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Endophytic fungi and silica content of different bamboo species in giant panda diet

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Abstract The giant panda (*Ailuropoda melanoleuca*), one of the most threatened mammalian species in the world, has adapted to herbivorous diet consisting mainly of bamboo (Poaceae: Bambusoidea). The most acute threats to the survival of the giant panda are habitat loss and fragmentation. However, changes in habitat may influence also the quality of giant panda diet through the bamboo species composition as well as their symbiotic leaf endophytes and plant chemical properties. Here we explore species composition and frequency of endophytic fungi and silica content in different bamboo species in the range of giant panda habitat in relation to panda food preference. Silica content of the bamboos varied from 3.7 g/kg to 45.7 g/kg and did not correlate with panda preference and altitudinal gradient. Systemic and vertically in seeds transmitted fungal endophytes or bacterial endophytes were not detected in bamboo leaves. Nearly half of the identified endophytic fungi belonged to genus *Arthrinium*. Pandas preferred bamboo species naturally occurring in higher altitudes. Furthermore, the total amount of endophytes tended to be lower in samples collected from bamboos in higher altitudes. This draws attention to the importance of more detailed studies on the endophytic fungi-bamboo-panda trophic interactions and the effect of land use and climate change on conservation programs of giant panda.

Keywords Bamboo · Endophytic fungi · Giant panda · Silica

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1 Introduction

The decreasing population sizes of giant panda (*Ailuropoda melanoleuca*) due to habitat loss and fragmentation (Peng et al. 2001) and climate change (Tuanmu et al. 2012) render it one of the most threatened mammalian species in the world. Giant panda has adapted to herbivorous diet and depends almost exclusively on bamboo (Poaceae: subfamily Bambusoideae), although it taxonomically belongs to bears (*Ursidae*) and has the digestive system of a carnivore. As the digestive system of the panda is not effective in digesting cellulose (Dierenfeld et al. 1982; Zhu et al. 2011), giant pandas must consume bamboo continuously, up to 12–18 kg daily (Schaller et al. 1985).

Large areas of natural forests in pandas' original range, throughout southwestern and central Asia, have been cleared for agriculture and timber (Loucks et al. 2001). Thus, the giant panda populations have been pushed into forest zones between 1,200 m a.s.l. and 3,400 m a.s.l.. Meanwhile the increasing human population has occupied the richer habitats such as river valleys at lower elevations. Panda habitats are continuing to disappear as human settlements are established higher up the mountain slopes (Liu et al. 1999; Linderman et al. 2006) narrowing the range of bamboo species available as a food source for the panda. Within these disappearing habitats the conservation programs of the giant panda are further challenged because differences among bamboo species

and environmental conditions greatly affect the nutritional value of diet to giant panda, and mast flowering and consequent withering of clones of many bamboo species across large areas at intervals of several decades force pandas occasionally to move to new areas (Reid and Jinchu 1991).

Physiological and chemical plant characteristics such as carbon, nitrogen and defensive metabolites determine the nutritional value of the diet to herbivores. Physiological defence mechanisms in graminoids (Poaceae, including bamboos) include structures containing high amounts of silica (Si) (Carnelli et al. 2001; Hodson et al. 2005), which inhibits both feeding and digestibility by mammalian grazers (Cotterill et al. 2006; Massey and Hartley 2006). Indeed, silica is proposed to be the primary mode of defense against herbivore damage in grasses, which are lacking secondary defense compounds such as alkaloids (Massey et al. 2007). Bamboo species eaten by giant panda have been detected to contain only low amounts of phenolic secondary compounds (Keski-Saari et al. 2008), while it has been suggested that high silica levels in bamboo leaves in spring may deter feeding also in giant pandas (Schaller et al. 1985).

Instead of producing secondary compounds against herbivores by the plant itself, some plant species are well known to live in a mutualistic relationship with endophytic micro-fungi producing a wide array of potentially herbivore deterring toxic compounds: alkaloids, steroids, terpenoids, phenolic compounds and peptides (Saikkonen et al. 1998; Hyde and Soytong 2008; Aly et al. 2010; Saikkonen et al. 2010). Particularly systemic and vertically in seeds transmitted grass-endophytes are known to be able to either directly produce toxins or synthesize them together with the host plant. In addition to systemic endophytes all terrestrial plants host a wide array of non-systemic and horizontally by spores transmitted endophytic fungi which are found to be particularly diverse in tropics (Arnold et al. 2002). Defense of the host plant via endophyte-derived mycotoxins appears to be fungal species- and genotype specific, and dependent on environmental conditions (Saikkonen 2007; Saikkonen et al. 2004). The ecosystems of diverse bamboo species range from temperate evergreen valleys to high mountains with a well-defined cold season and snow. Different species have occupied habitats from different altitudes. Thus, the endophytic fungi in bamboos presumably vary between the bamboo species and the environments, which are largely defined by the altitude.

Our aim is to study species specific and environmental variation in fungal endophyte occurrence and silica content in bamboos. Micro-fungi of bamboos are extensively studied because bamboos are economically important, highly diverse and globally distributed in tropical, subtropical and temperate regions (Hyde et al. 2002), and the bamboo associated fungi have economic importance as pathogens, degraders in bamboo structures in houses and utensils, and medicinal use (Ying et al. 1987). Although approximately 500 species of micro-fungi have been detected in bamboo only in Asia (Hyde et al. 2002), ecological aspects of endophytic fungi in bamboos are poorly studied. In this study we examine species diversity and quantity of endophytic fungi and the silica content of bamboos because their occurrence may be correlated with each other and they may both, separately or in concert, affect the food quality of giant panda. All the described endophytic fungi in bamboos have been non-systemic and horizontally transmitted, but systemic and vertically transmitted endophytes should not be ruled out in bamboos because they are commonly found in other grasses (Saikkonen et al. 2004, 2007). We expect the total amount and species diversity of endophytes to be high and context dependent decreasing along the altitudinal gradient because of harsher conditions in higher altitudes. Furthermore, we expect pandas to prefer bamboo species with low frequencies of endophytes and silica.

2 Materials and methods

We sampled bamboos in the Tang Jiahe Panda Reserve in Minshan Mountains and Da Fengding Reserve in Mount Liang area, China in July 2010. Nine bamboo species were examined to detect their endophytes and 12 species to examine their silica contents at altitudes between 900 m.a.s.l. and 2,600 m.a.s.l. (Table 1). Out of these bamboo species, we determined both endophytes and silica from the same plant individuals of *Bashania fangiana*, *Yushania ailuropodina*, *Y. brevipaniculata*, *Y. dafengdingensis*, and *Y. mabianensis*. Both silica and endophytes were examined only from the bamboos growing higher than 2,000 m.a.s.l. (see Table 1). Samples of each studied bamboo species were collected from five individual plants (see Table 1 for exceptions) growing several hundred meters apart to avoid sampling from the same clone. Panda preferences ranking bamboo species as their food choice were assigned according to Fu et al. (2011). The pandas kept in captivity were allowed to choose from selection of bamboo species and the eaten proportion (%) of each bamboo species was recorded.

Endophytes To detect endophytic fungi in bamboo leaves, we selected nine bamboo species (*Bashania fangiana*, *B. fargesii*, *Fargesia denudata*, *F. rufa*, *F. scabrida*, *Yushania ailuropodina*, *Y. brevipaniculata*, *Y. dafengdingensis* and *Y. mabianensis*) for the study. Four leaves were cut at the height of one meter from different compass directions of each of the five sampled plant per bamboo species. The leaves of individual plants were placed in plastic bags and transported in cool boxes for further handling within 48 h.

We then cut a 5 cm long piece from the basal part of each leaf. To eliminate spores and mycelia of epiphytic fungi from the leaf surface, we used an effective surface sterilization method. The leaves were dipped to 75 % ethanol for 30 s,

Table 1 Bamboo species analyzed for their fungal endophytes and silica content. (n=number of individual plants, X=analysed, n/a=not analyzed)

Species	n	Location	Altitude	Panda preference	Endophytes	Silica
Bambusa blumeana	5	Da Fengding reserve (Mount Liang)	900 m	n/a	n/a	X
Bashania fangiana	5	Tang Jiahe reserve (Mount Minshan)	2,600 m	9 %	X	X
Bashania fargesii	10	Tang Jiahe reserve (Mount Minshan)	1,150 m	0 %	X	n/a
Chimonobambusa quadrangularis	5	Da Fengding reserve (Mount Liang)	1,600 m	n/a	n/a	X
Chimonobambusa szechuanensis	5	Da Fengding reserve (Mount Liang)	1,900 m	3 %	n/a	X
Fargesia denudata	5	Tang Jiahe reserve (Mount Minshan)	2,300 m	25 %	X	n/a
Fargesia rufa	5	Tang Jiahe reserve (Mount Minshan)	1,600 m	23 %	X	n/a
Fargesia scabrida	5	Tang Jiahe reserve (Mount Minshan)	2,100 m	16 %	X	n/a
Phyllostachys bissetii	4	Da Fengding reserve (Mount Liang)	900 m	2 %	n/a	X
Phyllostachys heteroclada	3	Da Fengding reserve (Mount Liang)	900 m	n/a	n/a	X
Phyllostachys nigra	1	Da Fengding reserve (Mount Liang)	900 m	0 %	n/a	X
Qiongzhuea macrophylla	5	Da Fengding reserve (Mount Liang)	1,900 m	15 %	n/a	X
Yushania ailuropodina	5	Da Fengding reserve (Mount Liang)	2,200 m	13 %	X	X
Yushania brevipaniculata	5	Da Fengding reserve (Mount Liang)	2,600 m	18 %	X	X
Yushania dafengdingensis	5	Da Fengding reserve (Mount Liang)	2,600 m	11 %	X	X
Yushania mabianensis	5	Da Fengding reserve (Mount Liang)	2,500 m	n/a	X	X

4 % NaOCl for 5 min and to 75 % ethanol for 1 min. The leaf pieces were set to air dry and cut to three sequential 1 cm× 1 cm pieces from the middle of each leaf using a sterile razorblade. Leaf pieces were placed on 2 % (weight/volume) malt extract agar (MEA) in Petri-dishes, which were then sealed with parafilm.

The Petri-dishes were checked for growing endophyte colonies 1, 4 and 8 weeks after sampling. Emerging fungal endophyte colonies were counted and isolated to fresh MEA. Some Petri-dishes (total 18 %) were discarded because of microbial contamination and overgrowth, and consequently our results might underestimate the fungal diversity.

We identified the isolated fungal endophyte species based on both morphological characteristic and DNA amplification using fungus-specific primers and sequencing. Using both morphological and molecular approach to identify endophyte fungal species decreases the probability for wrong species identification (Ko et al. 2011). We grouped the cultures to operational taxonomic units (OTUs) based on culture morphology. Micromorphology of all cultures or at least three cultures was examined if the number of cultures assigned to an OTU was ≤ 3 or > 3, respectively. Cultures were identified using standard identification books if spores or conidia were present. The internal-transcribed-spacer regions (ITS) of the rDNA of at least one culture per OTU were amplified using primers prITS1 and prITS4 (White et al. 1990). Cultivation, DNA extraction and PCR were performed as described previously (Grünig et al. 2003, 2007). Sequencing of purified PCR products was performed by Microsynth (Balgach, Switzerland) using primer prITS4. ITS sequences were compared with those deposited in GenBank using the BLAST.

Silica We analysed silica (Si) content of five bamboo species (*B. fangiana*, *Y. ailuropodina*, *Y. brevipaniculata*, *Y. dafengdingensis* and *Y. mabianensis*) from the same five bamboo individuals as the endophyte samples. In addition we analysed four other bamboo species (*Phyllostachys bissetii*, *P. heteroclada*, *P. nigra* and *Qiongzhuea macrophylla*) for their silica content. The samples consisted of leaf and culms (bamboo stems) taken at the height of one meter of the plant. They were freeze-dried, ground and analyzed for their silica content in Chemical Laboratory, MTT Argifood Research Finland. Samples were digested in a mixture of concentrated nitric acid, hydrogen peroxide and hydrofluoric acid with microwave (CEM MARS5). After digestion 10 ml of 0,8 M boric acid was added and heated and diluted into 50 ml (Friedlund et al. 1994). All materials used during analyzes were silica free. Silica from samples was measured with plasma emission spectrometry (ICP-OES). Plant certified reference material of known silica content was employed for quality control measures (NJV 94–4 'Energy Grass'; National Analysis Center for Iron and Steel, China) in every batch of samples.

For correlation analyses panda preference data was arcsine-transformed and total endophyte and silica content data ln+1 transformed to meet the assumptions of normality.

3 Results

All the studied bamboo species were inhabited by local infections of horizontally transmitted fungal endophytes. By contrast, we did not detect bacterial endophytes or systemic and

vertically in seeds transmitted grass-endophytes in any of the samples. Systemic endophytes were ruled out, because the same fungal species were not growing out from sequential leaf parts.

We were able to isolate 265 fungal endophyte colonies from 49 out of 50 examined plants belonging to nine bamboo species. Total amount of isolated endophyte colonies tended to decrease in higher altitudes ($r = -0.2521$, $n = 49$, $p = 0.0806$) varying from 0.3 to 4.7 colony forming units (CFU) per square centimeter on average being lowest in *Y. brevipaniculata* and highest in *F. scabrida* (Fig. 1). Contrast to our presumption, however, the species diversity of endophytes did not decrease along the altitudinal gradient (Fig. 1; Table 2).

Out of the 265 fungal colonies we were able to identify 165. Cultures were assigned to 24 operational taxonomic units, OTUs, based on culture morphology and the ITS regions (Table 3). Amplification and sequencing of the ITS regions was successful for most OTUs except four. Since these OTUs could be identified easily based on micromorphology sequencing was not repeated, i.e. *Curvularia brachyspora*, *Nodulisporium*-anamorph of *Hypoxylon fragiforme*, *Pestalotiopsis palustris* and *Xylaria* sp..

Arthrinium species (OTU 2; Table 3) comprised 48 % of all the identified fungi. We detected them in all bamboo species except *Yushania brevipaniculata*. The most frequent species was *Arthrinium* sp. 1 which probably is conspecific with *A. sacchari*. The conidia formed by *Arthrinium* sp. 1 correspond with those of *A. sacchari* (5.5–7.0×3.8–4.0 μm), but the ITS sequences of *Arthrinium* sp. 1 matched by maximally 95 % with those published previously in GenBank (Table 3). The ITS sequences of the three sequenced strains of *Arthrinium* sp. 1 deviated by up to 5.2 % from each other indicating that *Arthrinium* sp. 1 (*A. sacchari*) probably is a complex of several cryptic species. The conidia of *Arthrinium* sp. 2 (10.0–13.0×7.2–7.5 μm) are significantly bigger than those of *Arthrinium* sp. 1 and fit the conidial sizes of *A. phaeospermum*. Due to inserts in the ITS1, the sequence of *Arthrinium* sp. 2 is almost 30 bp longer than that of *Arthrinium* sp. 1. It matched the sequence of *A. phaeospermum* by 97 %. In contrast, the closest match of *Arthrinium* sp. 3 had a similarity of only 88 % (Table 3). Compared to *Arthrinium* sp. 1, *Arthrinium* sp. 3 has inserts of almost 70 bp in the ITS1, and possesses big lemon-shaped conidia measuring more than 15 μm in diameter. *Arthrinium* sp. 3 probably is an undescribed species.

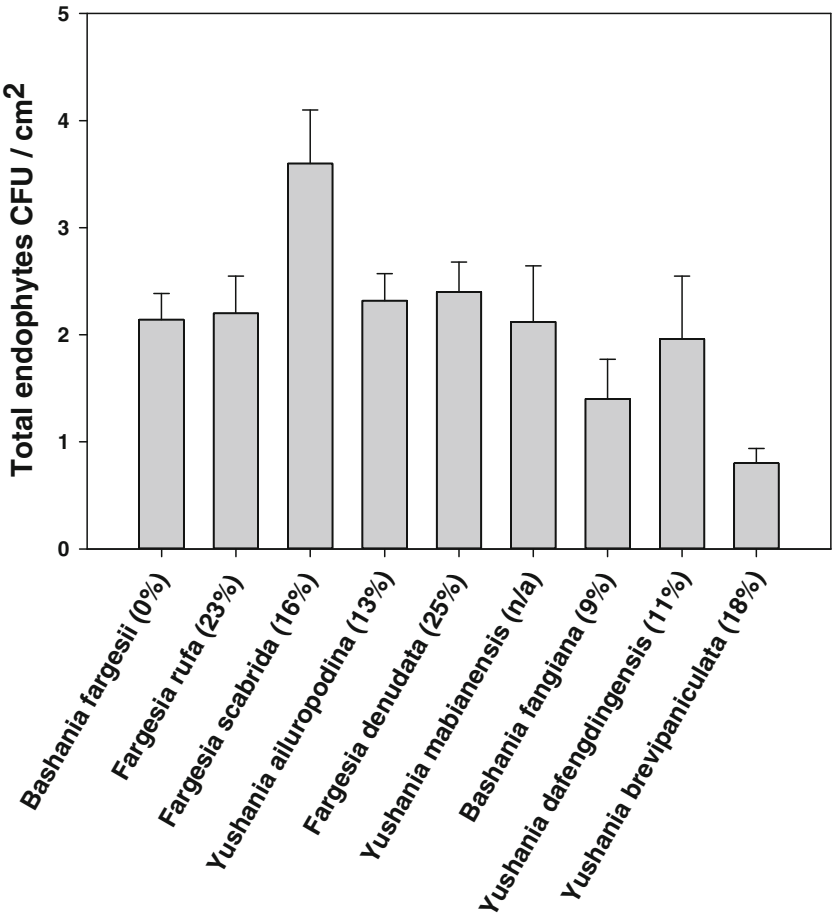

Fig. 1 Total endophyte frequencies (CFU/cm^2=colony forming units of fungi growing out from bamboo leaf area) of different bamboo species organized in increasing order of their sampling altitude. The panda preference (according to Fu et al. 2011) of the bamboo species is marked after the species name. n/a=not analyzed

Table 2 Endophyte species/genera detected from different bamboo species. Specific information about the OUTs (operational taxonomic units) according to strain labels is provided in Table 3

Bamboo species	Endophyte species/genera	OTU	Strain label
Bashania fangiana	*Arthrinium phaeospermum*	2	28-1′
	Alternaria alternata	1	
	Cladosporium cladosporoides	7	
	Penicillium sp.		
	Rhizopus sp.		
Bashania fargesii	*Arthrinium sacchari*	2	
	Cochliobolus sativus	11	22–2
	Colletotrichum sp.	8	
	Diaporthe phaseolorum	3	17–3
	Gibberella avenacea	5	24–3
	Leptostroma sp.	13	20-2′
	Nemania diffusa	17	20-1′
	Periconia sp.	24	16-3′
	Phoma sp.	10	20–2
Fargesia denudata	*Arthrinium sacchari*	2	2–3 and 3–1
	Arthrinium phaeospermum	2	
	Aspergillus cervinus		
	Asteromella sp.	5	1-1′
	Chaetomium sp.		
	Creosphaeria sassafras	4	
	Diaporthe phaseolorum	3	1–2
	Nemania diffusa		
	Trichoderma viride		
Fargesia rufa	*Alternaria alternata*	1	
	Arthrinium sacchari	2	
	Diaporthe phaseolorum	3	
	Gibberella pulicaris	6	13-3′
	Nemania diffusa	16	14-1′
Fargesia scabrida	*Arthrinium phaeospermum*	2	
	Arthrinium sacchari	2	
	Hypoxylon fragiforme		
	Nemania diffusa	15	6–1
	Pestalotiopsis palustris		
Yushania ailuropodina	*Alternaria alternata*	1	
	Arthrinium sacchari	2	
	Cladosporium cladosporoides	7	41–3
	Nemania diffusa	18 and 19	38–1 and 38–2
Yushania brevipaniculata	*Aspergillus oryzae*		
	Chaetomium globosum	6	33–2
	Cladosporium cladosporoides	7	
	Glomerella cingulata	12	33–3
	Rhizopus sp.		
Yushania dafengdingensis	*Arthrinium* sp.	2	51–1
	Creosphaeria sassafras	4	
	Penicillium sp.		
Yushania mabianensis	*Arthrinium phaeospermum*	2	46–3
	Arthrinium sacchari	2	
	Chaetomium globosum	6	
	Creosphaeria sassafras	4	43-2
	Curvularia brachyrospora		

We observed quite frequently pure white colonies partly covered with black streaks or patches reminiscent of *Xylaria* (*Hypoxylon*) species. Macromorphology among colonies was highly variable, and no spores or conidia were formed in any of the colonies. Therefore, we considered most colonies separate OTUs and sequenced each of them. Two types of ITS sequences were found which differed by 3.2 %. *Xylaria* sp. 2 (Table 3, strain 6–1) and *Xylaria* sp. 7 (Table 3, strain 38–2) had one type and all the other *Xylaria* spp. the other type. Interestingly, independently on the ITS type all *Xylaria* strains showed a 98 % or 99 % match with sequences of *Nemania diffusa* which is the current name of *Hypoxylon unitum* (Table 3).

We assigned four strains of *Phomopsis* to the same OTU based on micro- and macromorphology. However, the ITS of the two sequenced strains 1–2 and 17–3 deviated by 9.5 % from each other (Table 3), i.e. the two strains probably represent different species of *Phomopsis*. Nevertheless, both sequences were to 99 % similar to sequences of different strains of *Diaporthe phaseolorum*, the teleomorph of *Phomopsis phaseoli*.

Similarly to endophytes, silica contents were highly variable among the bamboo species ranging from 3.7 g/kg to 45.7 g/kg on average being lowest in *Qiongzhuea macrophylla* and highest in *B. blumeana* (Fig. 2). However, total amount of fungal endophytes and silica contents (examined from the same plant individuals of *B. fangiana*, *Y. ailuropodina*, *Y. brevipaniculata*, *Y. dafengdingensis* and *Y. mabianensis*) were not correlated ($r=-0.1689$, $n=24$, $p=0.4302$; Figs. 1 and 2), and contrast to silica content ($r=-0.0090$, $n=52$, $p=0.9497$; Fig. 2), total amount of endophytes tended to decrease along the altitudinal gradient ($r=-0.2521$, $n=49$, $p=0.0806$; Fig. 1).

Panda preference was not correlated with total endophytes ($r=-0.0244$, $n=44$, $p=0.8752$) or silica content ($r=0.2002$, $n=34$, $p=0.2562$). However, pandas preferred bamboo species occurring in higher altitudes (panda preference vs. altitude, $r=0.6546$, $n=12$, $p=0.0209$; Fig. 3) where the total amount of endophytes tended to lower (altitude vs. total endophytes, $r=-0.2521$, $n=49$, $p=0.0806$).

Table 3 Identification of OTUs using morphology and ITS sequencing. (n.a=not analyzed)

OTU	Strain label	Morphology-based identification[a] Taxon name	DNA-sequence-based identification							Proposed taxon name
			Closest matching taxon in GenBank according to BLAST	Query coverage	Maximum identy	Host, substrate, country	GenBank accession of closest match	GenBank accession of sequenced strain	Length [bp] of ITS regions of sequenced strain	
1	28-1′	*Alternaria alternata*	*Alternaria alternata*	99	100	*Ocimum basilicum*, Italy	HQ540552.1	KC354568	497	*Alternaria alternata*
2	3-1	*Arthrinium* sp. 1	*Arthrinium sacchari*	100	95	Bamboo, China	HQ647334	KC354569	508	*Arthrinium sacchari*[b]
2	2-3	*Arthrinium* sp. 1	*Arthrinium sacchari*	100	95	Bamboo, China	HQ647334	KC354570	508	*Arthrinium sacchari*
2	12-1	*Arthrinium* sp. 1	*Arthrinium sacchari*	100	95	Bamboo, China	HQ647334	KC354571	508	*Arthrinium sacchari*
2	46-3	*Arthrinium* sp. 2	*Arthrinium phaeospermum*	99	97	Culms of *Phragmites australis*, The Netherlands (CBS 463.83)	AJ279447.1	KC354572	535	*Arthrinium phaeospermum*[b]
2	51-1	*Arthrinium* sp. 3	*Arthrinium phaeospermum*	100	88	n.a.	FJ462766.1	KC354573	573	*Arthrinium* sp.
5	1-1′	*Asteromella* sp.	*Pleosporales*	98	99	Orchid, Ecuador	HQ207041.1	KC354574	479	*Asteromella* sp.[c]
6	33-2	*Chaetomium* sp.	*Chaetomium globosum*	100	100	n.a.	JF826005.1	KC354575	503	*Chaetomium globosum*
7	41-3	*Cladosporium cladosporioides*	*Cladosporium cladosporioides*	100	99	n.a.	JN031062.1	KC354576	479	*Cladosporium cladosporioides*
8	16-2	*Colletotrichum* sp.	*Gaeumannomyces graminis* var. *graminis*	100	89	n.a.	JF710374.1	KC354577	468	*Colletotrichum* sp.
9	40-1	*Coniochaeta* sp.	*Coniochaeta* sp.	100	99	n.a.	HM595513.1	KC354578	492	*Coniochaeta* sp.
10	20-2	*Phoma* sp.	*Pleosporales*	92	88	*Saccharum officinarum*	HQ631059.1	KC354579	467	*Phoma sp*.[d]
11	22-2	*Drechslera* sp.	*Cochliobolus sativus*	99	100	n.a.	HM195261.1	KC354580	513	*Cochliobolus sativus*
5	24-3	*Fusarium graminearum*	*Gibberella avenacea*	97	99	n.a.	EU255804.1	KC354581	477	*Gibberella avenacea*[e]
6	13-3′	*Fusarium sambucinum*	*Gibberella pulicaris*	100	98	n.a.	U34579.1	KC354582	463	*Gibberella pulicaris*[f]
12	33-3	*Glomerella* sp.	*Glomerella cingulata*	99	99	*Salix* sp.	AJ301952.1	KC354583	519	*Glomerella cingulata*
4	43-2	*Libertella* sp.	*Creosphaeria sassafras*	100	99	*Lindera glauca*, China	JF502457.1	KC354584	513	*Creosphaeria sassafras*[g]
4	52-3	*Libertella* sp.	*Creosphaeria sassafras*	99	99	*Lindera glauca*, China	JF502457.1	KC354585	402	*Creosphaeria sassafras*
13	20-2′	*Leptostroma* sp.	fungal endophyte	100	100	*Artemisia* sp., China	EU054412.1	KC354586	545	*Leptostroma* sp.
24	16-3′	*Periconia cookei*	Ascomycete	100	99	root of *Holcus lanatus*	FN392304.1	KC354587	508	*Periconia* sp.
3	17-3	*Phomopsis* sp.	*Diaporthe phaseolorum*	100	99	*Vitis vinifera*, China	EU030368.1	KC354588	512	*Diaporthe phaseolorum*[h]
3	1-2	*Phomopsis* sp.	*Diaporthe phaseolorum*	100	99	*Arctium lappa* (soybean), Croatia	HM347705.1	KC354589	506	*Diaporthe phaseolorum*
15	6-1	*Xylaria* sp. 2 (sterile culture)	*Nemania diffusa*	99	97	bark of *Castanopsis carlesii* var. *sessilis*, Taiwan	GU292817.1	KC354590	505	*Nemania diffusa*[i]

Table 3 (continued)

OTU	Strain label	Morphology-based identification[a] Taxon name	DNA-sequence-based identification							Proposed taxon name
			Closest matching taxon in GenBank according to BLAST	Query coverage	Maximum identy	Host, substrate, country	GenBank accession of closest match	GenBank accession of sequenced strain	Length [bp] of ITS regions of sequenced strain	
16	14-1′	*Xylaria* sp. 3 (sterile culture)	*Nemania diffusa*	98	100	*Taxus chinensis* var. *mairei*, China	JN198514.1	KC354591	402	*Nemania diffusa*
17	20-1′	*Xylaria* sp. 5 (sterile culture)	*Nemania diffusa*	98	100	Taxus chinensis var. mairei, China	JN198514.1	KC354592	507	*Nemania diffusa*
18	38-1	*Xylaria* sp. 6 (sterile culture)	*Nemania diffusa*	98	100	*Taxus chinensis* var. *mairei*, China	JN198514.1	KC354593	507	*Nemania diffusa*
19	38-2	*Xylaria* sp. 7 (sterile culture)	*Nemania diffusa*	99	97	bark of *Castanopsis carlesii* var. *sessilis*, Taiwan	GU292817.1	KC354594	505	*Nemania diffusa*
20	24-2	*Xylaria* sp. 8 (sterile culture)	*Nemania diffusa*	99	99	n.a.	FJ438909.1	KC354595	507	*Nemania diffusa*

[a] Name of the fructification formed in culture

[b] see text

[c] *Asteromella* are anamorphic forms of many species of the *Pleosporales*, i.e. the name *Asteromella* is retained for this OTU

[d] The match of the sequences in GenBank is low and *Phoma* are anamorphic forms of many species of the *Pleosporales*, i.e. the name *Phoma* is retained for this OTU

[e] *Gibberella* species are teleomorphs of *Fusarium* species. It is impossible to distinguish *F. graminearum* and *F. avenaceum* based on morphology, i.e. *Gibberella avenacea* is a good name for this OTU

[f] *Gibberella pulicaris* is the teleomorph of *Fusarium sambucinum*

[g] *Creosphaeria sassafras* (=*Hypoxylon sassafras*) has a *Libertella*-anamorph. In culture, only the *Libertella*-anamorph is formed and since many different species of partly only distantly related ascomycetes possess

Libertella-anamorphs a correct identification is impossible based solely on colonial morphology. The two *Libertella* strains 43–2 and 52–3 had identical ITS sequences

[h] see text

[i] see text

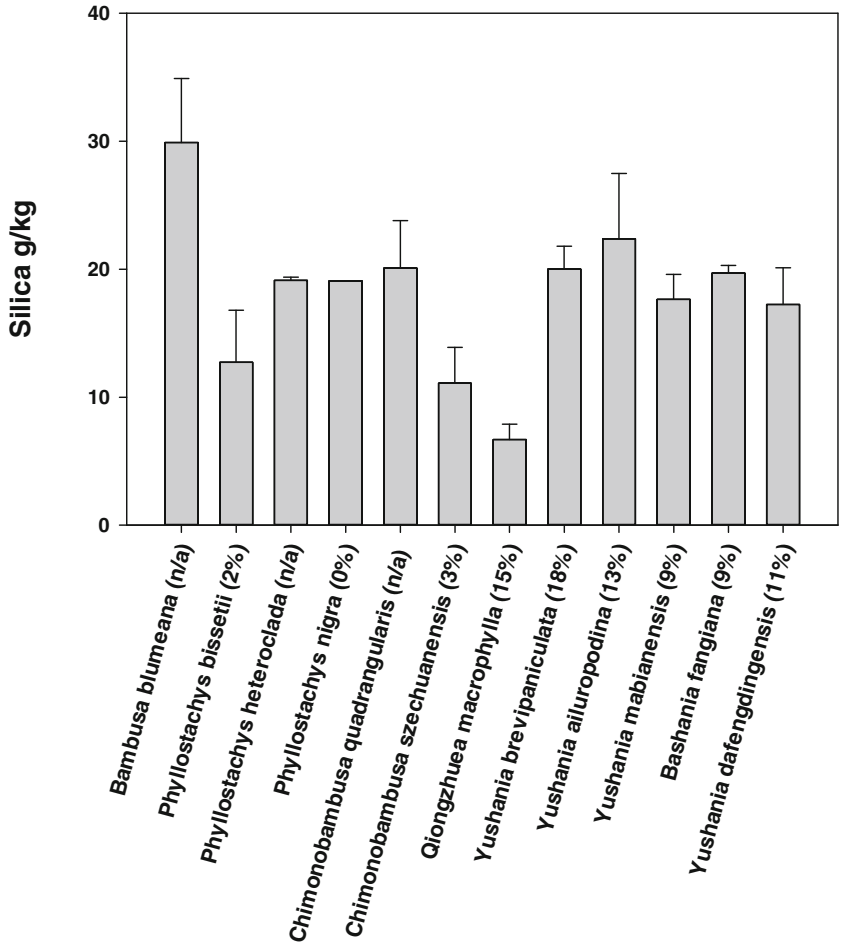

Fig. 2 Silica (Si) content (silica g/bamboo kg) of different bamboo species organized in increasing order of bamboo sampling altitude. The panda preference (according to Fu et al. 2011) of the bamboo species is marked after the species name. n/a=not analyzed

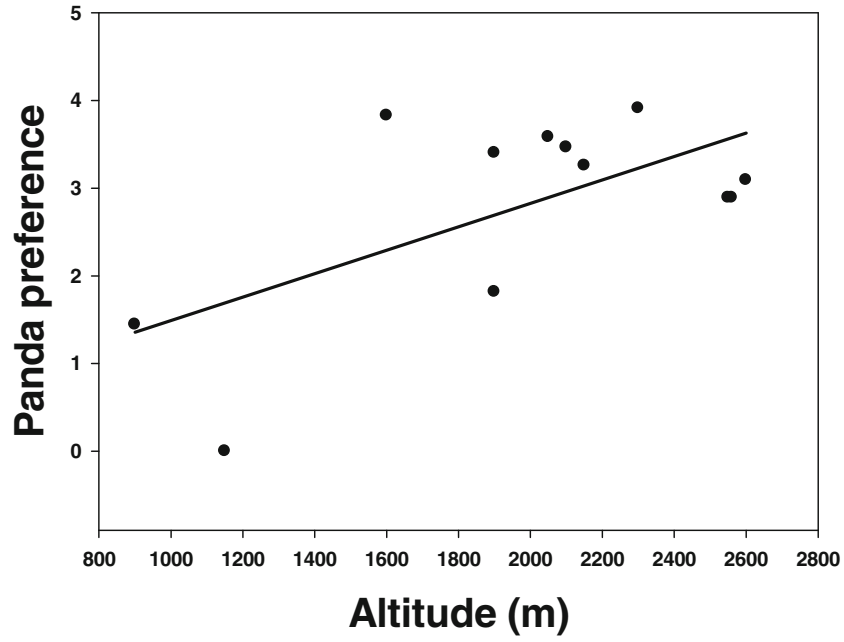

Fig. 3 Significant correlation ($r=0.6546$, $n=12$, $p=0.0209$) between bamboo habitat altitude and panda bamboo species preference (according to Fu et al. 2011). Preference is arcsin-transformed

4 Discussion

We detected only local, horizontally from plant to plant transmitted fungal endophytes colonizing our bamboo samples (Saikkonen 2007). Compared to perennial cool season grasses commonly infected by systemic fungal endophytes (Saikkonen et al. 2010), bamboos grow faster and larger in size, the majority of them are semelparous, and have unusual extended reproduction cycle from 10 to 120 years (Janzen 1976). The reproduction of many bamboo species is characterized by synchronized mast flowering before death of conspecifics across large area (Janzen 1976). Similarly to woody plants, fast growth, and age of sexual maturity and semelparity of bamboos provide fewer opportunities for systemic growth and subsequent successful vertical transmission to endophytic fungi than to other perennial grasses (Saikkonen et al. 2004).

Arthrinium species were the most commonly detected endophytic fungi in bamboos comprising almost half of the identified isolates and they were detected in all but one (*Yushania brevipaniculata*) studied bamboo species. Many *Arthrinium* species (e.g. *A. phaeospermum*) are commonly detected pathogens in bamboos and capable of producing toxins (Li et al. 2013). According to our study, *Arthrinium* species are common in healthy bamboo leaves as well, and thus probably mediating giant panda diet. Thus, we propose that their mycotoxin production potential in different bamboo species and environmental conditions should be taken into account in future studies.

We expected pandas to prefer bamboo species with low frequencies of mycotoxin producing endophytes and silica. Contrary to expectations, however, panda preference was not correlated with total endophytes or silica.

This controversy might be due to research technical reasons. Our data is inadequate to draw strong conclusions of the relationships between panda preference, endophyte infections and silica content of the bamboos for two reasons. First, panda preference data came mainly from variable sets of field observations and experiments with captive pandas without any direct measurements of chemical quality of bamboos. Second, the bamboos in this study were low in number of replicates and incomplete in species comparisons. For example, correlations between the total amount of endophytes and silica content did not include *Y. brevipaniculata* and *F. scabrida* with the lowest and highest detected amounts of total endophytes (Fig. 1) and *Q. macrophylla* and *B. blumeana* with the lowest and highest detected silica contents, respectively (Fig. 2).

Alternatively, our results may suggest that panda preference and endophyte abundance and diversity may be caused by (1) genetic differences in bamboo quality for endophytic fungi and pandas, or (2) genetic differences in responses to environmental conditions as suggested in previous studies on other plant-herbivore model systems (see e.g. Ahlholm et al. 2002a, b). Interestingly, we detected that pandas preferred bamboo species occurring in higher altitudes where the total amount of endophytes tended to be lower. This may indicate positive genetic correlation structure in genetically determined bamboo susceptibility to endophytic fungi and food quality for giant panda having importance for evolution of bamboo resistance to fungi and herbivores.

Thus, we propose that these interactions should be examined more thoroughly to fully understand the consequences of land use (Peng et al. 2001), multispecies coevolution and trophic interactions, and climate change (Tuanmu et al. 2012) on conservation programs of giant panda.

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