelic frequencies of the population and the error rate per locus: COLONY v.2.0.5.0 (http://www.zsl.org/science/software/colony) and FRANz v.2.0.0 (http://www.bioinf.uni-leipzig.de/Software/FRANz/). COLONY implements a maximum likelihood method to assign sibship and parentage jointly, using individual multilocus genotypes at a number of co-dominant or dominant marker loci. FRANz reconstructs pedigrees (family trees) using polymorphic, co-dominant markers. [See Suppl. material 1 (Parameters used for parentage analysis) for the settings used].

Two different statistical models were used aiming to obtain reliable results from the analysis of the Low Template-DNA (LT-DNA) from the swab samples: the "classical" biological model (Caragine et al. 2009, Benschop et al. 2011, Pfeifer et al. 2012) and the statistical or probabilistic model (Curran et al. 2005, Gill et al. 2008, Gill and Buckleton 2009, Gill and Haned 2013, Benschop et al. 2015).

Following the biological model, the genetic profiles from the four swabs were interpreted, not individually, but in an integrated manner, by comparing the results obtained from the four independent replicates of each swab. Scientific literature describes two main approaches, both of which were applied to the evaluation of the genetic profiles of the single trace:

- The consensus method (Gill et al. 2000, Benschop et al. 2011, Benschop et al. 2013) which provides that, for each locus, an allele, that meets pre-specified acceptability criteria, can be considered reliable and it can contribute to a virtual consensus profile, only if it is confirmed in the replicates. The authors considered reliable only the alleles that appeared in at least half of the replicated genetic profiles. This approach allowed the authors to partially reduce the drop-in phenomenon because only the alleles consolidated in the replicates were considered (Benschop et al. 2011).

- The composite method (Bright et al. 2012, Pfeifer et al. 2012) which provides that, for each locus, each allele considered responsive to predetermined acceptability criteria and observed in the replicates may contribute to a virtual composite profile, which consists of the sum of all observed alleles. This approach allowed the authors to partially reduce the drop-out phenomenon because all the alleles presented in the replicates were considered (Pfeifer et al. 2012).

The results from the biological model analyses were compared with the results obtained by the statistical model. There are three groups of statistical methods to evaluate the Weight-Of-Evidence from the traces through the calculation of the likelihood value (Likelihood Ratio – LR), based on different algorithms and classified as: binary models (traditional methods of calculation), semi-continuous models and continuous models (Gill et al. 2015). All the statistical methods cited are based on the LR calculation, an adimensional value that, on the basis of the genetic profiles obtained and the assumptions formulated, objectively identifies which one of two possible and mutually exclusive hypotheses is the more likely in a given scenario. The use of the binary statistical methods is not recommended in cases of LT-DNA traces, in which stochastic events may occur. In addition, to date, the continuous statistical methods that use all the quantitative information (intensity of allelic signals) have found a restricted application in the LT-DNA analysis, because they can provide misleading information when the signals are of low intensity, there are stochastic phenomena and the analytical noise background is relevant (Gill et al. 2007, Benschop et al. 2011, Taylor et al. 2013). In this study, an algorithm has been applied of a semi-continuous calculation that evaluates, in terms of LR, the possibility that one or more comparison subjects contributed with their own genetic material to the genetic profiles detected in the traces, including consideration of possible stochastic events of drop-out (Pr(D) – drop-out probability) and drop-in (Pr(C) drop-in, or contaminations, probability).

Specifically in the case of conspecifics, the low amount of DNA mixtures can be treated as the DNA traces usually found in a crime scene. For the interpretation of the low-level complex DNA mixtures with the statistical model, an open-source software was used: LRmix STUDIO (version 2.1.3-CommunityEdition, 2013–2016 Netherlands Forensic Institute, freely available at http://lrmixstudio.org). This software is dedicated to the semi-continuous approach and explicitly accommodates for uncertainty in the DNA profile from the allelic drop-out (ADO) and drop-in (contaminations) phenomena. LRmix STUDIO estimates these quantities from the available data and uses those estimates to generate LR. LRmix STUDIO was used to compute the LR for each suspected male (reference DNA profile) and to compare the global consensus profile and the global composite profile, obtained from the comparison of each trace, with all the reference profiles. The authors performed:

- A LR calculation defining the prosecution (Hp) and the defence (Hd) hypotheses. Under each hypothesis, the authors defined the contributors (Hp: victims and 1 suspect, Hd: victims and 1 unknown), the drop-out probabilities (victims: 0.01, suspect and unknown: 0.6, afterwards replaced with the average value of drop-out, calculated over all suspects, by the drop-out estimation of the sensitivity analysis), the Pr(C) and the rare alleles frequency (the default values: 0.05 and 0.001 respectively), the allelic frequencies of the population (calculated by GenAlEx on the reference database) and the Theta correction (0.03 for small and isolated populations);

- A Sensitivity Analysis (SA) that plots the log10 LRs along with the likelihoods of the Hp and Hd. The SA, showing the variation of the LR value when $0 \leq Pr(D) \leq 0.99$, allows the verification of the range of the most likely values of Pr(D) (from the 5th to 95th percentile) using a Monte-Carlo simulation method (Haned et al. 2015). The corresponding most likely range of LR values is compared with the LR value initially obtained. The drop-out estimation is a qualitative estimator of the Pr(D) of the whole profile, based on the average number of alleles observed in the profile. The ADO value obtained is an interval of the plausible range of drop-out, plotted on the SA, but also displayed as a highlighted area in the plot (see Suppl. material 2 and Suppl. material 3 for detailed LRmix STUDIO results on Global Consensus and Global Composite respectively);
- A Non-contributor test for better understanding the case specific LR (Gill and Haned 2013). This test consists of the evaluation of the LR value when the subject of interest (the suspected male) is replaced by N subjects taken at random (N = No. of iterations), in which the genetic profile is simulated based on the allelic frequencies. The distribution of LR for N iterations is shown in a barplot where the case-specific log10 LR is displayed in red (LR(POI)) and the minimum, the maximum, the 1-50-99 percentiles (LRs) are displayed in grey (see Suppl. material 2 and Suppl. material 3 for detailed LRmix STUDIO results on Global Consensus and Global Composite respectively). If the LR calculation model worked efficiently and if Hp is true, then the LR(POI) will be >1 and LRs will be <1.

Results

Reliable genotypes were obtained from all the hair samples collected from the three corpses. The DNA profiles obtained from each victim were compared with the profiles in the reference database: 93 genotypes (9 founders and 84 offspring in 15 years) of which 45 are females and 48 are males. This comparison allowed the identification of the mother (a female called BJ1), while the cubs (one male and one female) were unknown and these were added them to the reference database, with the names of M33 and F22 (Table 1). The low PID values at 15 loci ($PID_{unb}=3.7\times10^{-13}$ and $PID_{sibs}=6.6\times10^{-06}$) indicated that a shadow effect is unlikely, given the size of the studied population.

BJ1 was genetically identified as the mother of M33 and F22. Moreover, both software used for parentage analysis agree in identifying a known male, MJ4, as the father of the cubs (Table 2a, COLONY results; Table 2b, FRANz results).

Table 1. List of samples with matching multilocus genotypes at all loci.

Sample	cxx20		G10M		G10P		G10X		G1D		Mu11		Mu15		Mu23		Mu50		Mu59		G10C		G10H		G10L		Mu09		Mu10	
M22	134	134	123	123	165	171	133	139	106	108	80	86	136	146	118	120	102	106	123	123	94	106	253	253	151	151	193	195	118	130
Positive Control	134	134	123	123	165	171	133	139	106	108	80	86	136	146	118	120	102	106	123	123	94	106	253	253	151	151	193	195	118	130
BJ1	118	118	117	119	151	171	139	143	102	116	88	88	136	142	120	122	98	104	103	123	94	106	253	253	151	151	177	185	128	130
Victim 1 (mother)	118	118	117	119	151	171	139	143	102	116	88	88	136	142	120	122	98	104	103	123	94	106	253	253	151	151	177	185	128	130
Victim 2 (male cub – M33)	118	118	117	123	171	171	133	139	102	106	78	88	136	146	120	122	98	98	111	123	94	94	253	253	151	151	185	185	118	128
Victim 3 (female cub – F22)	118	118	119	123	171	171	133	143	102	116	88	88	136	146	120	122	102	104	101	103	94	94	253	253	151	153	185	193	118	130

Table 2. Parentage analysis. (**a**) Colony results; (**b**) FRANz results.

(a)

Offspring ID	Inferred Mum	Prob. Mum	Inferred Dad	Prob. Dad
M33	BJ1	1.000	MJ4	1.000
F22	BJ1	1.000	MJ4	1.000

(b)

Offspring	Parent 1	Parent 2	LOD	Posterior	Mismatches	n_f	n_m	Pair LOD Parent 1	Pair LOD Parent 2	Posterior Parent 1	Posterior Parent 2
M33	BJ1	MJ4	2.104E+01	1.000	0	14	12	9.377E+00	9.835E+00	1.000	1.000
F22	BJ1	MJ4	2.298E+01	1.000	0	14	12	1.126E+01	7.903E+00	1.000	1.000

Due to the small quantities of DNA extracted from the swabs, only 10 loci ($PID_{unb}=3.0\times10^{-10}$ and $PID_{sibs}=1.3\times10^{-04}$) were typed. Although drop-in phenomena cannot be excluded, the presence in most of the STR loci of more than two alleles suggests a genetic mixture, produced from organic material from at least two individuals. The presence of the allelic signal Y (male-specific), appreciable both in Amelogenin and in SRY, in three out of four swabs, suggests that the contribution to the formation of the traces is derived from at least one male, in addition to the dominant genetic component originating from the blood of the mother. As a whole, for the extrapolation of genetic profiles, all the replicates of the traces were considered useful and these were all used for comparison (Table 3).

Comparison of the results obtained using the biological model provides quantitative information regarding the degree of concordance or discordance between each genetic profile of comparison (potential infanticidal males) and the genetic profiles from the traces, based on the consensus and the composite methods (Table 3, see Suppl. material 4 for detailed results of the biological model). For each comparison, the authors calculated, excluding molecular sexing: (1) the ratio of the number of alleles present in each suspect's genetic profile to the number of alleles found in the genetic profile from the single traces (No (%) of matching alleles); (2) the ratio of the number of alleles from the single traces to the number of alleles not found in the genetic profile of each suspect (No (%) of divergent alleles). The results obtained were presented in terms of percentage of concordances and discordances with a histogram to allow a more intuitive graphical display (Figure 1). The bear that most likely contributed to the analysed traces is the one that receives the lowest values of percentage of discordances. Apparently, the culprit seems to be MJ4, but this is the father of the cubs and the high value of allelic concordance and the low value of allelic discordance can be explained by the fact that he shares half of his genetic heritage with the killed cubs. Previous studies have demonstrated, through DNA analysis, that infanticidal males are unrelated to the infants they attack (Bellemain et al. 2006a). The putative perpetrator is, therefore, the second one: M7. Applying the consensus method, there is present only one discrepancy between the alleles of M7 and those from the traces: at the locus cxx20, alleles of M7 have not been detected in the genetic profiles of the traces.

About the results obtained using the statistical model, the Hp was tested by comparing the values of $Log10(Pr(E|Hp))$ and $Log10(Pr(E|Hd))$ for every suspect (Figure 2): if $Log10(Pr(E|Hp))>Log10(Pr(E|Hd))$ and the difference (Δ) between these two values is greater than zero, it is more likely that the DNA, belonging to the individual tested, is present in the analysed traces. The mean value of ADO was corrected for the data presented in Figure 2, based on the results of the Drop-out Estimation calculated during the SA (0.65 for Global Consensus and 0.55 for Global Composite). The bear that most likely contributed to the analysed traces is the one that achieves the highest values: the biostatistical analysis allowed the authors to observe some compatibility between the genetic profile of one male (M7) and the genetic profiles from the traces and a high degree of incompatibility of the genetic profiles of the remaining suspects

Table 3. Results of typing of the four replicates for each swab sampled on the female's injuries.

		Genetic profiles										Sexing	
		cxx20	G10M	G10P	G10X	G1D	Mu11	Mu15	Mu23	Mu50	Mu59	AMG	SRY
I SWAB	I	118/118	117/119	151/171	139/143	102/116	88/88	136/142	120/122	98/104	103/123	158/212	75/75
	II	118/118	117/119	151/171	139/143	102/116	88/88	136/142	120/122	84/98/104	103/123	158/212	75/75
	III	118/118	117/119	151/171	139/143	102/116	88/88	136/142	120/122	84/98/104	103/123	158/212	75/75
	IV	118/118	117/119	151/171	139/143	102/102	88/88	136/142	120/122	84/98/104	103/123	158/212	75/75
Biological model results	I Consensus	118/118	117/119	151/171	139/143	102/116	88/88	136/142	120/122	84/98/104	103/123	158/212	75/75
	I Composite	118/118	117/119	151/171	139/143	102/116	88/88	136/142	120/122	84/98/104	103/123	158/212	75/75
II SWAB	I	118/118	119/123	171/171	133/143	102/102	88/88	136/146	120/122	102/104	101/103	212/212	0
	II	118/118	119/123	171/171	133/143	102/116	88/88	136/146	120/122	102/104	101/103	212/212	0
	III	118/118	119/123	171/171	133/143	102/116	88/88	136/146	120/122	102/104	101/103	212/212	0
	IV	118/118	119/123	171/171	133/143	102/116	88/88	136/146	120/122	102/104	101/103	212/212	0
Biological model results	II Consensus	118/118	119/123	171/171	133/143	102/116	88/88	136/146	120/122	102/104	101/103	212/212	0
	II Composite	118/118	119/123	171/171	133/143	102/116	88/88	136/146	120/122	102/104	101/103	212/212	0
III SWAB	I	118/118	111/117/119	151/171	139/143	102/102	88/88	0	120/122	98/104	0	158/212	0
	II	118/134	111/117/119	151/171	139/143	102/102	78/88	0	120/122	84/98/104	103/103	158/212	75/75
	III	118/118	111/117/119	171/171	139/143	102/102	78/88	0	122/122	84/98/104	103/103	212/212	75/75
	IV	0	111/117/119	151/171	139/143	102/102	78/88	0	120/122	84/98/104	103/103	158/212	0
Biological model results	III Consensus	118/118	111/117/119	151/171	139/143	102/102	78/88	0	120/122	84/98/104	103/103	158/212	75/75
	III Composite	118/134	111/117/119	151/171	139/143	102/102	78/88	0	120/122	84/98/104	103/103	158/212	75/75
IV SWAB	I	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75
	II	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75
	III	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75
	IV	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75
Biological model results	IV Consensus	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75
	IV Composite	118/118	117/123	171/171	133/139	102/106	78/88	136/146	120/122	98/98	111/123	158/212	75/75

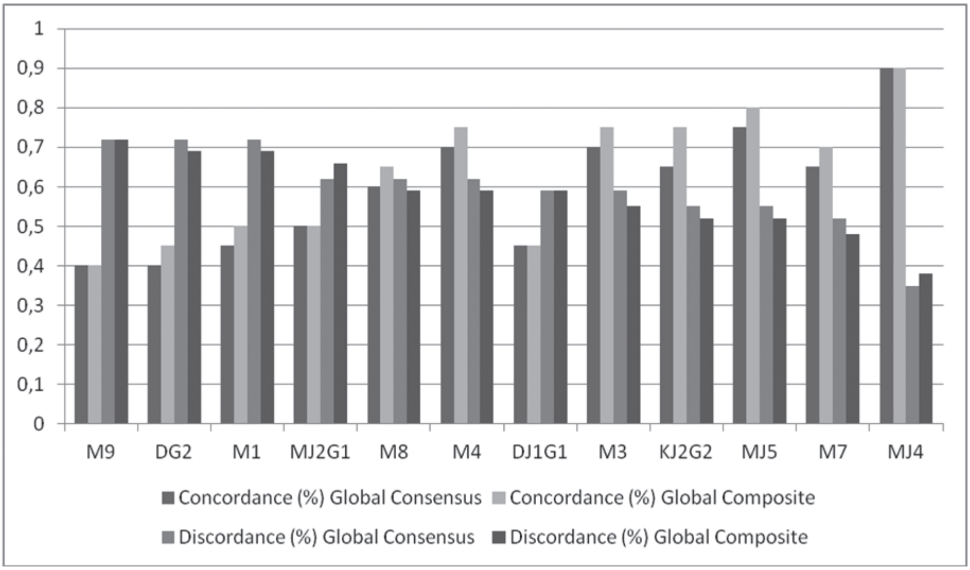
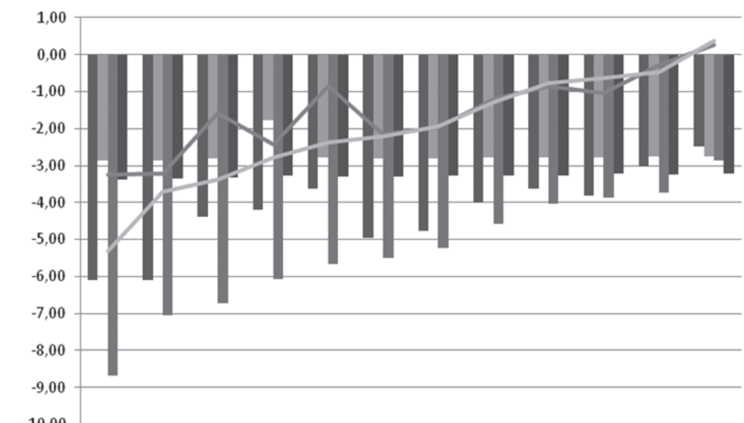


Figure 1. Classical biological model. Biological model results obtained in terms of percentage of concordances and discordances for each suspected male.



	M9	DG2	MJ2G1	MJ4	DJ1G1	M4	MJ5	M8	M3	KJ2G2	M1	M7
Global Consensus (ADO 0.65) Log10(Pr(E|Hp))	-6,10	-6,09	-4,39	-4,20	-3,63	-4,96	-4,77	-4,01	-3,63	-3,82	-3,03	-2,49
Global Consensus (ADO 0.65) Log10(Pr(E|Hd))	-2,86	-2,87	-2,81	-1,78	-2,78	-2,82	-2,81	-2,79	-2,78	-2,79	-2,76	-2,75
Global Composite (ADO 0.55) Log10(Pr(E|Hp))	-8,69	-7,06	-6,73	-6,07	-5,67	-5,49	-5,22	-4,57	-4,04	-3,86	-3,72	-2,86
Global Composite (ADO 0.55) Log10(Pr(E|Hd))	-3,39	-3,36	-3,34	-3,28	-3,29	-3,29	-3,28	-3,27	-3,27	-3,22	-3,24	-3,23
Δ Consensus	-3,23	-3,23	-1,58	-2,42	-0,85	-2,15	-1,96	-1,21	-0,85	-1,04	-0,27	0,27
Δ Composite	-5,30	-3,70	-3,39	-2,78	-2,38	-2,20	-1,94	-1,30	-0,78	-0,64	-0,48	0,36

Figure 2. Test for the veracity of the prosecution hypothesis. Likelihood ratio of the prosecution hypothesis (Log10(Pr(E|Hp))) versus the defense hypothesis (Log10(Pr(E|Hd))) for each suspected male. The Δ value gives an idea of the veracity of the hypothesis tested.

Figure 3. Non-contributor test results in comparison to the Overall LR value for each tested subject. **a** Global Consensus with ADO 0.65 **b** Global Composite with ADO 0.55.

(Figure 3). Allelic components attributable, consistently and coherently, to neither M7 nor any of the victims are absent in the traces. Suppl. materials 2 and 3 describe the detailed results of LRmix STUDIO (SA, Non-contributor test and LR values obtained for each suspect, for single STR locus and for the entire genetic profile – Overall LR).

Discussion

A comparative analysis of the multilocus genotypes of the 12 adult males considered present in the PAT during 2015 and the genetic profiles found in the analysed traces (biological model) has highlighted a variable percentage of discrepancies depending on the suspect, both in the consensus and in the composite profile (from 0.35 to 0.72). In particular, for some subjects (DG2, MJ2G1, M1, M4, M8 and M9), there is clear evidence about the absence of a genotype consistent with the alleles of the suspects on almost all of the STR loci from the traces (percentage of divergent alleles >0.60). These findings are an important support to the hypothesis of exclusion of these subjects as contributors to the genetic traces found on the swabs. As expected, alleles from MJ4, the father of the killed cubs, have been detected in the biological traces, not because the infanticidal male is the father (which is highly unlikely, Bellemain et al. 2006a-b), but because the swabs were probably contaminated with the cubs' blood, which shares half of their alleles with the father. It is difficult to determine with certainty the dynamics of what happened, but it is clear that the struggle lasted for several minutes, during which time the perpetrator repeatedly attacked both the cubs and the mother. The male, therefore, was contaminated with the blood of both. Samples were collected from the mother's injuries, which were caused by the male during the fight with the cubs, so it is highly probable that the male wounded the female and cubs alternately, leaving traces of the cubs' blood also on the mother. Moreover, comparison between the genetic profile of the suspects and the genetic profiles of the analysed traces has highlighted numerous concordances with the male M7. Actually, with the exception of only six alleles not observed on the swabs (allele 120 on locus cxx20, allele 165 on locus G10P, allele 108 on locus G1D, alleles 118/124 on locus Mu23 and allele 113 on locus Mu59), there is a full correlation between the alleles of the genetic profile of M7 and the alleles of the consensus and the composite genetic profile from the traces. Given that: (i) the traces appear in conditions of LT-DNA and are likely affected by stochastic phenomena (primarily drop-in and drop-out) and therefore are to be evaluated with extreme caution, in agreement with international guidelines (Gill et al. 2007), (ii) the discrepancies found on locus cxx20, that emerge applying the consensus method, can reasonably be due to ADO and (iii) the discrepancies found are anyway subjected to biostatistical evaluation with the probabilistic method of interpretation described above, according to the latest guidelines ISFG (Gill et al. 2012), the results presented indicate that M7 contributed with its biological fluids to the composition of the swabs. The remaining allelic components not attributable to M7 in more than one STR locus, suggest a genetic mixture condition and all of them derive from the blood of the victims (mother and cubs).

LRmix STUDIO (statistical model) estimates the likelihood and the Weight-Of-Evidence by comparing two hypotheses: the accusatory hypothesis (Hp) and the defensive hypothesis (Hd). Each one of the 12 adult males considered in the population during 2015 was tested individually. LRmix STUDIO tests what is the probability that DNA of the suspect contributing to the formation of the traces (DNA mixture extracted from swabs). The alternative hypothesis (Hd), is that the subject did not contribute to the formation of the traces. The calculation of the LR value is the result of the statistical analysis and it gives an estimate of the weight of the two hypotheses that were explored: the subject is present in the traces or the subject is absent. A high LR value indicates that Hp is much more reasonable than Hd; on the contrary, a low LR value indicates that the Hd is preferred. The LR values, obtained from the statistical analysis, range between 5.94×10^{-4} and 1.87 in the case of Global Consensus (Pr(D) = 0.65) and between 1.2×10^{-5} and 2.07×10^{-1} in the case of the Global Composite (Pr(D) = 0.55). The only suspect that obtains values of LR greater than 1 is M7. These values, considered with the results of the Sensitivity Analysis and Non-contributor test (see Suppl. material 2, and Suppl. material 3 for detailed LRmix STUDIO results on

Global Consensus and Global Composite respectively), exclude the hypothesis that the genetic material of MJ4, MJ5, DG2, KJ2G2, MJ2G1, DJ1G1, M1, M3, M4, M8 and M9 is present in the genetic traces, confirming the assessment expressed by applying the biological model and raising the inference that M7 has contributed to the traces with its own biological material.

The use of the statistical model is certainly preferable to the use of the classical model, as it eliminates the bias due to the presence of the father of the killed cubs between the suspected bears. Numerically, the LR value can range between 0 (absolute non-involvement) and +∞ (certain identification) and can express three consequences: (i) LR>1 means "strong support for the hypothesis of identification"; (ii) LR~1 means "neutrality" (the result of the genetic analysis does not allow support for either Hp or Hd, since it has not yielded useful results, i.e. it was inconclusive); (iii) LR<1 means "strong support for the hypothesis of exclusion", in a manner much more accentuated as LR tends to 0; if LR=0, the non-identity between the suspect and the perpetrator can be assured.

Finally, in light of the evaluations expressed, the question can be answered: Who is the perpetrator of the killing of BJ1 and her cubs? The genetic analysis conducted on the four swabs showed the presence of a very small amount of genetic material, resulting from the contribution of more subjects, which led to considering the samples in complex analytical conditions. The genetic typing, carried out with multitube protocol procedure on the traces, allowed the authors to obtain four genetic profiles largely overlapping amongst them and, on the whole, suitable for comparisons. The comparison was carried out for each adult male considered in the bears' population during 2015 and the genetic results were obtained from the traces, interpreting the results on the basis of both the biological model and the statistical model, in accordance with the strictest and updated protocols of interpretation, drawn from international scientific literature. The outcome of this comparison excludes the hypothesis that the genetic material of MJ4, MJ5, DG2, KJ2G2, MJ2G1, DJ1G1, M1, M3, M4, M8 and M9 is present in the analysed traces and that, therefore, these subjects may have contributed to the genetic traces; on the contrary, the overall assessment of the interpretative analysis carried out supports the hypothesis that the genetic material of M7 is present in the traces. Therefore, M7 is probably the killer of M33, F22 and BJ1.

Conclusions

Infanticide occurs in brown bear populations and it is an important cause of mortality, which can affect even the demographic evolution of the population (Gosselin et al. 2015). Similarly to that which was reported by the authors, in Sweden it was found that none of the four infanticidal males that have been genetically identified, was the father of the killed cubs (Bellemain et al. 2006a), likely due to the fact that infanticidal males can distinguish their own cubs from those of others, possibly because they recognise the mother (Bellemain et al. 2006b). Moreover, in Sweden, it has been seen that, in two of the eight cases of infanticide studied, the male also killed the mother (Bellemain et al. 2006a). Furthermore, in support of SSI theory, in Cantabria (Spain), it has been found that all the confirmed cases of infanticide occurred during the mating season, between April and June (Palomero et al. 2011). In the evolutionary scenario, females of brown bear have developed strategies to limit the consequences of infanticidal behaviour. One of these is sexual promiscuity, as a mechanism to confuse males and generate uncertainty about future paternity, in the brown bears as in other animals (Wolff and Macdonald 2004, Bellemain et al. 2006b). In the Italian alpine population of brown bear, the authors have genetically determined only one case of multipaternity: in 2006, the female MJ2 gave birth to two cubs, one of these being a cub of the then dominant male (Joze) and the other being a cub of the only other adult male, which started to reproduce exactly in 2006 (Gasper). Following Joze's disappearance, Gasper became the dominant male until 2015, the year of his death.

Analogously to what happens in bear populations subject to hunting pressure (LeCount 1987, Wielgus 2002, Wielgus and Bunnell 1994, 2000), in Trentino, the case of a reported infanticide occurred when the dominant male disappeared. Death or disappearance of older adult males frequently coincided with an influx of younger immigrant males, which apparently contributed with SSI to low reproductive rate and population decline (Wielgus and Bunnell 1994). M7, indeed, is a male born in 2009 by female DJ3 and male Gasper. During the juvenile dispersal, M7 moved outside the brown bear core area in Trentino: it was sampled in Lombardia (Bergamo and Sondrio provinces) from 2011 to 2013. In late 2013, it returned to Trentino, where it was sampled in 2014 and 2015, the year of the death of his father, the dominant male Gasper. Around 10 May 2015, the date of discovery of the corpses of BJ1 and her cubs, M7 was sampled near the site of infanticide. M7 probably returned to the core area after years of absence and, to increase its reproductive opportunities, has eliminated the cubs of an adult female. The mother's reaction, however, brought M7 to killing her and failing in his attempt at copulation. There is no evidence to suggest that M7 has reproduced until now.

Future monitoring actions should allow the supervision of the behaviour of infanticidal males (e.g. using radiotelemetry) and, in the case of risk of repeated infanticide, should facilitate suitable conservation actions (e.g. deterrence plans that can include some level of active and passive dissuasion activities). In small and isolated populations, in fact, behaviour that leads to the killing of cubs and adult females could lead to a further decrease in the Ne and a potential reduction in the population growth rate. Wildlife managers should be cautious when dealing with small populations of vulnerable and threatened species. The small populations, in fact, must be studied to understand their dynamics. The monitoring of litters is a fundamental tool for the management of bear populations: it has allowed the authors to genetically confirm the existence of cases of infanticide and in the future may facilitate the retrieval of information necessary to assess the impact of SSI on demographic trends. In the Italian Alps, although infanticide does not seem to be a serious problem and the population seems to be in progressive and continuous growth, it is imperative to continue to gather further information.

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Supplementary material 1

Text S1. Parameters used for parentage analysis

Authors: Francesca Davoli, Mario Cozzo, Fabio Angeli, Claudio Groff, Ettore Randi

Data type: statistical analysis

Link. https://doi.org/10.3897/natureconservation.25.23776.suppl1

Supplementary material 2

Text S2. Detailed results of LRmix STUDIO for each suspected male: Global Consensus (ADO 0.65)

Authors: Francesca Davoli, Mario Cozzo, Fabio Angeli, Claudio Groff, Ettore Randi

Data type: statistical analysis

Link. https://doi.org/10.3897/natureconservation.25.23776.suppl2

Supplementary material 3

Text S3. Detailed results of LRmix STUDIO for each suspected male: Global Composite (ADO 0.55)

Authors: Francesca Davoli, Mario Cozzo, Fabio Angeli, Claudio Groff, Ettore Randi

Data type: statistical analysis

Link. https://doi.org/10.3897/natureconservation.25.23776.suppl3

Supplementary material 4

Table S1. Detailed results of the biological model (consensus and composite)

Authors: Francesca Davoli, Mario Cozzo, Fabio Angeli, Claudio Groff, Ettore Randi

Data type:statistical analysis

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DATA PAPER

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BioNNA: the Biodiversity National Network of Albania

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Abstract

Recently, the Albanian Government started the process to join the European Union. This process also involves matching the EU parameters in protecting its biodiversity. In order to support the Albanian authorities, the Italian Ministry of Foreign Affairs, General Directorate for Development Cooperation (DGCS) and the International Union for Conservation of Nature (IUCN) joined efforts in the project "Institutional Support to the Albanian Ministry of Environment, Forest and Water Administration for Sustainable Biodiversity Conservation and Use in Protected Areas". This project aims at identifying priority needs in safeguarding ecosystem services and biodiversity conservation. Another project funded by the EU – "Strengthening capacity in National Nature Protection – preparation for Natura 2000 network" – started in 2015 with the aim to raise awareness for assisting local and national Albanian institutions to better exploit the potential of protected areas. One of the main issues encountered during these projects was the need for a national biodiversity data repository. The Biodiversity National Network of Albania (BioNNA) has been created to aggregate occurrence records of plants and animals and aims at becoming the most relevant source of information for biodiversity data as far as Albania is concerned. In this paper, the authors detail structure and data of BioNNA, including the process of data gathering and aggregation, taxonomic coverage, software details and WebGIS development. BioNNA is a milestone on the path towards Albania's inclusion in the EU and has also a relevant potential social relevance for improving people's awareness on the importance of biodiversity in the country.

Keywords
Albania, biodiversity, database, occurrence records, species

Introduction

Recently, the Albanian Government started the process to match European Union parameters in biodiversity conservation, as part of a larger process which is expected to lead to its inclusion in the Union. This process is highlighted by the recent expansion of the Albanian protected area system, as well as by the efforts to gather and aggregate biodiversity data (fundamental for reporting to the European Union and to other international conventions). In order to support the Albanian authorities, the International Union for Conservation of Nature (IUCN) implemented a project funded by the Italian Ministry of Foreign Affairs, General Directorate for Development Cooperation (DGCS), entitled "Institutional Support to the Albanian Ministry of Environment, Forest and Water Administration (currently Ministry of Environment - MoE) for Sustainable Biodiversity Conservation and Use in Protected Areas" (hereafter the "Institutional Support" project). This five-year project aimed at identifying priority needs in safeguarding ecosystem services and biodiversity conservation. The project focused on two protected areas (PAs), the Buna River Protected Landscape and the Shebenik-Jabllanicë National Park, which were selected, amongst other criteria, for the relevance of the ecosystem services they provide to local communities. The objective of the project was to start a capacity building process, which would lead the MoE to adopt a systemic approach in planning and managing protected areas. This included *inter alia* the participatory preparation of the two PAs management plans, training of local MoE staff, NGOs and university post graduate students on ecological monitoring, implementation of concrete activities (such as building and renovation of PAs ecotourism facilities) and carrying out awareness-raising actions.

Another project funded by the EU, "Strengthening capacity in National Nature Protection - preparation for Natura 2000 network" (NaturAL), is being implemented in Albania since 2015, aiming at a) raising awareness for assisting local and national Albanian institutions to better exploit the potential of protected areas and b) further promoting the partnership between the Italian Cooperation and the Albanian Ministry of Environment. Its main objective is to stop the loss of biodiversity in Albania by further improving the management practices of protected areas. This will be achieved by implementing five PAs' Management Plans and by preparing a preliminary list of Natura 2000 sites, with a view to their future submission to the European Commission by the Albanian authorities. A proper organisation and maintenance of, confidence on and accessibility to biodiversity data is mandatory for elaborating conservation policies and strategies, performing strategic assessments and identifying priorities of interventions, hence reducing the current rate of biodiversity loss (Wheeler et al. 2012, Costello et al. 2013). Especially since the Earth Summit of Rio in 1992, electronic access to biodiversity information has become a priority task worldwide, leading to great progress in the field of Biodiversity Informatics (European Commission 2011, Martellos and Attorre 2012, Costello et al. 2013). This, amongst other relevant results, led to the creation of global federated networks for the aggregation of biodiversity data (e.g. the Global Biodiversity Information Facility (GBIF) – http://www.gbif.org/ – and the BioCASE – http://www.biocase.org (Holetschek et al. 2012)). The GBIF especially, which aggregates more than 750 million occurrence data, was developed to accomplish one of the aims of the Convention on Biological Diversity, i.e. to make biodiversity data accessible online.

Hence, in the framework of the "Institutional Support" project, one of the strategic needs expressed by the Albanian institutions was the development of a national biodiversity data repository to aggregate, manage and make data available online. Such a tool was seen as mandatory for supporting Albanian Institutions in elaborating effective conservation strategies and reporting to relevant EU Conventions. Too often biodiversity data are unavailable to institutions, researchers and the general public in many countries or, if available, they are scattered in several different repositories which differ in platform, structure and data semantics (Edwards et al. 2000, Schuurman and Leszczynski 2008, Heidorn 2011, Martellos and Attorre 2012). Furthermore, biodiversity data are often collected in the framework and with the technical and financial support of international projects. Unfortunately, in absence of official data repositories, these data tend to disappear at the project's end. Several international projects have also a limited impact, since they are unrealistically complicated and impossible to maintain with limited local funding and human resources (Šašić et al. 2015). Furthermore, biodiversity data collected in extemporaneous efforts are often incomplete, with gaps for many taxa and habitats (Qiriazi and Sala 2000, Mullaj et al. 2007).

Albania has maintained many of its traditional agrosilvicultural practices, thus preserving most of its rich biodiversity, also in the absence of a formal system of protected areas. However, a process of over-exploitation of natural resources started in the last decades (since the country opened to external influences (Šašić et al. 2015)), together with the need for achieving several targets mandatory for being part of the EU, prompted the creation of an official network of protected areas. However, planning the development of an effective network of protected areas requires a thorough knowledge of local biodiversity. While Albania hosts a rich biodiversity (Qiriazi and Sala 2000, Mullaj et al. 2007, Mizsei et al. 2017, Szabolcs et al. 2017), only few data exist in the digital domain (e.g. Corine Biotopes (https://www.eea.europa.eu/data-and-maps/data/clc-2012-raster), Emerald (http://cdr.eionet.europa.eu/al/coltswjhw/Emerald/envtncdca), Red Lists of Flora and Fauna of Albania (http://www.nationalredlist.org/files/2015/06/Red-list-of-Albanian-flora-and-fauna-2013-MO-1280-20-11-2013.pdf)) and are often difficult to access, mainly due to scarcity of ICT resources and the absence of long-term vision and strategy in the past. Hence, it was planned to transfer to Albania previous experiences (Martellos et al. 2011, Holetschek et al. 2012), aiming at the development of a sustainable infrastructure for the aggregation of biodiversity data, the Biodiversity National Network of Albania (BioNNA). BioNNA was developed for supporting the National Biodiversity Strategy and Action Plan of the Albanian MoE for the period 2000–2015 and it will serve as a data repository according to international standards of biodiversity data collected by future projects as well as from citizen science initiatives. The Action Plan defines the main directions for preserving biodiversity and habitats, the identification and institution of protected areas and the protection of species inside and outside these areas. Due to BioNNA, biodiversity data collected from different sources are made available in a standard format to institutional decision-makers and planners, researchers, eco-tourists and the general public. Data originating from several different initiatives and projects are aggregated and validated by using standardised procedures by national and international experts in botany, zoology and biodiversity informatics.

In the present paper, the architecture of BioNNA, the data it currently aggregates and its WebGIS platform are detailed.

Methods

Data and taxonomic coverage

BioNNA aggregates primary biodiversity data, i.e. occurrences of plants and animals recorded in Albania. They have been obtained by Albanian and international researchers from three different sources: i) field data collected in the years 2015–2016 during the Institutional Support and the NaturAL projects, ii) data published in the scientific literature and iii) field data collected in the last 10 years by international experts or local researchers involved in the Institutional Support and the NaturAL projects. Field data have been collected mostly during June–October 2012/2013/2016 field campaigns in 7 protected areas, which were selected because of their relevance in terms of species and habitats diversity: Buna River-Velipoje Protected Landscape, Shebenik-Jabllanica National Park, Lake Shkodra Managed Nature Reserve, Korab-Koritnik Managed Nature Reserve, Divjaka-Karavasta National Park, Tomorri Mountain National Park, and Bredhi i Hotoves-Dangelli National Park (Fig. 1).

While a detailed description of the methodologies applied during field work is beyond the scope of this paper, it is important to mention at least the approaches used for each taxonomic group. Botanical data have been collected according to the phytosociological methods of Braun-Blanquet (1964) and Westhoff & van der Maarel (1978). The list of species occurring in scattered plots, selected in relation to the homogeneity of physical features, vegetation structure and species dominance, has been reported in order to identify vegetation communities and habitats.

During faunistic surveys, fish population sampling was undertaken following the CEN 14757 standardised protocol, using benthic multi-mesh gillnets and pelagic multi-mesh gillnets. For amphibians and reptiles, Visual Encounter Surveys (VES)

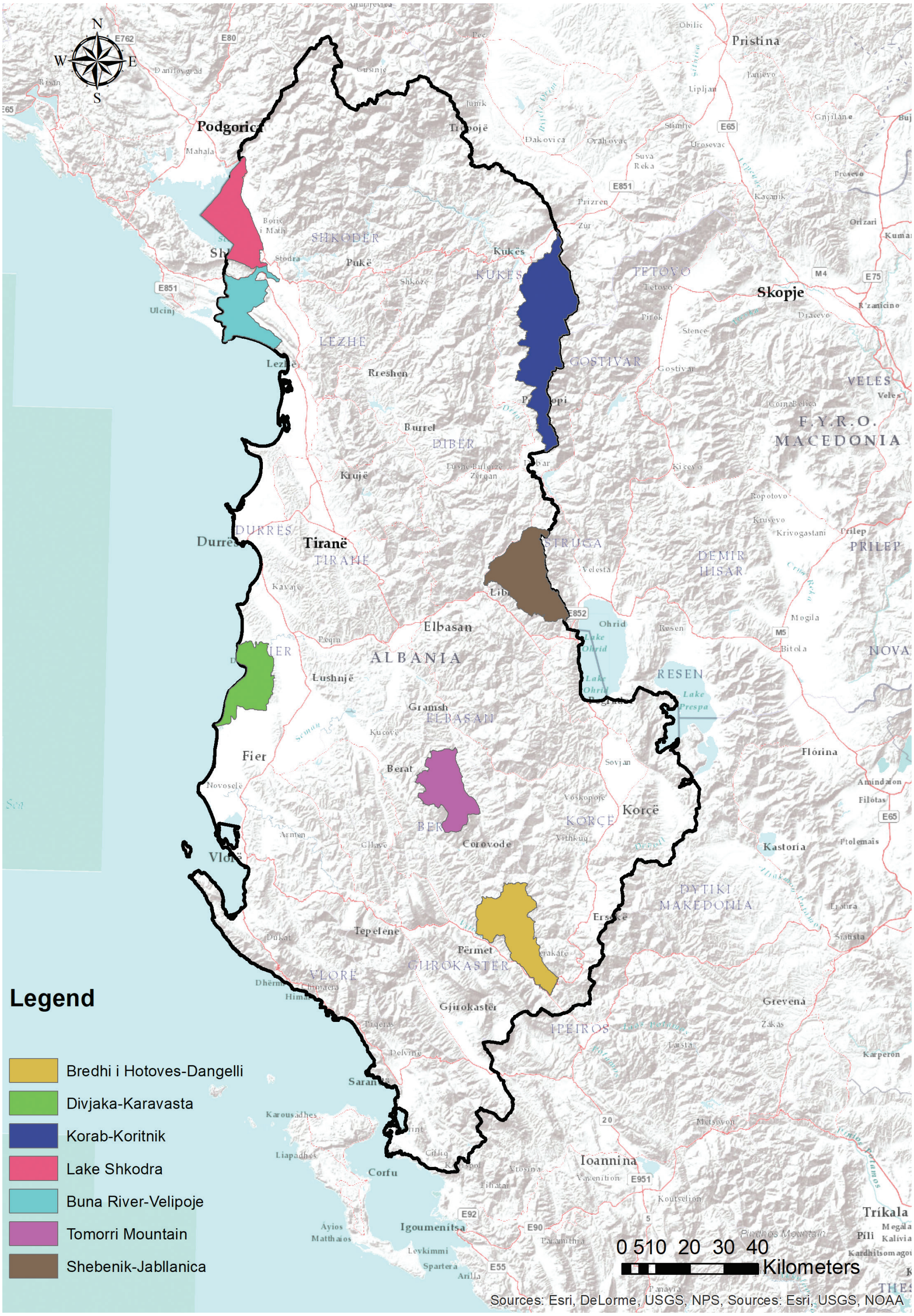


Figure 1. Distribution of the 7 protected areas in which field campaigns have been undertaken during the "Institutional Support to the Albanian Ministry of Environment, Forest and Water Administration for Sustainable Biodiversity Conservation and Use in Protected Areas" and NaturAL projects.

have been applied through transects according to the habitat requirements of each taxon. The censuses of birds were undertaken by using binoculars, bird calls and spotting scopes. Counting points were chosen as a function of accessibility, visibility and the best coverage of the area. Four different methods have been used for mammals: 1) Sherman traps, for capturing live specimens of small terricolous mammals, 2) camera traps, located in selected sites to sample large and medium-sized mammals, 3) tracks and signs observed along transects and 4) mist nets and bat detectors to study the presence of bats, mostly in caves and disused bunkers.

In Albania, 411 species of plants and 1206 species of animals are listed in the IUCN Red List of Threatened Species (Zeneli et al. 2014). Although the country is known to host 3629 species of plants and 1751 of animals (Meço and Mullaj 2015, Olšavská et al. 2016), these figures are likely to increase, since some areas in the Albanian territory are largely unexplored (Olšavská et al. 2016), especially in the north- and south-eastern portions of the country and some taxa, particularly animals, are still understudied even in well-known areas (Bego et al. 2018). To date, BioNNA has aggregated 9967 occurrence records of plants (1452 infrageneric taxa) and 11376 occurrence records of animals (637 infrageneric taxa). Although insects are known to be the largest group of animals on the planet, the number of records for this taxonomic group (266, 93 infrageneric taxa) are very scarce, mostly because of a lack of local entomologists.

Software

The core of BioNNA is a federated database system of primary biodiversity data (i.e. data obtained from floristic and faunistic observations and from specimens of natural history collections), which uses the BioCASE protocol for allowing the communication amongst the nodes of the federation and ABCD as data standard (Martellos et al. 2011, Holetschek et al. 2012, Martellos and Attorre 2012). When a user queries the system, the output is produced by sending the query to all federated nodes and returning an answer in the standard format ABCD. The BioCASE provider software is a free package supplied by the BioCASE project (http.//www.biocase.org), supported by the Botanic Garden and Botanical Museum of Berlin-Dahlem. The Web-App was the part of the system which required the highest effort, since it was developed from scratch. It allows the use of BioNNA in two different modalities; as a Standard User, which is allowed to query and retrieve data and as a Professional User, which also has access to several editing tools for managing the databases. In order to improve users' experience, the Web-App was developed by using innovative libraries from DEVEXPRESS, within a Microsoft Visual Studio development environment. All the functions of the Web-App are provided with automated input control tools, in order to prevent any erroneous use, in particular in data cleansing and updating processes.

BioNNA is hosted in a web portal which, other than the Web-App, hosts:

- Introductive Pages, where information about BioNNA and the dataset can be retrieved;

- Species Fact Sheets, where it is possible to list the taxa and their biological features. This section also links to the Encyclopaedia of Life (EOL), for retrieving information on taxonomy, distribution and conservation status of the species;
- Species Data pages, where it is possible to list the occurrence points. From the Species Data page, it is also possible to access the Web-GIS viewer;
- Web-GIS viewer, which is used for visualising the spatial distribution of each infrageneric taxon. This tool has been specifically developed for the BioNNA system, making use of open source and open code tools;
- Protected Areas page, which contains Socio-Ecological Fact Sheets (data and technical information) about the current situation of the Albanian protected areas.

A well as being a federated network of data providers, BioNNA was also planned as a data provider for the BioCASE and GBIF networks, in order to make the biodiversity heritage of Albania available worldwide.

Results and discussion

BioNNA: datasets and policy

BioNNA is currently the largest aggregator of primary biodiversity data in Albania and it is planned to grow further by aggregating new datasets. However, the aggregation of a new dataset into the federation is not an automated process. Each dataset must have a certain level of data quality, as well as a minimum mandatory set of data: a unique ID for each observation record, the scientific name of the taxon, latitude and longitude. For consistency purposes, it is suggested that the coordinates follow the WGS84 geographic system in decimal degrees. These minimum requirements are evaluated by the Scientific Committee of BioNNA. In addition to the minimum mandatory dataset, many other data can potentially be included. Those which have been already implemented in the Web-App of BioNNA are: date of survey or collection, collector or observer, locality of collection or observation, community in which the organism was surveyed and references. Furthermore, the use of ABCD, which lists ca. 1200 concepts, permits far more data for each observation record to be aggregated in the BioNNA federation.

BioNNA is also storing data on sensitive taxa. In this case, a relevant issue is given by showing the exact locations of occurrence, providing the risk of misuse of those sensitive data by parties with commercial interests. On the other hand, the exact location of sensitive taxa (e.g. species classified as threatened by the IUCN – critically endangered, endangered or vulnerable – e.g. important nursery colony of bats, den site of bears, otter holts etc.) is an important source of information for decision-makers, such as the Ministry of Environment, the National Environmental Agency (NEA), the National Agency of Protected Areas (NAPA) and other Ministries and development agencies. Hence, sensitive data will not be displayed online, but provided under strict safeguard policy, in order to avoid their misuse.

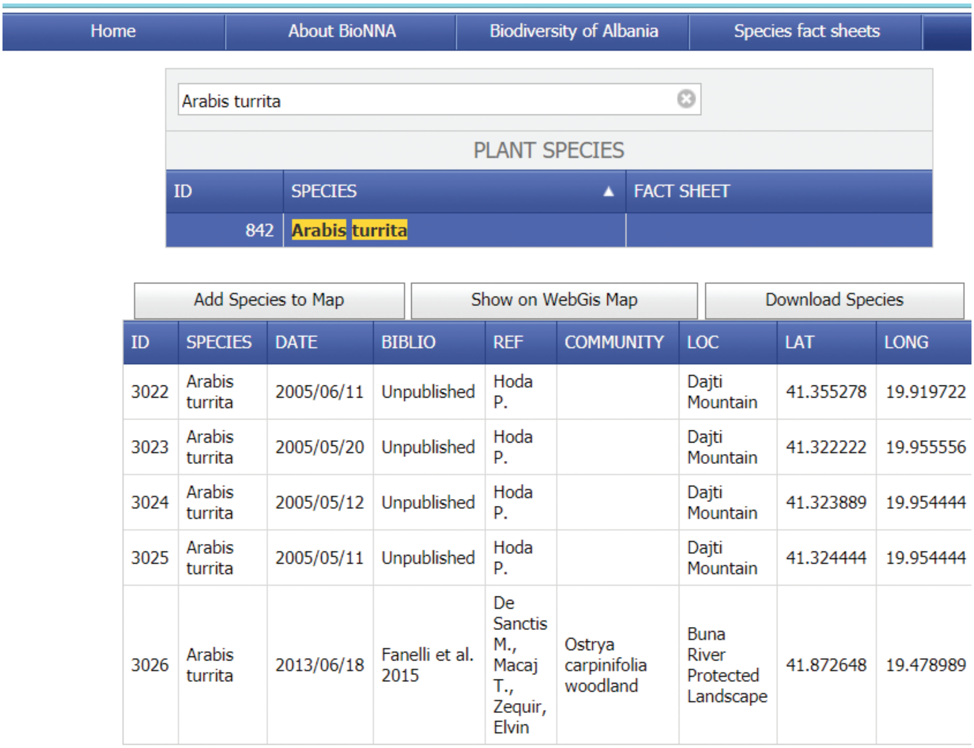
Currently, BioNNA aggregates not only data of taxa listed in the Birds and Habitats Directives, but also of several other taxa and potentially of all those which are known to occur in the country. This is of pivotal importance for Albania, since the data aggregated in BioNNA could provide evidence of the need for protection of some endemic/rare taxa or highlight areas of high biodiversity or relevance, deserving some degree of protection.

Using BioNNA

The web portal of BioNNA (www.bionna.al) provides users with several ways to access the data. The query system hosts two tools, one for plants and one for animals. In the 'Species data' section, in addition to the data sheets, users can visualise a data table with all the occurrence records for a species. For each occurrence, ID number, latitude and longitude, as well as the optional details are reported (when available: date of data entry, number of individuals, type of data collected, references, vegetation community (only for plants) and locality in which the individual has been found; Fig. 2). In this section, the user can also create a spatial reference file (.shp) for each selected species and visualise it by using the WebGIS viewer, which is one of the most relevant features of BioNNA. It displays occurrence points on a satellite map of Albania, evidencing protected areas, as well as counties borders (Fig. 3). From the 'Species data' page, it is possible, using the button "Add Species to Map", to load the occurrences of a taxon into the Web-GIS Viewer. This can be done for a single taxon or for a selection of taxa. The viewer displays the points on the map, hence allowing a preliminary analysis of the spatial distribution of the taxa. Furthermore, it is possible to access the metadata associated with the shapefile of each taxon. From the 'Species data section' it is also possible to download a data table of occurrence records in Excel format (.xls), which was chosen since it can be imported into several modelling and analysis tools (e.g. MaxEnt (Elith et al. 2011) and GeoCAT (Bachman et al. 2011)).

Relevance of BioNNA and future development

BioNNA aims at supporting Albanian Institutions in managing data on the biodiversity of the country. Several stakeholders will potentially benefit from it, especially whether it will receive regular updates and grow with the aggregation of other datasets. Practitioners will benefit from knowing which taxa are known to occur in the areas they manage, in order to plan species- or habitat-specific conservation measures. A detailed and up-to-date knowledge of the distribution of "problematic" taxa, e.g. those which prey on livestock (e.g. wolf, bear, fox, jackal) will help reducing the conflicts between human needs and wildlife conservation. Depicting the areas in which charismatic species live will also improve tourism, providing economic benefits to local communities, hence leading to an increased awareness of the values provided by wildlife conservation amongst local populations.



Home	About BioNNA	Biodiversity of Albania	Species fact sheets

Arabis turrita

PLANT SPECIES

ID	SPECIES	FACT SHEET
842	Arabis turrita	

Add Species to Map | Show on WebGis Map | Download Species

ID	SPECIES	DATE	BIBLIO	REF	COMMUNITY	LOC	LAT	LONG
3022	Arabis turrita	2005/06/11	Unpublished	Hoda P.		Dajti Mountain	41.355278	19.919722
3023	Arabis turrita	2005/05/20	Unpublished	Hoda P.		Dajti Mountain	41.322222	19.955556
3024	Arabis turrita	2005/05/12	Unpublished	Hoda P.		Dajti Mountain	41.323889	19.954444
3025	Arabis turrita	2005/05/11	Unpublished	Hoda P.		Dajti Mountain	41.324444	19.954444
3026	Arabis turrita	2013/06/18	Fanelli et al. 2015	De Sanctis M., Macaj T., Zequir, Elvin	Ostrya carpinifolia woodland	Buna River Protected Landscape	41.872648	19.478989

Figure 2. The BioNNA search interface.

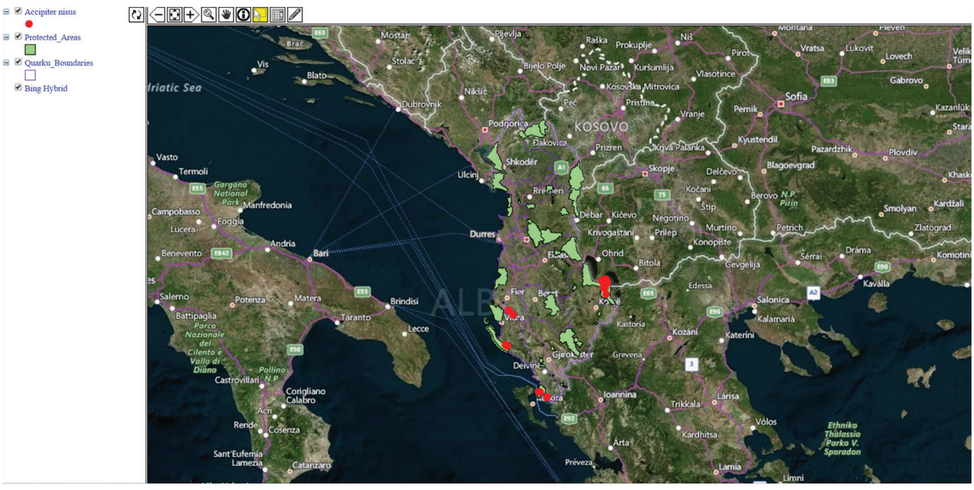


Figure 3. BioNNAWebGIS interface.

BioNNA will also be useful to scientists in depicting gaps in knowledge of the biodiversity of Albania, hence driving new sampling efforts. A large portion of Albania still remains under-investigated and it is likely that several interesting/rare taxa occurring in the country are still to be reported (Bego et al. 2018). Academics will have a fundamental role in the development of BioNNA, since they will be involved in supporting the Albanian Ministry of Environment for its future growth. Therefore, a pool of outstanding local researchers has been selected as the Scientific Committee of BioNNA, with the aim of:

1. Validating the dataset before aggregating them in the federated database system;
2. Deciding whether to provide data on sensitive species to interested parties. Monitoring the use of the sensitive data should be mandatory, in order to ensure the protection of sensitive taxa;
3. Promoting the use of BioNNA to elaborate effective conservation strategies and defining the main strategies for its further development (i.e. through the implementation of citizen science initiatives – e.g. initiatives such as the CSMON-LIFE project, BioBlitzes, The Great Nature, Project BudBurst, Nature's Notebook (Van Vliet and Moore 2016).

The Scientific Committee will be supported by international scientists during its early stages. The active cooperation of local scientists with the Albanian Ministry of Environment will be fundamental to improve BioNNA and make it a reliable reference point for Albanian biodiversity data. Other projects (e.g. "Conservation and sustainable use of biodiversity at Lakes Prespa, Ohrid and Shkodra/Skadar") currently aim at monitoring wild species in Albania and the occurrence data collected by scientists and the staff of protected areas may well be integrated within the BioNNA database.

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RESEARCH ARTICLE

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Planning priority conservation areas under climate change for six plant species with extremely small populations in China

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Abstract
The concept of Plant Species with Extremely Small Populations (PSESP) has been employed to guide conservation of threatened plant species in China. Climate change has a high potential to threaten PSESP. As a result, it is necessary to integrate climate change effects on PSESP into conservation planning in China. Here, ecological niche modelling is used to project current and future habitat distributions of six PSESP in China under climate change scenarios and conservation planning software is applied to identify priority conservation areas (PCAs) for these PSESP based on habitat distributions. These results were used to provide proposals for in-situ and ex-situ conservation measures directed at PSESP. It was found that annual precipitation was important for habitat distributions for all six PSESP (with the percentage contribution to habitat distributions ranging from 18.1 % to 74.9 %) and non-climatic variables including soil and altitude have a large effect on habitat suitability of PSESP. Large quantities of PCAs occurred within some provincial regions for these six PSESP (e.g. Sichuan and Jilin for the PSESP *Cathaya argyrophylla*, *Taxus cuspidata*, *Annamocarya sinensis* and *Madhuca pasquieri*), indicating that these are likely to be appropriate areas for in-situ and ex-situ conservation measures directed at these PSESP. Those nature reserves with large quantities of PCAs were identified as promising sites for in-situ conservation measures of PSESP; such reserves include Yangzie and Dongdongtinghu for *C. argyrophylla*, Songhuajiangsanhu and Changbaishan for *T. cuspidata* and Shiwandashanshuiyuanlian for *Tsoongiodendron odorum*. These results suggest

that existing seed banks and botanical gardens occurring within identified PCAs should allocate more resources and space to ex-situ conservation of PSESP. In addition, there should be additional botanical gardens established for ex-situ conservation of PSESP in PCAs outside existing nature reserves. To address the risk of negative effects of climate change on PSESP, it is necessary to integrate in-situ and ex-situ conservation as well as climate change monitoring in PSESP conservation planning.

Keywords

PSESP, climatic change, systematic conservation planning, China, in-situ and ex-situ conservation measures

Introduction

Climate change has a large potential to threaten plant diversity from species to biomes, as well as hinder endangered species protection (Thuiller et al. 2005, Bellard et al. 2012, Diez et al. 2012, Grimm et al. 2013, Watson et al. 2013). Climate change may result in the migration, vulnerability or extinction of plant species by causing species distributions to shift, habitat fragmentation to increase, population sizes to decrease and genetic diversity to decline (Thuiller et al. 2005, Heller and Zavaleta 2009, Bellard et al. 2012, Diez et al. 2012, Grimm et al. 2013, Watson et al. 2013). Furthermore, other human impacts are likely to cause additional habitat loss and threaten plant species (Tilman and Lehman 2001, Kier et al. 2005, Vásquez et al. 2015). In particular, threatened plants with narrow niche width and small population sizes may fail to adapt to novel climatic conditions and thus become endangered or even extinct (Bellard et al. 2012, Botts et al. 2013, Slatyer et al. 2013). Future climate may change rapidly and enhance loss of threatened plant species stemming from vulnerability to climate change, which is influenced by species' sensitivity and adaptive capacity, as well as the degree of exposure (Thuiller et al. 2005, Bellard et al. 2012, Diez et al. 2012, Watson et al. 2013). Plant conservation faces great uncertainty as a result of climate change; decreasing this uncertainty is a challenge for conservation biologists and government managers (Lavergne et al. 2004, Heller and Zavaleta 2009). There is a need to integrate climate change into conservation planning for threatened plants.

As many of its species are currently threatened or on the brink of extinction, China is one of the highest priorities for biodiversity conservation globally (López-Pujol et al. 2006, Wang et al. 2015, Zhang et al. 2015, Feng et al. 2017). It is urgent for China to take effective measures to conserve threatened plant species. A list of 120 wild plant species was recently identified as the first set of species in the nation to receive urgent protection (Ren et al. 2012, Volis 2016). These 120 species are labelled Plant Species with Extremely Small Populations (PSESP) due to: 1) the limited number of mature individuals in the wild; 2) restricted distribution ranges; 3) recognition as national or regional endemic species in China; and 4) economic development or scientific value (Ma et al. 2013, Chen et al. 2014, Wade et al. 2016, Volis 2016, Wang et al. 2017). To conserve the 120 PSESP, several national and regional conservation strategies have been implemented and such strategies will be expanded across China (Ren et al. 2012, Ma et al. 2013). In-situ and ex-situ conservation methods have been widely employed for PSESP (Ren et al. 2012, Ma et al. 2013, Wade et al. 2016, Volis 2016). These approaches have the advantage of being cost-efficient, flexible and capable of supplementing other conservation measures (Wade et al. 2016, Volis 2016). However, due to the vulnerability of PSESP to rapid climate change, the effectiveness of in-situ and ex-situ PSESP conservation actions may be decreased by climate change (Chen et al. 2014, Wang et al. 2017). Planning long-term in-situ and ex-situ conservation strategies for the protection of PSESP under scenarios of climate change can be challenging (Wang et al. 2017).

Identifying priority conservation areas (PCAs) is a useful step in making climate change adaptation strategies for the conservation of PSESP. Recently, many conservation biologists and ecologists have used ecological niche modelling (ENM) in combination with conservation planning software to identify PCAs for endemic, threatened and endangered plant species under climate change conditions (Pérez and Font 2012, Wan et al. 2014, 2015, Adams-Hosking et al. 2015, Wang et al. 2015). ENMs, which are based on occurrence records and climatic variables, are widely used to predict spatial distribution patterns of species diversity (Merow et al. 2013, Adams-Hosking et al. 2015, Wang et al. 2015). Such models are used to generate proposals for biological conservation actions and to examine their probable feasibility (Adams-Hosking et al. 2015, Wang et al. 2015, Abrahms et al. 2017, Reside et al. 2017). For example, ENMs may enable conservation practitioners to predict previously unknown locations of species (Pearson et al. 2007). Conservation planning software is commonly used to generate a spatial conservation framework that can be used to prioritise large-scale conservation projects that involve numerous species or to identify the most effective conservation areas that will capture target species, as predicted by the results of ENMs (Moilanen 2007, Di Minin and Moilanen 2012, Lehtomäki and Moilanen 2013, Wan et al. 2014, Abrahms et al. 2017). The ability of existing or proposed nature reserves to protect threatened plants can be evaluated using ENMs and conservation planning software and new conservation areas could be designated in order to respond to climate change effects (Wan et al. 2014, 2015, 2017, Wang et al. 2016). In this way, the plant conservation effectiveness of a network of nature reserves can be maximised under climate change conditions (Wan et al. 2015, Wang et al. 2016, Abrahms et al. 2017). For example, Wang et al. (2015) used ENM coupled with conservation planning software to identify PCAs for threatened plants in China under climate change. The habitat distributions of PSESP are related to the climatic variables temperature and precipitation and therefore may be affected negatively by future climate change (Wang et al. 2017). Hence, there is a need to identify PCAs for PSESP in China under climate change conditions and to provide a simple protection assessment system for either in-situ or ex-situ conservation measures.

PSESP as a designation is not only important for conservation prioritisation in China, but also may be a useful framework in conservation efforts for threatened plants around the world (Wang et al. 2017). As a consequence of climate change, species will respond by shifting their distributional ranges and some populations may shrink to the point of extinction (Thuiller et al. 2005, Mawdsley et al. 2009, Bellard et al. 2012, Grimm et al. 2013, Watson et al. 2013). Hence, it is important for conservation biologists and governmental managers to integrate the impacts of climate change on habitat distributions of plants into conservation planning for PSESP (Mawdsley et al. 2009, Bellard et al. 2012). Here, PCAs are delineated and potential sites identified for conservation of PSESP in China under climate change conditions (Chen et al. 2014, Wade et al. 2016, Volis 2016, Wang et al. 2017). Conservation of PSESP in China requires an integrated approach, encompassing both in-situ and ex-situ conservation measures and their methodologies, as well as establishing effective evaluation systems for PSESP (Wade et al. 2016, Volis 2016, Wang et al. 2017). In-situ conservation measures, which may occur within nature reserves and other types of scenic locations, can be used to maintain the evolutionary and biological reproductive potential of the ecological system (Wade et al. 2016, Volis 2016). Ex-situ conservation measures, in which parts of the population are placed in a new location, can be used to identify suitable living environments for species for the future and to retain existing populations (Wade et al. 2016, Volis 2016). By definition, PSESP have a limited number of individuals and small population sizes in China (Wade et al. 2016, Volis 2016). Natural regeneration of PSESP is poor and some species have no chance of survival (Ren et al. 2012, Ma et al. 2013). There is a need for increased research on reproduction, wild endangered population dynamics, conditions conducive to growth and seed bank establishment to facilitate ex-situ conservation of PSESP (Wade et al. 2016, Volis 2016).

The primary objective of this study is to identify PCAs for PSESP in China under climate change conditions. To achieve this objective, six PSESP were selected as study species and ENM used to model the habitat distributions of these PSESP under current and future climate scenarios and the environmental variables that contribute significantly to the habitat distributions of the focal PSESP were explored. Then, conservation planning software was used to identify PCAs for PSESP in China under projected climate change conditions based on the species' habitat requirements. Finally, the regions were identified with high potential to serve as effective conservation sites for the focal PSESP based on identified PCAs and suggestions were developed for in-situ and ex-situ conservation measures of PSESP.

Materials and methods

Study species and occurrence records

The State Forestry Administration of China has been concentrating on management of PSESP through its "Conservation Programme for Wild Plants with Extremely Small Populations in China (from 2011 to 2015)" (http://www.forestry.gov.cn/portal/main/s/72/content-540092.html). This plan identifies PSESP as species comprising fewer than 5,000 individuals and restricted to known localities (Ren et al. 2012, Ma et al. 2013). *Cathaya argyrophylla*, *Taxus cuspidata*, *Annamocarya sinensis*, *Ulmus elongata*, *Tsoongiodendron odorum* and *Madhuca pasquieri* were selected as the study species for the present analysis. Occurrence records were obtained from the State Forestry Administration of China and were also obtained from a number of reference resources (e.g. China's State Forestry Administration and the Institute of Botany, Chinese Academy of Sciences, 2013; Flora of China (http://foc.eflora.cn/), Fang et al. 2009, Ren et al. 2012, Wang et al. 2017). Occurrence points were recorded in 10 arc-minute grid cells to avoid errors in georeferencing, obvious misidentifications and duplicate species records in each grid cell (Wang et al. 2016). The number of occurrence records used as inputs into the ENMs ranged from 10 to 49 per species (Pearson et al. 2007, Merow et al. 2013, Table 1).

Environmental variables

Spatial data were obtained for 14 environmental variables at a 10-arc-min resolution including eight soil, one topographic, one natural state and four climate variables (Suppl. material 1: Table S1; Wang et al. 2016). Multi-collinearity was tested amongst variables using Pearson correlation coefficients and variables were excluded with a cross-correlation coefficient absolute value exceeding 0.85. These 14 environmental variables may influence the current distribution and physiological performance of threatened plant species and can therefore be used in ENMs to infer the current climate suitability of PSESP (Wang et al. 2017).

To model the future habitat distributions of PSESP in the 2080s (i.e. 2070–2099), the average projection maps generated under four global climate models were used (i.e. bcc_csm1_1, csiro_mk3_6_0, gfdl_cm3 and mohc_hadgem2_es) and two greenhouse gas concentration scenarios as representative concentration pathways (RCPs) of 4.5 (mean, 780 ppm; range, 595 to 1005 by 2100) and 8.5 (mean, 1685 ppm; range, 1415 to 1910 by 2100), representing low and high gas concentration scenarios, respectively (http://www.ccafs-climate.org/).

Modelling the distributions of PSESP

Using Maxent (a commonly-used ENM software) and the 14 environmental variables, the current and future species distributions for the six focal PSESP were modelled with maximum entropy (Phillips et al. 2006, 2017, Merow et al. 2013). Then, RCPs 4.5 and 8.5 were used to project distributions of PSESP under low and high greenhouse gas concentration scenarios. These projections kept the non-climatic variables constant into the future, with only the climate variables changing in accordance with these scenarios (Wang et al. 2016). Maxent is appropriate for this type of modelling for a variety of reasons: (1) it can be used with small sample sizes, which drastically impact both the performance and the adjustment of ENM (Pearson et al. 2007, Merow et al. 2013, Fourcade et al. 2014, Proosdij et al. 2016); (2) It is insensitive to multicollinearity amongst predictors, which can impede the analysis of species-environment relationships

Table 1. Characteristics of the six focal PSESP and Maxent performance test results.

Name	Form	Altitude (m)	Individual	Record	Training AUC	Test AUC	Training Omission	Ecoregion
Cathaya argyrophylla	Tree	900–1900	4484	10	0.983	0.980	0.00±0.00	TBMF
Taxus cuspidata	Tree	500–1000	42700	24	0.997	0.996	0.03±0.04	TBMF
Annamocarya sinensis	Tree	500–2500	472	19	0.993	0.987	0.04±0.02	TBMF
Ulmus elongata	Tree	500–900	1430	11	0.995	0.993	0.03±0.04	TSMBF
Tsoongiodendron odorum	Tree	500–1000	6548	49	0.989	0.985	0.04±0.04	TSMBF
Madhuca pasquieri	Tree	0–1100	6429	23	0.992	0.990	0.05±0.04	TBMF

Individual: the number of saved individuals; Record: the number of occurrence records as an input of Maxent; TBMF: Temperate Broadleaf & Mixed Forests; TSMBF: Tropical & Subtropical Moist Broadleaf Forests.

in multiple regression settings; finally, (3) it provides the relative contribution of each variable as an output (Pearson et al. 2007, Merow et al. 2013, Phillips et al. 2017). All grid cells were assumed to be possible distribution space with maximum entropy (Phillips et al. 2006, Merow et al. 2013). Maxent predicted habitat suitability across maps wherein pixel values of 1 indicated the highest scores of habitat suitability and values of 0 indicated the lowest habitat suitability (Phillips et al. 2006).

For modelling the distributions of PSESP, the Maxent sets were as follows: 1) the regularisation multiplier (beta) was set to two to produce a smooth and general response that could be modelled in a biologically realistic manner (Radosavljevic and Anderson 2014); 2) a 10-fold cross-validation approach was used to remove bias from recorded occurrence points (Oke and Thompson 2015); 3) the maximum number of background points was set to 10,000 (Phillips et al. 2006); 4) the jackknife method was used to determine the response curves of environmental variables to habitat suitability (Merow et al. 2013); 5) The cloglog was used as the output of modelling, giving it a stronger theoretical justification than the logistic transformation (which it replaces by default) (Phillips et al. 2017); and 6) other settings were identical to those described in Phillips et al. (2006). The variable jackknife was used to evaluate the percentage contribution (PC) of environmental variables to distribution modelling for each species (Merow et al. 2013). The threshold PC of habitat suitability was set at 15%; environmental variables exceeding this level of PC were considered important for each species (Oke and Thompson 2015).

The analysis produced a receiver operating characteristic (ROC) curve, which established each value of the prediction results as a possible judging threshold; the corresponding sensitivity and specificity of the predicted results were obtained (Phillips et al. 2006). The performance of the model was evaluated by calculating the area under the ROC curve (AUC). Models were graded as poor (AUC<0.7), fair (0.7<AUC<0.8), good (0.8<AUC<0.9) or very good (0.9<AUC<1.0) (Swets 1988). However, AUC alone is not sufficient to evaluate the model performance (Lobo et al. 2008). The training omission rate is the proportion of the training occurrence localities that fall in pixels of predicted absence based on binomial probabilities (Phillips et al. 2006, Anderson and Gonzalez 2011). These are 1-sided tests of the null hypothesis that test points are no better predicted than random (Phillips et al. 2006, Anderson and Gonzalez 2011).

Binomial probabilities were based on three thresholds: Fixed cumulative value 10, 10th percentile training presence and Equal training sensitivity and specificity, used by default by Maxent (Phillips et al. 2006). An average training omission rate of less than 17% is considered good for the model (Anderson and Gonzalez 2011).

Prioritising conservation areas for PSESP

The Zonation conservation planning software (http://cbig.it.helsinki.fi/software/) was used to prioritise conservation areas for PSESP under conditions of climate change (Lehtomäki and Moilanen 2013; Wan et al. 2017). Zonation is usually used as a spatial conservation prioritisation framework for large-scale conservation planning directed at multiple biodiversity features (e.g. species; Lehtomäki and Moilanen 2013). The highest priorities for conservation, namely protection of hot-spot areas, were confirmed by identifying the top-ranking cells after computation in Zonation (Moilanen 2007, Di Minin and Moilanen 2012). To decrease conservation uncertainty due to climate change, the geographic distance between the current and future distributions of each PSESP was minimised and the influence of climate change on species distributions was considered when selecting potential sites in Zonation for reserves (Lehtomäki and Moilanen 2013, Wang et al. 2015).

The distributions of each species under current, low and high gas concentration scenarios, as assessed by the Maxent value of each grid cell, were used as input feature maps for the Zonation software (Wang et al. 2015). The present distributions of the target species were weighted as 1 and future distributions were weighted as 0.5 when input into Zonation (Adams-Hosking et al. 2015). The core-area Zonation solutions were used to optimally capture the areas of the distribution of PSESP at each removal step (Lehtomäki and Moilanen 2013; Wan et al. 2017). The 'warp factor' was set to 1 (i.e. the single worst pixel was removed at each iteration) to maintain the reliability of the output. Default settings were used for 'edge removal' (i.e. pixels were removed preferentially from the edges of distributions; Lehtomäki and Moilanen 2013).

As limited resources rarely allow all potential habitats to be conserved, the top 10% of grid cells of distributions were extracted (referred to as the grid cells ranking in the top 10% in the following), based on PCAs for each PSESP according to realised ecoregional ranges of species as presented in Wang et al. (2017), Olson et al. (2001) and Xu et al. (2017). *Ulmus elongata* and *T. odorum* belong to the Tropical & Subtropical Moist Broadleaf Forests ecoregion and *C. argyrophylla*, *T. cuspidata*, *A. sinensis* and *M. pasquieri* belong to the Temperate Broadleaf & Mixed Forests ecoregion (Olson et al. 2001, Wang et al. 2017).

Identifying potential regions for conservation of PSESP

First, grid cells of PCAs were downscaled from 10 arc-minutes to 2.5 arc-minutes and the number of grid cells was used to quantify the size of PCAs in order to improve the precision of the assessment (Araújo et al. 2011). Then, the grid cells were identified where PCAs occurred in each provincial region and those occurring within existing nature reserves belonging to each provincial region (Araújo et al. 2011). Data for the cities Beijing and Tianjin and data for Hebei Province were combined, as were data for Shanghai with Zhejiang Province, Chongqing with Sichuan Province and both Hong Kong and Macau with Guangdong Province (Axmacher and Sang 2013). This allowed the identification of potential regions (including nature reserves) with high potential to conserve PSESP (Wang et al. 2015). The map of ecoregions used for this purpose was downloaded from http://www.worldwildlife.org/ and the map of nature reserves was obtained from the World Database on Protected Areas (WDPA; http://www.wdpa.org/; Fig. 1). Finally, proposals were developed for in-situ and ex-situ conservation of PSESP based on this process of delineation of PCAs.

Results

All ENMs had AUC values greater than 0.7 for both the training and test data sets and the training omission rates were less than 17 %, indicating a high level of accuracy for each model (Table 1). Annual precipitation was important for the distributions of all six PSESP (with PCs ranging from 18.1 % to 74.9 %; Table 2). The annual precipitation response curves of distributions of *T. cuspidata*, *A. sinensis* and *T. odorum* were single peak in shape (Fig. 2b, c, e). Response curves indicated that with increasing annual precipitation, habitat suitability of *C. argyrophylla*, *U. elongata* and *M. pasquieri* is likely to increase and then remain stable (Fig. 2a, d and f). Temperature seasonality contributes substantially to the distribution of *T. cuspidata* and precipitation seasonality was the most important variable influencing distributions of *C. argyrophylla* and *U. elongata* (Table 2). Soil variables, such as bulk density, cation exchange capacity and sand as a fraction of soil texture, exert a large effect on distributions of *C. argyrophylla*, *T. cuspidata* and *U. elongata*. Habitat suitability of *A. sinensis* was affected by altitude (PC = 18.4%; Table 2).

Out of all provinces, the greatest total area of PCAs for the studied PSESP occurred in Sichuan and Jilin; PCAs in these provinces included those of *C. argyrophylla*, *A. sinensis* and *M. pasquieri* and *T. cuspidata* (Fig. 3). Overall, PCAs of *C. argyrophylla* occurred in Anhui, Henan, Hubei, Hunan, Jiangsu and Jiangxi (Fig. 3). For *T. cuspidata*, PCAs occurred in Heilongjiang, Jilin and Liaoning; PCAs for *A. sinensis* occurred in Sichuan, Hubei, Shaanxi and Tibet; PCAs for *U. elongata* occurred in Jiangxi, Fujian and Hunan; PCAs of *T. odorum* occurred in Guangdong and Guangxi; and PCAs for *M. pasquieri* occurred in Sichuan, Tibet and Jiangxi (Fig. 3).

The nature reserves with largest capacity to conserve the focal PSESP included Yangzie, Songhuajiangsanhu, Changbaishan, Dongdongtinghu and Shiwandashanshuiyuanlian (Figs 1, 3; Suppl. material 1: Table S2). Specifically, PCAs indicate that Yangzie and Dongdongtinghu are highly suitable for *C. argyrophylla* and Songhuajiangsanhu and Changbaishan demonstrate high PCA occurrence for *T. cuspidata* (Figs 1, 3; Suppl. material 1: Table S2). There is high overlap between PCAs of *A. sinensis* and the

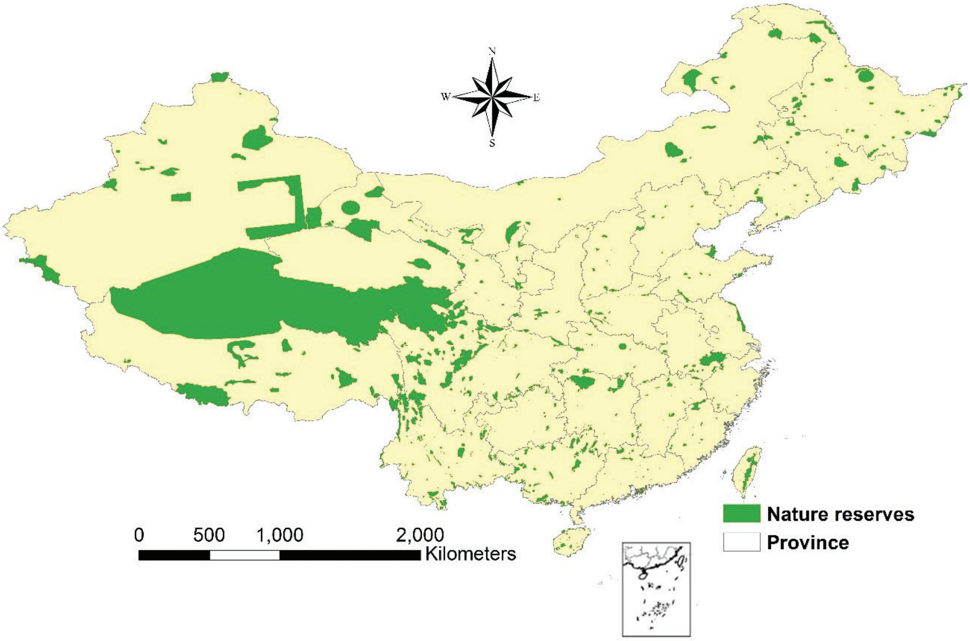

Figure 1. Nature reserves in China.

existing nature reserves Baishuijiang and Cuiyunlanggubai, between PCAs of *U. elongata* and the reserves Jiangxiwuyishan and Mountwuyi, between PCAs of *T. odorum* and the reserves Shiwandashanshuiyuanlian and Xishuangbanna and between PCAs of *M. pasquieri* and the reserves Cuiyunlanggubai and Jinyunshan (Figs 1, 3; Suppl. material 1: Table S2).

Discussion

These results indicate that some provincial regions (e.g. Sichuan and Jilin) contain large areas of habitat as identified by the PCAs for the six PSESP. As such, the outputs serve as tools to identify potential areas for the conservation of PSESP. Figure 3 may be regarded as an important reference for determining promising locations for in-situ and ex-situ conservation efforts directed at PSESP. In-situ and ex-situ conservation are effective approaches for protecting PSESP (Wade et al. 2016, Volis 2016, Wang et al. 2017). Via in-situ conservation, protection in each region is increased, establishing local protected zones for PSESP that conserve the natural environment. In China, many nature reserves have been established for conservation of threatened plant species (Wade et al. 2016, Volis 2016). For example, the aim of the nature reserves in Muling was to protect *T. cuspidata*. Based on these results, it is suggested that the Songhuajiangsanhu and Changbaishan nature reserves also have high potential to be effectively used for conservation actions for *T. cuspidata*. Furthermore, Yangzie and Shiwandashanshuiyu-

Table 2. Percentage contribution of environmental variables to predicted distributions of PSESP.

Name	Alt	Bio1	Bio4	Bio12	Bio15	BLD	CEC	CLYPPT	CRFVOL	OCSTHA	PHIHOX	SLTPPT	SNDPPT	HF
Cathaya argyrophylla	2.36	1.5	1.06	20.88	16.39	52.72	0.26	0.23	0.13	0	4.47	0	0	0
Taxus cuspidata	6.41	1.84	33.6	18.12	0.13	19.91	0.63	0	0.91	0.44	1.3	0.28	16.28	0.14
Annamocarya sinensis	18.65	0.16	3.4	63	0	4.31%	0.3	0.57%	5.13%	2.16%	0.6	0.72	0.92	0.09
Ulmus elongata	2.54	0	1.72	22.11	21.81	23.06	22.53	1.18	0.52	0.02	4.21	0.31	0	0
Tsoongiodendron odorum	7.58	5.13	1.39	74.91	0.28	1.08	1.17	0.15	5.91	0.01	0.33	1.67	0.16	0.23
Madhuca pasquieri	6.91	3.83	9.72	61.74	0.01	2.32	12.62	1.01	1.46	0.07	0.02	0.25	0	0.05

The code was the same as in Suppl. material 1: Table S1.

Table 3. Priority conservation areas of PSESP across the provinces.

Province	*Cathaya argyrophylla*	*Taxus cuspidata*	*Annamocarya sinensis*	*Ulmus elongata*	*Tsoongiodendron odorum*	*Madhuca pasquieri*	Total
Anhui	98999	0	7122	0	0	4946	111067
Fujian	0	0	0	44356	9013	0	53369
Gansu	0	0	14403	0	0	0	14403
Guangdong	0	0	0	7970	77130	0	85100
Guangxi	0	0	0	4303	74487	0	78790
Hainan	0	0	0	0	19843	0	19843
Hebei	0	800	0	0	0	0	800
Heilongjiang	0	112413	0	0	0	0	112413
Henan	72	0	4828	0	0	0	4900
Hubei	60968	0	37233	159	0	16691	115051
Hunan	50584	0	3484	38545	0	14745	107358
Inner Mongolia	0	2400	0	0	0	0	2400
Jiangsu	22032	0	0	0	0	6798	28830
Jiangxi	55232	0	9043	54250	839	70620	189984
Jilin	0	168579	0	0	0	0	168579
Liaoning	0	62161	0	0	0	0	62161
Shaanxi	0	0	38544	0	0	0	38544
Shandong	0	0	0	0	0	205	205
Shanxi	0	0	215	0	0	0	215
Sichuan	7032	0	189237	0	0	177506	373775
Taiwan	0	0	0	7350	12078	0	19428
Tibet	0	0	36198	0	919	31169	68286
Yunnan	0	0	494	0	7465	494	8453
Zhejiang	51113	0	6197	45862	0	22938	126110

The values represent the number of grid cells containing priority conservation areas.

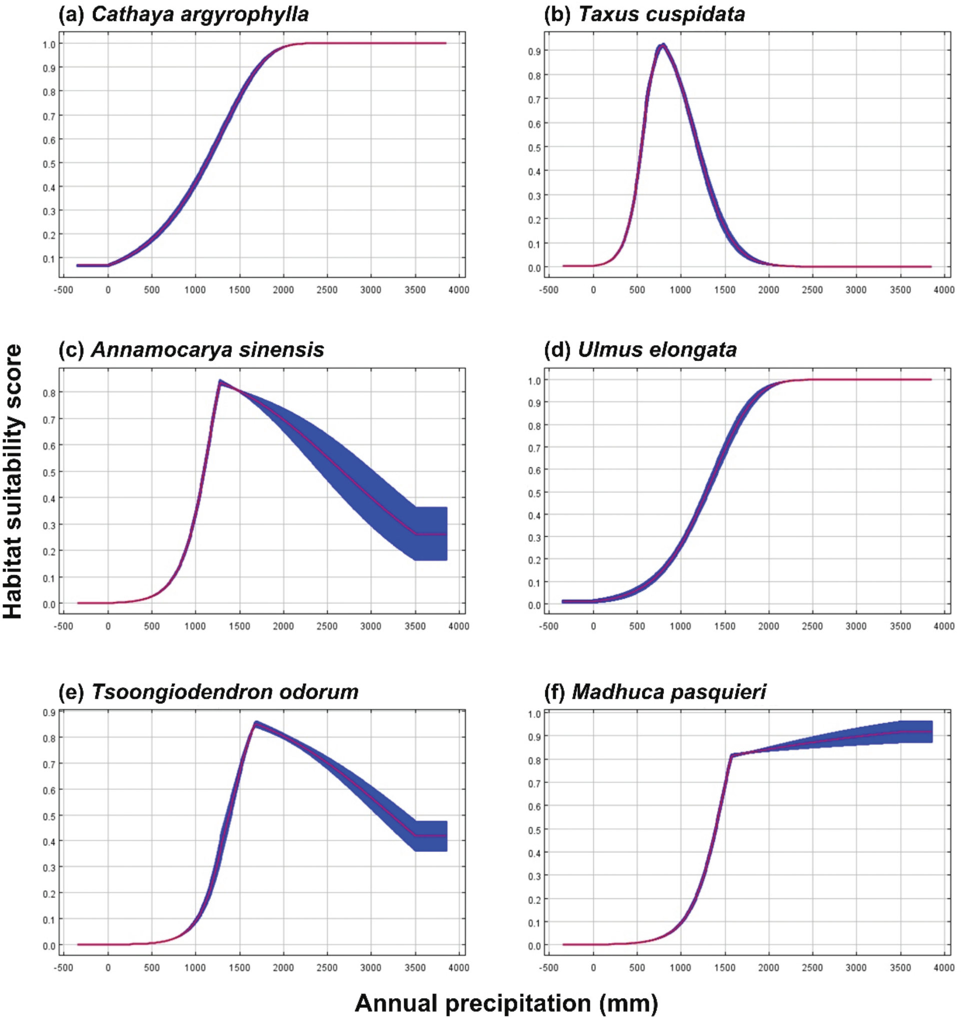

Figure 2. Response curves of annual precipitation to habitat suitability for six PSESP.

anlian have the potential to play an important role in the protection of *C. argyrophylla*. This study identifies key existing nature reserves for in-situ conservation of *C. argyrophylla*, *T. cuspidata*, *A. sinensis*, *U. elongata*, *T. odorum* and *M. pasquieri*. Within these reserves, the construction of small nature reserves, eco-orchards and forest eco-stations for in-situ conservation should be bolstered (Wade et al. 2016, Volis 2016). However, climate factors, including temperature and precipitation, should be regarded as important monitoring indicators for in-situ conservation of PSESP because future climate change may alter suitable sites for PSESP (Suppl. material 1: Figure S1). Just as existing habitats are predicted to be disrupted by climate change, in some cases forcing the use of ex-situ conservation, newly established conservation sites may be impacted in the future (Wang et al. 2017).

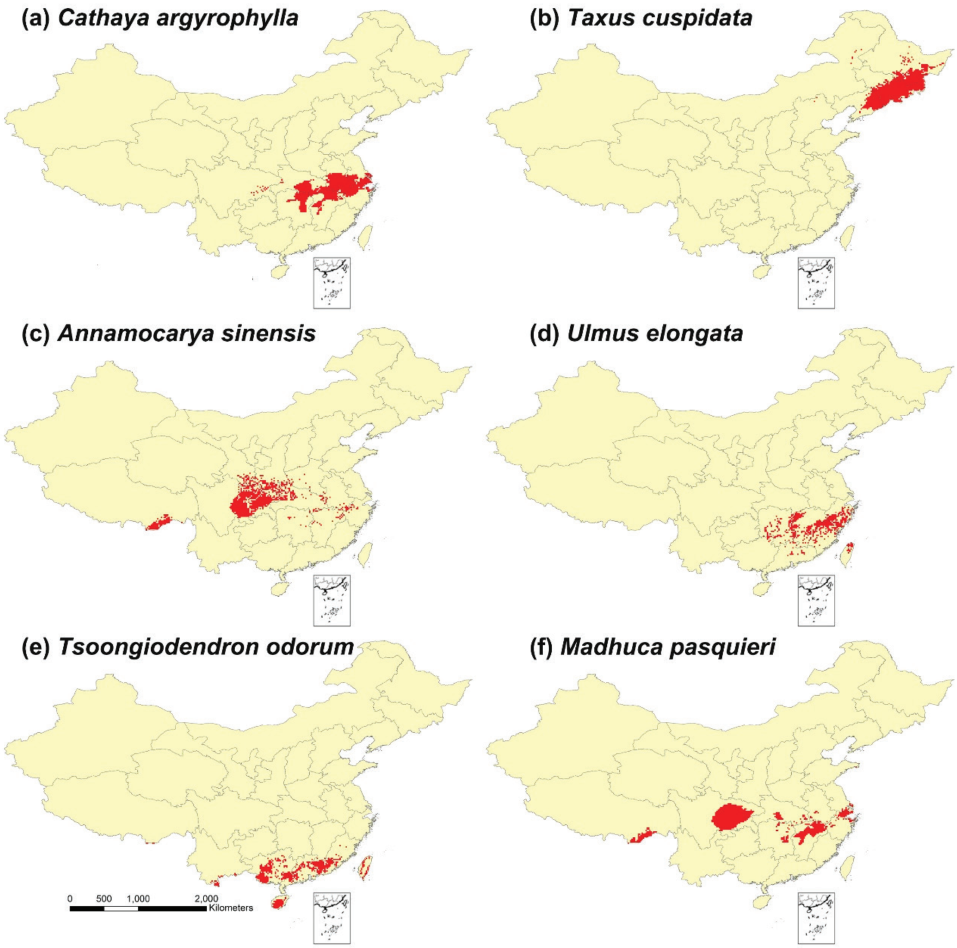

Figure 3. Priority conservation areas for six PSESP in China under climate change.

In an attempt to take into account these future scenarios, current and future suitable distributions were integrated into PCA predictions in order to consider where and how ex-situ conservation could be used in PCAs for PSESP (Wang et al. 2015). Vulnerability of PSESP to climate change must be adopted as the most important indicator that the species is really endangered due to climate change effects. As shown in Fig. 3, PCAs often included areas outside the network of established nature reserves, indicating that ex-situ conservation or the establishment of new protected areas with less vulnerability to predicted climate change may be appropriate for species currently reliant on nature reserves (Wang et al. 2016; Wan et al. 2018). For instance, Cuiyunlanggubai and Jinyunshan appear to be suitable experimental areas for future research on *M. pasquieri* and Baishuijiang and Cuiyunlanggubai are likely to be key nature reserves for ex-situ conservation for *A. sinensis*. The construction of conservation areas for PSESP should be increased in PCAs outside existing nature reserves (Wade et al.

2016, Volis 2016). For example, few seedlings have been observed in natural populations of *A. sinensis* and climate change has a large potential to decrease the distribution probability; thus, both range and population sizes are projected to decline for *A. sinensis* (Wade et al. 2016, Volis 2016). To conserve *A. sinensis*, it is necessary to mitigate the impacts of climate change by establishing areas of ex-situ conservation with the ability to adapt to future climate change for *A. sinensis* (Wade et al. 2016, Volis 2016). Existing seed banks and botanical gardens occurring within PCAs should allocate more resources and space to PSESP. Usage of integrated ex-situ/in-situ approaches must become the norm for PSESP.

It was found that climatic variables and, particularly annual precipitation, were important for distributions of the six focal PSESP in China (Table 2), indicating that there is a need to consider climate change when planning PSESP conservation efforts via in-situ and ex-situ measures. PSESP with a high protection value, such as plants with high scientific research values and ornamental plants, are threatened by over-exploitation and utilisation, habitat fragmentation and the small sizes of their wild populations in broad-leaved forests and bush fallows (Wang et al. 2017). For example, for *T. odorum* and *M. pasquieri*, habitat fragmentation is very severe in China (Wang et al. 2017). Population persistence and growth are at high risk for *T. odorum* and *M. pasquieri*. As discussed for *A. sinensis*, above, populations of PSESP that are already this vulnerable may be further impacted by rapid climate change (Bellard et al. 2012, Wang et al. 2016, 2017). Future climate change has a large potential to impact populations, individuals and habitats of PSESP in China. Wang et al. (2017) has shown that high temperatures and low temperature seasonality could influence the occurrence of suitable habitats for the PSESP in China. For example, these results demonstrate that temperature seasonality could affect distributions for *T. cuspidata* (Table 2). However, these models found different distribution responses to climatic variables for different PSESP, suggesting that different conservation strategies will be necessary for the different PSESP. The importance of each climatic factor may vary depending on the PSESP of interest (Fig. 2). There is a need to monitor the patterns of responses of habitat suitability to environmental variation for these six PSESP. However, it is also important for protection efforts to consider non-climatic factors, such as soil, vegetation types, slope, aspect and elevation etc., so that protection areas can be chosen appropriately (Parmesan et al. 2005, Schwartz et al. 2006, Austin and Van Niel 2011, Oke and Thompson 2015). Altitude and soil variables, including bulk density, cation exchange capacity and fraction of sand as a component of soil texture, have a large contribution to distributions of PSESP (Table 2).

To address the negative effects of climate change on PSESP, there is a need to integrate in-situ and ex-situ conservation measures and climate change monitoring into conservation planning for the six focal PSESP. The delineation of PCAs may be used for providing in-situ and ex-situ conservation measures for PSESP populations and habitats. Monitoring of environmental variation is essential for successful in-situ and ex-situ conservation management of PSESP (Wade et al. 2016, Volis 2016; Wan et al. 2018). However, limits to this study include a need for more detailed empirical data collection. Future studies must take future land use and land cover into account in conservation planning and consider conservation management needs of more PSESP under future global change. Furthermore, PSESP as a designation is likely to be globally useful and it is recommended that global assessments of species be selected based on PSESP criteria.

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Supplementary material 1

Table S1, S2; Figure S1, S2

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Data type: (measurement/occurence/multimedia/etc.)

Explanation note:

Table S1. Environmental variables used in this study.

Table S2. Potential nature reserves overlapped with priority conservation areas for PSESP.

Figure S1. Distribution probabilities of PSESP under current, low and high greenhouse gas concentration scenarios. The colour from yellow to blue represents increasing distribution probability for PSESP.

Figure S2. Priority conservation rank of PSESP. The colour from yellow to blue represents increasing priority conservation rank for PSESP.

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