

A peer-reviewed version of this preprint was published in PeerJ on 8 December 2016.

[View the peer-reviewed version](https://peerj.com/articles/2781) (peerj.com/articles/2781), which is the preferred citable publication unless you specifically need to cite this preprint.

Horn T, Häser A. 2016. Bamboo tea: reduction of taxonomic complexity and application of DNA diagnostics based on *rbcL* and *matK* sequence data. PeerJ 4:e2781 <https://doi.org/10.7717/peerj.2781>

Adulteration of herbal products: Bamboo tea authentication

Thomas Horn^{Corresp., 1}, Annette Häser¹

¹ Molecular Cellbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany

Corresponding Author: Thomas Horn
Email address: thomas.horn@kit.edu

Background. Names for "substances" used in food products are rarely precise. The term bamboo (*Bambusoideae*, *Poaceae*) comprises over 1600 distinct species of which only few are well established sources for food products on the European market (i.e. bamboo sprouts).

Methods. We analysed bamboo species and tea products containing an exotic ingredient (bamboo leaves) using anatomical leaf characters and DNA sequence data. Our primary concern was to determine the taxonomic origin of bamboo leaves to establish a baseline for EU legislation, to introduce a simple PCR based test to distinguish bamboo from other *Poaceae* leaf components and to assess the diagnostic potential of DNA Barcoding markers to resolve taxonomic entities within the bamboo subfamily and tribes.

Results. Based on anatomical and DNA data we can pinpoint the taxonomic origin of genuine bamboo leaves used in commercial products to the genera *Phyllostachys* and *Pseudosasa* from the temperate "woody" bamboo tribe (*Arundinarieae*). We detected adulteration by carnation in 4 of 8 tea products and, after adapting our objectives, could trace the taxonomic origin of the adulterant to *Dianthus chinensis* (*Caryophyllaceae*), a well known traditional Chinese medicine with counter indications for pregnant women.

Adulteration of Herbal Products: Bamboo Tea Authentication

Thomas Horn¹ and Annette Häser²

^{1,2}Molecular Cell Biology, Botanic Institute, Karlsruhe Institute of Technology, Kaiserstraße 2, D-76128 Karlsruhe, Germany

ABSTRACT

Background. Names for "substances" used in food products are rarely precise. The term bamboo (*Bambusoideae*, *Poaceae*) comprises over 1600 distinct species of which only few are well established sources for food products on the European market (i.e. bamboo sprouts).

Methods. We analysed bamboo species and tea products containing an exotic ingredient (bamboo leaves) using anatomical leaf characters and DNA sequence data. Our primary concern was to determine the taxonomic origin of bamboo leaves to establish a baseline for EU legislation, to introduce a simple PCR based test to distinguish bamboo from other *Poaceae* leaf components and to assess the diagnostic potential of DNA Barcoding markers to resolve taxonomic entities within the bamboo subfamily and tribes.

Results. Based on anatomical and DNA data we can pinpoint the taxonomic origin of genuine bamboo leaves used in commercial products to the genera *Phyllostachys* and *Pseudosasa* from the temperate "woody" bamboo tribe (*Arundinarieae*). We detected adulteration by carnation in 4 of 8 tea products and, after adapting our objectives, could trace the taxonomic origin of the adulterant to *Dianthus chinensis* (*Caryophyllaceae*), a well known traditional Chinese medicine with counter indications for pregnant women.

Keywords: Food Diagnostics, Food Fraud, ARMS, Bamboo, Bambusoideae, Lemongrass, Carnation, Dianthus, DNA Barcoding, Character Based DNA Diagnostics, Consumer Rights

INTRODUCTION

People are used to acquire and consume food products without having to confront themselves with complex topics like biological systematics and effects of globalisation. The European Union (EU) introduced the Novel Food Regulation (NFR) to protect consumers from products containing unknown potentially dangerous ingredients. To market an exotic food component within the EU, business operators are required to prove that it had been consumed to a considerable amount before 1997. If the component does not comply to this criteria, it has to be considered a novel food and further steps (i.e. safety evaluations) are required before it can be marketed.

Bamboo leaf tea is considered a delicious and healthy drink in Asian countries and has found its way into the European market. The current status of bamboo leaf as food ingredient in the EU, however, is less than clear. The Novel Food Catalogue currently (June 2016) contains entries of several taxa associated with the term "bamboo": *Bambusa* spec., *Dendrocalamus latiflorus*, *D. asper*, *Gigantochloa albociliata*, *G. levis*, *Phyllostachys pubescens* and *Sinocalamus oldhamii*. Except for the first entry, all relate to the use of the stem as food source. A note connected to the entry for *Bambusa* spec. states that the use of leaves as food source is not known to any member state and therefore if they were to be used as a food might be subject to the NFR and require a safety assessment.

Bamboo appears to be a very loose term that, particularly in respect of bamboo tea, requires us to ask the question: Which kind of bamboo are the leaves taken from that are used in bamboo tea?

Bamboos

Bamboos are herbaceous or "woody" plants from the subfamily *Bambusoideae* (*Poaceae*) diversified in temperate and tropical Asia, South America and Africa. They are extensively used by humans (e.g. *Phyllostachys* species in China and neighbouring countries) and cultivated beyond their natural distribution range. Many species are only known from cultivation (e.g. *Bambusa* spec.). Bamboo is industrially

46 used for construction, furniture and paper production. Domestically it is used as tool (e.g. farming,
47 hunting, fishing, eating, weaving). The leaves of *Gelidocalamus latifolius* and *Indocalamus* species
48 are used to wrap glutinous rice [Wu et al., 2006], those of broad-leaved species (e.g. *Sasa* species) are
49 cut during the first 5 weeks, cleaned, dried, roasted and used for bamboo tea. For ages bamboo tea
50 has been considered a delicious and healthy drink in the bamboo countries and is now spreading to
51 other regions (e.g. Europe). It contains neither theine nor caffeine and is rich in protein, calcium, iron,
52 magnesium and recommended for various pharmaceutical applications, particularly stomach pain [Liese,
53 2015]. In Japan the leaves of *Sasa* plants (*S. palmata*, *S. senanensis* and rarely *S. yahikoensis* and *S.*
54 *kurilensis*), which are called "Kuma-zasa", have been used to treat burns or urinary hesitancy [Sasaki
55 et al., 2007]. In China and Indonesia leaves of different species of *Bambusa*, *Phyllostachys*, *Fargesia*
56 and *Indocalamus* are used for medicinal purposes [http://www.bamboocentral.org/pharmacopoeia.html].
57 According to Subhuti Dharmananda (http://www.itmonline.org/arts/bamboo.htm, Hsu et al. [1986], Zhen
58 [1995]) the most frequently used leaves in Chinese herbal medicine are collected from the grass bamboo
59 (*Lophatherum gracile*). It is also mentioned that the leaf of the black bamboo (*Phyllostachys nigra*)
60 and of the grass bamboo is often confused both in China and the West [Jiao, 2003]. Taxonomically,
61 there are 1'641 bamboo species, 120 genera and 3 tribes [Soreng et al., 2015] making up the subfamily
62 *Bambusoideae* (*Poaceae*). In appearance bamboos are either "woody" (lignified) or herbaceous. The first
63 group can be divided into two distinct lineages - the temperate (*Arundinarieae*) and tropical (*Bambuseae*)
64 "woody" bamboos. Nested between the "woody" tribes are the herbaceous bamboos (*Olyreae*). Strictly
65 speaking, the earlier mentioned grass bamboo (*Lophatherum gracile*) is not a bamboo. Instead it belongs
66 to another *Poaceae* subfamily (*Panicoideae*), which also harbours members from the genus *Cymbopogon*
67 (lemongrass), another common herbal tea ingredient.

68 **Identification of Herbal Product Components**

69 The classic approach to identify herbal product components is based on described anatomical features of
70 involved plant parts. While characteristics may exist for each species, most of the time the features are
71 more general and can be used to distinguish species of a certain genera from other species of different
72 genera. With increased degree of processing, more and more features get lost due to progressing influence
73 of artefacts or the absence of tissue carrying those features.

74 Even if no tissue is available at all, DNA still is a viable source for taxonomic placement [Ward et al.,
75 2009, Newmaster et al., 2013]. The probably most prominent approach aiming to pinpoint the identity of
76 a specimen is DNA Barcoding [Hebert et al., 2003]. Ideally, a small standardized region of the (plant)
77 genome (the barcode) is used to determine the species name of a specimen by comparing its barcode to
78 records of verified species references. Using the information of the DNA, this approach is not limited to a
79 certain developmental stage or particular tissue characteristics and is also not biased by environmental
80 factors. However, it has been shown to be of limited use for species-level specimen identification in land
81 plants when using the officially proposed [CBOL Plant Working Group, 2009] chloroplast markers (rbcL
82 and matK). Identification success rates increase when using more variable marker regions [Federici et al.,
83 2013, Roy et al., 2010, Seberg and Petersen, 2009, Taberlet et al., 2007]. Most markers, however, have
84 been shown to be unable to resolve closely related taxa as single DNA Barcoding marker.

85 Besides DNA Barcoding, which is based on sequence information of well established marker regions,
86 there are other approaches [Wang et al., 2001, Lee et al., 2006, Dnyaneshwar et al., 2006, Huh and Bang,
87 2006, Marieschi et al., 2010, Torelli et al., 2014] using DNA fingerprinting techniques like Random
88 Amplified Polymorphic DNA (RAPD) or Amplified Fragment Length Polymorphism (AFLP) to develop
89 simple PCR tests based on new and fairly unknown marker regions. Achieving a similar goal but relying
90 on well established markers, PCR-RFLP and ARMS have been used to test the identity of specimens by
91 specific PCR fragment patterns [Newton et al., 1989, Yang et al., 2004, Li et al., 2007, Wang et al., 2007,
92 2010, Horn et al., 2012, 2013].

93 **Aim**

94 Our primary concern was to determine the taxonomic origin of bamboo leaf samples obtained from
95 commercial teas to establish a baseline concerning EU food law (food vs. novel food). We also aimed to
96 establish anatomical and DNA based differentiation methods for bamboo and similar components. Finally
97 our goal was to assess the diagnostic potential of selected DNA Barcoding markers. After discovering
98 the adulteration of corresponding tea products we naturally extended our objectives by including the
99 adulterant in all analyses.

100 MATERIAL AND METHODS

101 Reference Plants and Commercial Samples

102 Specimens of bamboo (*Bambusoideae*, table 1), lemongrass and Carnation (*Cymbopogon* and *Dianthus*,
 103 supplementary table 2) were acquired and cultivated in the botanical garden of the Karlsruhe Institute
 104 of Technology. *Bambusoideae* and *Cymbopogon* specimens were identified to genus level [Farrelly,
 105 1984, Wu et al., 2006, 2007]. At least one specimen of each *Dianthus* species was identified to species
 106 level using morphological markers [Wu et al., 2001, Jäger et al., 2008]. Specimen details including
 107 images are available through the Barcoding of Life Data Systems web site (project BBOCA, <http://boldsystems.org>). Commercial products were acquired from local and internet sources (Table 2).
 108

Table 1. Reference accessions (Acc) B1 - B13 of *Bambusoideae* species, their taxon names and Genbank sequence accessions of three plastid DNA regions (rbcLa, rbcLb and matK-KIM).

Acc	Taxon	rbcLa	rbcLb	matK-KIM
<i>Bambuseae</i>				
B1	<i>Bambusa multiplex</i>	KX146450	KX146413	KX146427
B2	<i>Dendrocalamus giganteus</i>	KX146452	KX146415	KX146429
<i>Arundinarieae</i>				
B3	<i>Phyllostachys aureosulcata</i>	KX146453	KX146416	KX146430
B4	<i>Phyllostachys edulis</i>	KX146454	KX146417	KX146431
B5	<i>Phyllostachys nigra</i>	KX146455	KX146418	KX146432
B6	<i>Phyllostachys violascens</i>	KX146456	KX146419	KX146433
B7	<i>Pseudosasa japonica</i>	KX146457	KX146420	KX146434
B8	<i>Sasa borealis</i>	KX146458	KX146421	KX146435
B9	<i>Sasa kurilensis</i>	KX146459	KX146422	KX146436
B10	<i>Sasa palmata</i>	KX146460	KX146423	KX146437
B11	<i>Sasa veitchii</i>	KX146461	KX146424	KX146438
B12	<i>Semiarundinaria fastuosa</i>	KX146462	KX146425	KX146439
B13	<i>Bergbambos tessellata</i>	KX146451	KX146414	KX146428

109

110 Morphological and Anatomical Evaluations

111 Small rectangle hand-sections were made in the centre and at the margin of the leaf-blades of the first fully
 112 developed dried leaves of reference plants. Leaf fragments were isolated from all commercial products.
 113 After visual inspection of specimens using a stereo microscope (Leica S6D) the adaxial and abaxial
 114 leaf surfaces were brightened with 60 % chloral hydrate (Carl Roth GmbH) and analysed using a light
 115 microscope (Leica DM750). Both instruments are equipped with a digital image system (Leica EC3) that
 116 was used to document macroscopic and microscopic leaf structures.

117 DNA based Evaluations

118 For DNA based evaluations we chose to retrieve sequence information from the ribulose-bisphosphate
 119 carboxylase oxygenase large subunit (rbcL) employing primers for rbcLa [Soltis et al., 1992, Kress et al.,
 120 2009] and rbcLb [Dong et al., 2014], maturase K (matK) employing primers for matK-KIM (Ki-Joong
 121 Kim, unpublished) and the Internal Transcribed Spacers (ITS) of nuclear ribosomal DNA employing
 122 primers ITS5 and ITS4 [White et al., 1990].

123 **DNA Isolation:** DNA was isolated from sterilized leaf samples of reference plants and leaf fragments
 124 selected from commercial products using the innuPREP Plant DNA Kit (Analytik Jena AG) following the
 125 vendor's instructions (SLS protocol). Products containing more than one leaf component (i.e. bamboo
 126 and lemongrass) were sampled twice, one bamboo sample for sequencing and one mixed sample for
 127 PCR diagnostics. Purity and concentration of isolated DNA was determined using a spectrophotometer
 128 (Nanodrop, Thermo Fisher Scientific, Germany).

Table 2. Product accessions (Acc) P1 - P8 of fruit (FT) or single component (SC) tea products available in Germany. Fruit teas are mixtures of fruit fragments and one or two leaf components. Genbank accession numbers of rbcLa, rbcLb, matK-KIM and ITS sequences generated in this study are also included.

Acc	Type	Leaf Component(s)	rbcLa	rbcLb	matK-KIM	ITS
P1	FT	bamboo, lemongras	KU722894	KU722852	KU722866	KU722880
P2	FT	bamboo	KU722893	KU722851	KU722865	KU722879
P3	FT	bamboo	KU722891	KU722849	KU722863	KU722877
P4	SC	bamboo whole leaf	KU722892	KU722850	KU722864	KU722878
P5 ^a	SC	bamboo	KX233507	KX233494	KX233503	-
P6	FT	bamboo	KX233506	KX233493	KX233502	-
P7	FT	bamboo, lemongras	KX233505	KX233492	KX233501	-
P8 ^a	SC	bamboo	KX233508	KX233495	KX233504	-

^a fine fragmented leaf material is contained in tea bags

Amplification and Sequencing: A 30 µL reaction volume containing 20.5 µL nuclease free water (Lonza, Biozym Scientific GmbH), 1-fold Thermopol Buffer from New England Biolabs GmbH (NEB), 1 mg / ml bovine serum albumin, 200 µmol dm⁻³ dNTPs (NEB), 0.2 µmol dm⁻³ of forward and reverse primer (see supplementary table 1), 100 - 150 ng DNA template and 3 units of Taq polymerase (NEB) was used to amplify marker sequences. The PCR reaction was subsequently evaluated by agarose gel electrophoresis (AGE) using NEEO ultra-quality agarose (Carl Roth GmbH). DNA was visualized using SYBR Safe (Invitrogen, Thermo Fisher Scientific Germany) and subsequent blue light excitation. The fragment size was determined using a 100 bp size standard (NEB). Amplified DNA was purified using a NucleoSpin® Gel and PCR Clean-up kit (MACHEREY-NAGEL GmbH). Sequencing was outsourced to MacroGen Europe (Netherlands).

Evaluation of Sequence Data: Sequencing results were assembled using a perl script. Raw data was converted to fasta (phred 20) and bi-directional reads merged to recover ambiguous characters (N). For additional quality control IUPAC consensus sequences were generated and inspected. Resulting sequences of product samples were used in a BLAST analysis to approximate taxonomic identity.

143 **Phylogenetic Diagnostics**

144 To assess the diagnostic power of the used marker regions in a phylogenetic framework, we combined
 145 reference plant sequences with sequences of relevant taxonomic groups retrieved from Genbank (sup-
 146 plementary table 3 and 4). Sequence collections of each marker were aligned (coding regions: Edgar
 147 [2004a,b], ITS: Katoh [2002], Katoh and Standley [2013] with L-INS-i), primer sites removed and
 148 trimmed using reference plant sequences. Subsequently each dataset was evaluated for its information
 149 content (alignment length, variable positions, parsimony information and singleton sites) and phylogenetic
 150 trees were computed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Maxi-
 151 mum Parsimony (MP) and Maximum Likelihood (ML) algorithms implemented in MEGA6 [Tamura
 152 et al., 2013]. For UPGMA the evolutionary distances were computed using the p-distance method [Nei
 153 and Kumar, 2000] with all ambiguous positions removed for each sequence pair. The MP tree was
 154 obtained considering all sites using the Subtree-Pruning-Regrafting (SPR) algorithm [Nei and Kumar,
 155 2000] with search level 1 in which the initial trees were obtained by the random addition of sequences
 156 (10 replicates). The evolutionary history inferred by using the ML method was based on substitution
 157 models in combination with evolutionary rate differences among sites that had the lowest BIC (Bayesian
 158 Information Criterion) scores determined by analysing each dataset using MEGA6. Details are summa-
 159 rized in table 3. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining
 160 (NJ) method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood
 161 (MCL) approach. All trees were bootstrapped [Felsenstein, 1985] using 500 replicates. Additionally,
 162 we computed UPGMA, MP and ML trees using concatenated datasets (rbcL = rbcLa and rbcLb; r+m =
 163 rbcLa, rbcLb and matK-KIM). The results were analysed by first collapsing branches corresponding to
 164 partitions reproduced in less than 50% bootstrap replicates and recording bootstrap support values for
 165 relevant monophyletic groups (sensu Soreng et al. [2015]). All datasets and trees have been deposited

166 in TreeBase (<http://purl.org/phylo/treebase/phylovs/study/TB2:S19113>). For representation the dataset
167 and algorithm that provided most support for relevant clades was edited using FigTree V1.4.2 [Rambaut, 2014].

Table 3. Substitution models (K2 = Kimura 2-parameter; T92 = Tamura 3-parameter; GTR = General Time Reversible) and evolutionary rates among sites (+G = discrete gamma distribution) used for ML analysis

Dataset	Model	Rates	BIC
rbcLa	K2	+G	5217
rbcLb	T92	+G	7177
rbcL	T92	+G	9380
matK-KIM	GTR	+G	10829
r+m	GTR	+G	19223

168

169 **Character based Diagnostics**

170 The PCR diagnostics approach had two objectives. Firstly, we aimed to establish a simple but efficient
171 PCR based differentiation between bamboo (= subfamily = *Bambusoideae*), the common secondary
172 component (lemongrass = genus = *Cymbopogon*) from the same family (*Poaceae*) and the adulterant from
173 the genus *Dianthus* (*Caryophyllaceae*). Secondly, we wanted to assess the diagnostic potential of the used
174 marker regions to resolve entities within the subfamily level and the adulterant genus.

175 **Differentiation of Bamboo Tea components and Adulterant:** For the PCR based differentiation
176 protocol we chose rbcLa which had been successfully used before to differentiate above the generic
177 level [Horn et al., 2012, 2013]. Using the rbcLa dataset, we designed primers to detect single nucleotide
178 polymorphisms [Newton et al., 1989, Ward et al., 2009] characteristic for bamboo, lemongrass and
179 carnation. Nucleotide differences between the mentioned components were determined and potential
180 diagnostic primer sequences extracted. One suitable primer for each group was chosen and destabilized
181 according to Newton et al. [1989] (supplementary table 1). The theoretical suitability of a diagnostic
182 primer was determined using primer3 [Untergasser et al., 2007, 2012] with default settings.

183 The diagnostic primers were evaluated in a multiplex PCR with the universal primer-pair (rbcLa).
184 For each diagnostic primer a separate set of 10 μL PCR reactions containing 6.5 μL nuclease free water
185 (Lonza, Biozym Scientific GmbH), 1-fold Thermopol Buffer (NEB), 1 mg / ml bovine serum albumin,
186 200 $\mu\text{mol dm}^{-3}$ dNTPs (NEB), 0.3 $\mu\text{mol dm}^{-3}$ of universal forward primer, 0.2 $\mu\text{mol dm}^{-3}$ of universal
187 and diagnostic reverse primer, 25 - 50 ng DNA template and 0.5 units of Taq polymerase (NEB) was
188 used. The PCR products were evaluated by gel electrophoresis using high resolution agarose (Carl Roth
189 GmbH).

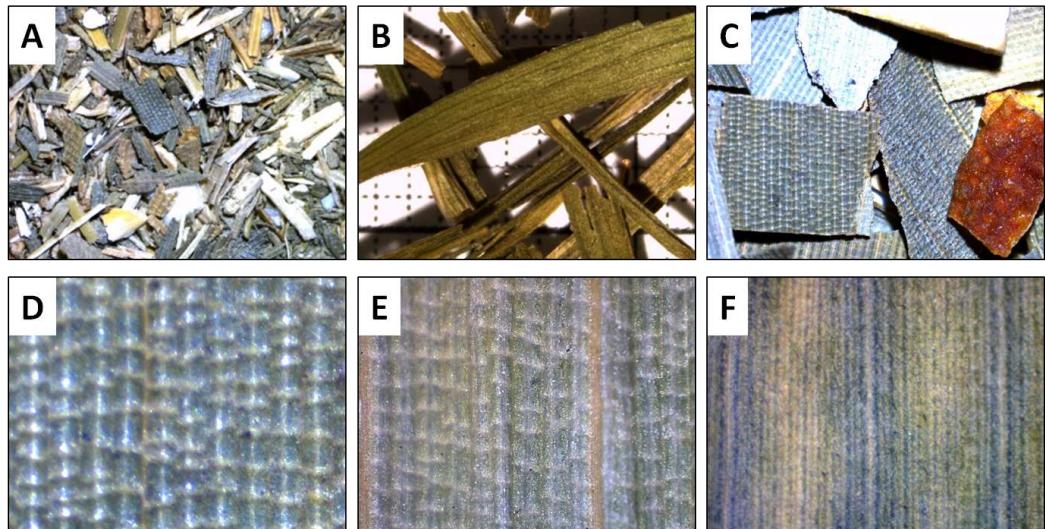
190 **Assessment of Diagnostic Potential:** To assess the diagnostic potential of DNA markers, we used a
191 character bases DNA Barcoding approach - Barcoding with LOGic [Weitschek et al., 2013, Bertolazzi et al.,
192 2009]. We prepared separate single and multi-locus datasets containing only sequences of *Bambusoideae*
193 and *Caryophyllaceae* respectively. Sequences were labelled according to specific taxonomic classes. For
194 the *Bambusoideae* dataset we tested tribe and genus as diagnostic entities. For *Dianthus* we only tested
195 the species as diagnostic entity. Since the general evaluation showed limited variation within rbcL in
196 *Dianthus* we chose to evaluate only matK-KIM as cytoplasmic marker. Additionally we included an
197 ITS dataset that contained all available Genbank *Dianthus* sequences regardless if data also existed for
198 the cytoplasmic markers. The BLOG algorithm was subsequently used with standard settings (except
199 padding=1, percslicing=100 and exclusivesfs=1) to find characters or character combinations by which
200 diagnostic entities can be classified.

201 **RESULTS**

202 **Anatomical Evaluation**

203 Morphology as the study of forms visible to the unaided eye, in food diagnostics is complemented by
204 anatomy, the study of cellular structures. For an intermediate between morphology and anatomy, in this
205 study we used the term "macroscopic". The magnification used does not yet allow to observe cellular

Figure 1. Macroscopic features of bamboo tea products (A-D) and bamboo leaf samples (E and F). Leaf fragments (10 x) of bamboo tea bag product (A), adulterant component (B) and of a bamboo fruit tea (C). Leaf surface (adaxial, 40 x) of bamboo tea component (D) in comparison to Arundinarieae (E, *Sasa palmata*) and Bambuseae (F, *Bambusa multiplex*) dried leaf samples.



206 structures in detail, but eases the study of their morphological manifestations. Both, microscopic and
 207 macroscopic anatomy are common techniques used in food diagnostics [Hohmann and Gassner, 2007].

208 **Macroscopic Features:** A characteristic of the bambusoid leaf is a mosaic pattern of longitudinal and
 209 transverse veinlets, so called tessellation. Our evaluation of leaf samples from bamboo reference plants
 210 supports the description of Farrelly [1984] wherein tessellation of the leaf is a visible characteristic of
 211 hardy, monopodial species (*Arundinarieae*, Figure 1E) and is hidden from the unaided eye by tissue in
 212 sympodial bamboos whose leaves are often more tough and leathery (*Bambuseae*, Figure 1F).

213 Evaluating the leaf samples taken from herbal tea products, tessellation was observed in samples P5 - P8
 214 (e.g. Figure 1C and D). While leaf fragments with tessellation always were fragments in longitudinal and
 215 transversal respect, leaf components of the remaining products P1 - P4 consisted of thin (approximately 4
 216 mm) linear to lanceolate leaves (Figure 1B), in some instances oppositely arranged at the fragment of a
 217 shoot. The observed arrangement of leaves is in direct conflict with the index of contents of corresponding
 218 products. *Poaceae* plants always have in two ranks alternately arranged leaves [Wu et al., 2006]. However,
 219 since bamboo tea is also available in a form where components are so small, that the arrangement of
 220 leaves cannot be determined (tea bags), microscopic features need to be considered.

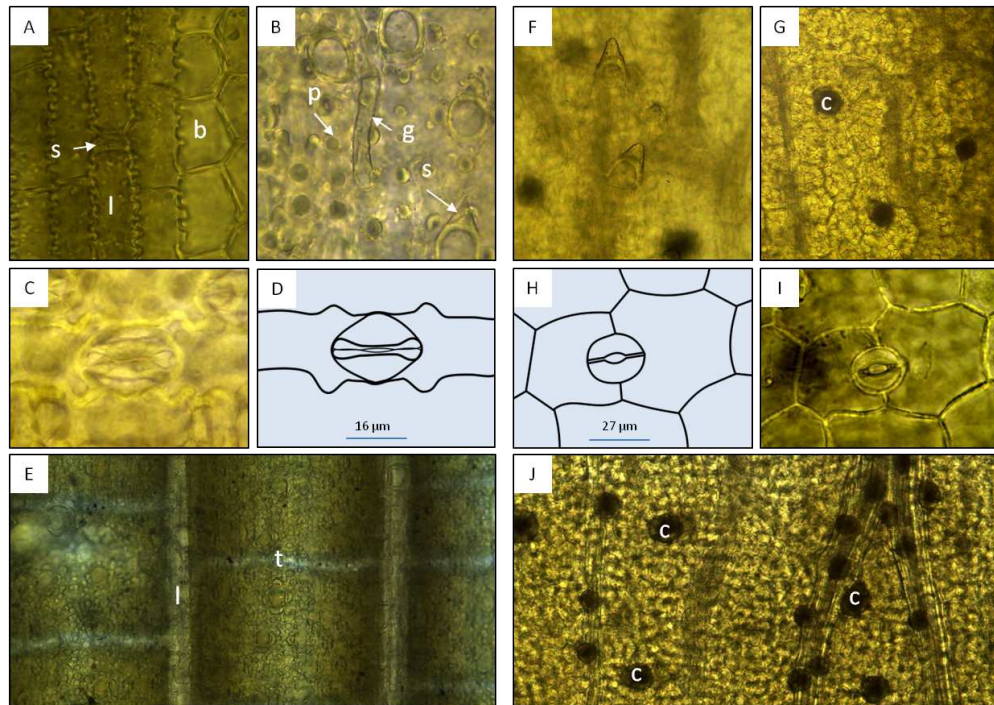
221 **Microscopic Features:** Using light microscopy (100 x), tessellation was observed in all bamboo
 222 reference plants. Additionally, characteristic structures of the bambusoid leaf [Wu, 1962, Vieira et al.,
 223 2002] were observed: epidermal cells - longitudinal bands composed of long rectangular cells with wavy
 224 lateral walls and alternating short rectangular cells, separated by bulliform cells [Beal, 1886, Alvarez
 225 et al., 2008] in the upper epidermis (Figure 2 A); and modified epidermal cells - stomata of the *Poaceae*
 226 type, microhairs, spines, papillae, bristles and silica cells (Figure 2 B - D).

227 The microscopic evaluation of commercial samples P5 - P8 was congruent with the results from
 228 bamboo reference plants, showing bambusoid features (e.g. tessellation: Figure 2 E). Samples P1 - P4 did
 229 not display any bambusoid characteristics but stomata of a different type than *Poaceae* (Figure 2 H and I)
 230 and crystal druses (Figure 2 J) along main veins and in intercostal regions. We recognised anomocytic
 231 stomata common in *Caryophyllaceae* and *Ranunculaceae* [Rohweder et al., 1971] predominated by the
 232 diacytic form. This suggests that samples P1 - P4 probably originated from a *Caryophyllaceae* plant.

233 DNA based Evaluation

234 All three cytoplasmic markers were retrieved with great success regarding PCR and sequencing results.
 235 ITS however turned out to be particularly problematic with bamboo samples. Preferential and co-
 236 amplification of ITS from fungal trace DNA prevented the retrieval of a complete dataset for bamboo

Figure 2. Microscopic features of the bambusoid leaf observed in reference specimens (A - D, 400 x) and product samples (E, 100 x), and microscopic features of *Dianthus chinensis* observed in reference specimens (F and G, 100 x; H and I, 400 x) and adulteration samples (J, 100 x). A: Adaxial epidermis of *Bambusa multiplex* showing longitudinal bands of long rectangular cells (l) with wavy lateral walls and alternating short rectangular cells (s) separated by bulliform cells (b). B: Abaxial modified epidermal structures of *Phyllostachys edulis* (p = papillae, g = geniculate hair, s = spine). C and D: Abaxial epidermis with *Poaceae* type stomata of *Sasa palmata*. E: Epidermis with longitudinal (l) and transverse veinlets (tessellation) observed in product samples. F: Leaf epidermis of *D. chinensis* showing unicellular trichomes. G: Mesophyll of *D. chinensis* showing crystal druses (c). H and I: Abaxial epidermis of *D. chinensis* with anomocytic stomata (here diacytic). J: Mesophyll with crystal druses (c) along main veins and in intercostal regions observed in product samples.



237 specimens. Similar problems have been reported by Zhang et al. [1997].

238 **General Assessments**

239 **BLAST Analysis of Product DNA Sequences:** Single locus markers (rbcLa, rbcLb and matK-KIM)
 240 were used in a BLAST analysis. Two groups could be distinguished: P1 - P4 returned hits indicating close
 241 relation to *Dianthus* (*Caryophyllaceae*) and P5 - P8 returned hits belonging to genera of *Bambusoideae*.

242 **Information Content:** Final single marker dataset alignments contained 553, 814 and 837 nucleotides
 243 for rbcLa, rbcLb and matK-KIM respectively. Combining rbcLa and rbcLb (rbcL) excluding redundant
 244 data, the alignment had 1'126 positions. The combination of rbcLa, rbcLb and matK-KIM had 1'963
 245 positions respectively. The *Dianthus* ITS dataset of reference plant accessions contained 611 nucleotides.
 246 Including Genbank accessions (supplementary table 4) the dataset was comprised of 85 sequences with 618
 247 positions. Information content (i.e. number and proportion of variable sites and parsimony informative
 248 positions) within *Bambusoideae* and *Dianthus* datasets is shown in table 4.

249 In both taxonomic groups most variation among single locus cytoplasmic markers was detected in the
 250 matK-KIM region. Considering parsimony information, rbcLa in bamboo and rbcLb in *Dianthus* show the
 251 highest proportion (57 and 100 % respectively). The combination of single locus data obviously contains
 252 all variation and informative sites but reduces the proportion in combined datasets. Among the *Dianthus*
 253 datasets the nuclear marker (ITS) contains the highest variation and thus delivers most information.

254 Sequence data of adulterated (P1 - P4) and genuine (P5 - P8) bamboo products as well as all reference
 255 plants are deposited in Genbank. Sequence accessions from other studies that were included in this study

Table 4. Information content of bamboo and *Dianthus* genetic marker datasets comprised of 43 and 14 DNA sequences respectively. Sequences were obtained from references (plant and Genbank sequence accessions) and product samples. Length (Len), conserved (Con), variable (Var), parsimony informative (PaI) and singleton (Sin) characters as well as the number of haplotypes (Hap) are listed for cytoplasmic markers (rbcLa, rbcLb and matK-KIM) and combinations of those (rbcL = rbcLa + rbcLb and r+m = rbcLa + rbcLb + matK-KIM). For *Dianthus* the same information is listed for a nuclear (ITS) marker, one simple dataset for comparison and one extended (^e) dataset consisting of 85 *Dianthus* sequences.

Bamboo									
Marker	Len	Con	Var	%	PaI	%	Sin	%	Hap
rbcLa	553	523	30	5.4	17	56.7	13	43.3	17
rbcLb	814	761	53	6.5	26	49.1	27	50.9	13
rbcL	1126	1057	69	6.1	35	50.7	34	49.3	22
matK	837	748	89	10.6	40	44.9	49	55.1	25
r+m	1963	1805	158	8.0	75	47.5	83	52.5	31
Dianthus									
Marker	Len	Con	Var	%	PaI	%	Sin	%	Hap
rbcLa	553	553	0	0.0	0	0.0	0	0.0	1
rbcLb	814	808	6	0.7	6	100.0	0	0.0	3
rbcL	1126	1120	6	0.5	6	100.0	0	0.0	7
matK	837	826	11	1.3	8	72.7	3	27.3	3
r+m	1963	1946	17	0.9	14	82.4	3	17.6	7
ITS	611	566	45	7.4	39	86.7	6	13.3	12
ITS ^e	618	498	120	19.4	72	60.0	48	40.0	87

^e, extended dataset

256 are contained within supplementary table 3 and 4.

257 **Phylogenetic Analysis**

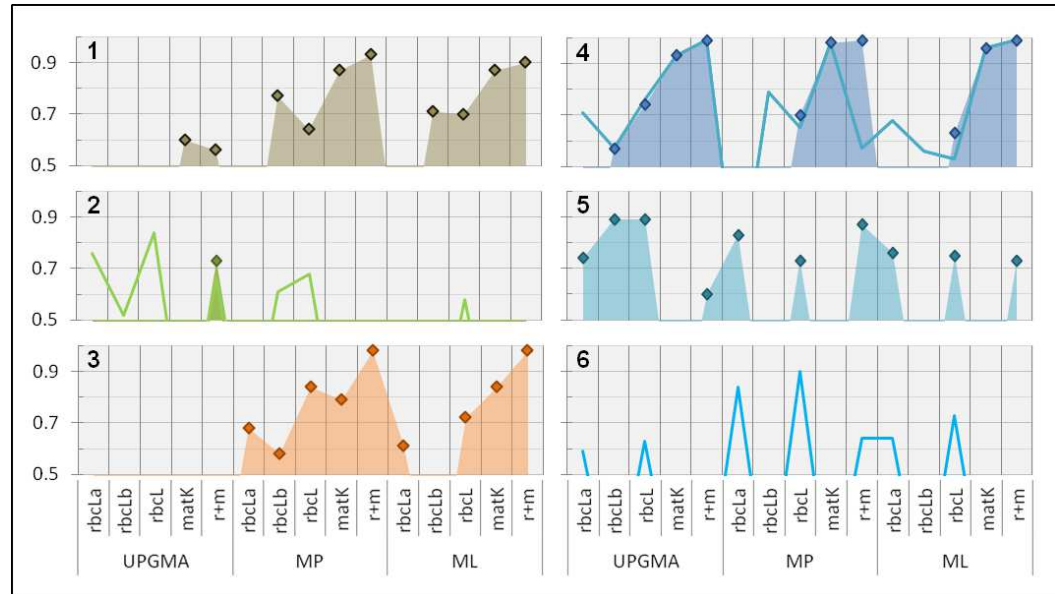
258 **Clade Support:** Comparing the support for relevant clades using different phylogenetic methods with
259 single and multi-locus datasets reveals several interesting aspects (Figure 3).

260 Sequence accessions of *Borinda* (*Arundinarieae*) and *Chusquea* (*Bambuseae*) cluster in the *Bam-*
261 *buseae* and *Arundinarieae* clade respectively. We therefore introduced additional evaluation classes:
262 *Arundinarieae* modified (mod.) and *Bambuseae* modified (mod.). For these classes the position of both
263 mentioned sequence accessions was ignored when assessing monophyly.

264 The bamboo subfamily (*Bambusoideae*, Figure 3-1) is supported with more than 50 % of replicates by
265 all marker regions except rbcLa, using MP and ML methodology. When using rbcLa the *Oryzoideae* clade
266 resides among the bamboo members making *Bambusoideae* a non-monophyletic clade. Support for the
267 bambusoid subfamily is constantly equal or above 70% except when using the combined rbcL-sub-regions
268 and the MP approach. With support between 50 and 60 % of two of five tested datasets (matK and r+m),
269 UPGMA only gives weak and inconsistent support for the subfamily.

270 Focusing on the three bambusoid tribes (*Arundinarieae*, figure 3-4; *Bambuseae*, figure 3-2; *Olyreae*,
271 figure 3-3) none of the markers and methods strongly support all corresponding clades at the same time.
272 Applying MP with matK or combined cytoplasmic data yields high support (>70 %) for *Arundinarieae*
273 and *Olyreae*. Both clades are also supported according to ML, using combined rbcL (>63 %), matK
274 (>84 %) and combined cytoplasmic (>98 %) datasets. The *Olyreae* clade (Figure 3-3) receives consistent
275 support using any dataset with the MP approach (rbcLb 58 % - r+m 98 %). Similarly, except when using
276 the rbcLb dataset, the ML approach offers high support (rbcLa 61 % - r+m 98 %). The *Arundinarieae*
277 clade (Figure 3-4) also is consistently supported by all three phylogenetic approaches, particularly when
278 using matK (UPGMA 93 % - MP 98 %) or the combined cytoplasmic dataset (99 %). Considering an
279 alternative taxonomic configuration (*Arundinarieae* mod., line in figure 3-4) some of the single datasets
280 offer support for the corresponding clade. However, a significant difference between the support for the
281 *Arundinarieae* clade (99 %) and the modified clade (57 %) can be observed when using the combined

Figure 3. Phylogenetic evaluation. The phylogenetic evaluation shows bootstrap support (y-axis) for relevant clades (1: *Bambusoideae*, 2: *Bambuseae* s.str. and mod., 3: *Olyreae*, 4: *Arundinarieae* s.str. and mod., 5: *Sasa*, 6: *Phyllostachys* mod.) using UPGMA, MP and ML methodology with single locus (rbcLa, rbcLb and matK-KIM) and multi-locus (rbcL: rbcLa + rbcLb; r+m: rbcL + matK-KIM) data. Lines indicate support for an alternative composition of the corresponding clade or taxonomic group. Please refer to the discussion for further information.



282 cytoplasmic dataset in a MP analysis. The *Bambuseae* clade (Figure 3-2) only once is supported above 50
 283 % (UPGMA: r+m) unless considering an alternative taxonomic configuration (*Bambuseae* mod., line in
 284 figure 3-2). In all cases where a *Bambuseae* mod. clade is supported, the *Chusquea* sequence accession
 285 fails to cluster (support >50 %) with other *Bambuseae* sequences. In every other instance where general
 286 support for *Bambuseae* is missing, the *Chusquea* sequence clusters with *Sasa* (MP, ML: rbcLb) and
 287 only some of the *Bambusaeae* sequences form supported clusters. A sister clade consisting of *Oatea*
 288 and *Olmecca* is consistently formed (UPGMA: matK; MP: rbcLa, matK, r+m; ML: rbcLa, rbcLb, r+m)
 289 along other *Bambuseae* sequences. In the ML analysis using matK the *Olyreae* clade resides within the
 290 *Bambuseae* clade resulting in the non-monophyly of the clade.

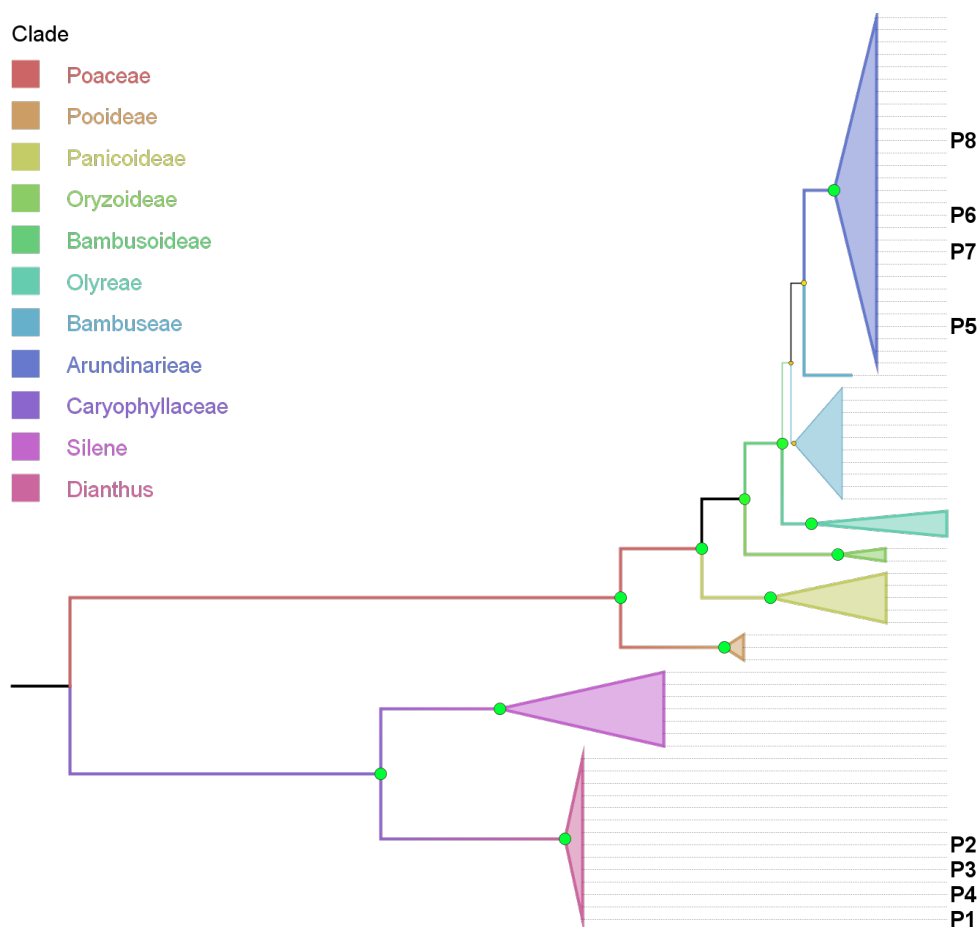
291 Support on the genus level is rare. Only *Sasa* (Figure 3-5) and *Thamnocalamus* form monophyletic
 292 clades. The *Sasa* clade can be observed in 10 of 15 cases, all based on rbcL data. A monophyletic
 293 *Thamnocalamus* clade can only be observed when using rbcLa data. Since product samples frequently
 294 clustered within a clade containing *Phyllostachys* we introduced another evaluation class: *Phyllostachys*
 295 modified (mod.). This class consists of all *Phyllostachys*, *Fargesia*, *Indocalamus* and *Drepanostachyum*
 296 sequence accessions. This clade can be observed using rbcLa and the combined rbcL dataset (UPGMA,
 297 MP and ML) as well as when using the combined cytoplasmic dataset (MP). Also in this case, support
 298 appears to be solely derived from rbcL data. Although rbcLb data does not offer direct support, its
 299 contribution to the combined dataset can clearly be observed by increased support values (e.g. up to
 300 almost 10 % in ML analysis).

301 All other *Poaceae* groups (i.e. *Bambusoideae* outgroups *Oryzoideae* and *Triticum*, and secondary
 302 component groups *Panicoideae* and *Cymbopogon*) receive consistent and strong (>85 %) support. One
 303 exception worth mentioning is the low (MP: 52 %) and missing support (UPGMA and ML) for *Panicoideae*
 304 (represented by *Cymbopogon* and *Lophaterum*) when using rbcLb data.

305 Support for the genus of particular interest (*Dianthus*, >= 72 %) as well as the corresponding family
 306 (*Caryophyllaceae*, 100 %) and outgroup (*Silene*, >= 64 %) are consistent and strong with rare low points,
 307 i.e. using matK data with ML (*Silene*) and using rbcLb data with ML (*Dianthus*).

308 **Phylogenetic Representation:** Using the combined cytoplasmic dataset with sequences recovered
 309 from product components and building a MP tree, basically visualizes the BLAST results within an

Figure 4. Phylogenetic tree based on combined cytoplasmic sequence data using Maximum Parsimony (MP). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are indicated by the size and colour of the nodes. The analysis involved 74 nucleotide sequences and 1999 positions in the final dataset.



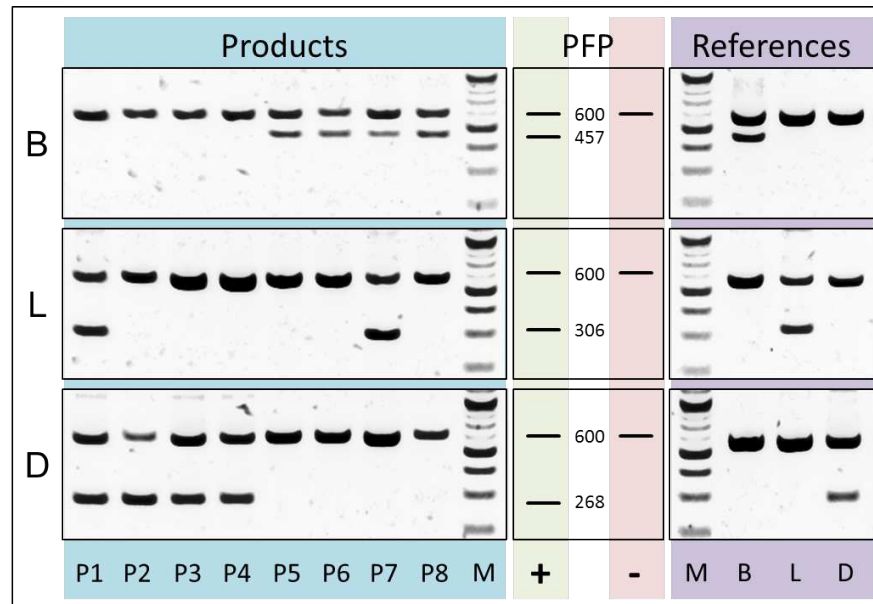
310 evaluated phylogenetic framework (Figure 4). Product samples P1 - P4 clearly are located within the
 311 *Dianthus* (*Caryophyllaceae*) clade and product samples P5 - P8 are located within the *Arundinarieae*
 312 (*Bambusoideae*, *Poaceae*) clade.

313 **Diagnostic Analysis**

314 **Differentiation of Tea Components and Adulterant:** Based on a *rbcLa* dataset containing bamboo,
 315 lemongrass and *Dianthus* sequences we designed three reverse ARMS primer (supplementary table 1)
 316 with diagnostic nucleotides located at position 407, 254 and 223 respectively. The evaluation of multiplex
 317 PCRs, applying these specific primers in separate reactions together with *rbcLa* universal primers (Figure
 318 5), shows sufficient specificity and amplification of diagnostic fragments (bamboo, 457 bp; lemongrass
 319 306 bp; *Dianthus* 268 bp) to differentiate the three leaf components present in commercial tea products.
 320 Products P1 - P4 show diagnostic fragments of size 268 bp indicating the presence of *Dianthus* (figure
 321 5-D) and are lacking bamboo diagnostic fragments (figure 5-B). Products P5 - P8 show the exact opposite
 322 pattern, no diagnostic fragments specific for *Dianthus* but for bamboo. Additionally, the presence of
 323 lemongrass in products P1 and P7 is shown by diagnostic fragments of the corresponding size (figure 5-L,
 324 306 bp). All reference plants of the corresponding groups have been tested for positiv reaction using the
 325 diagnostic primer and negativ (null) reaction using any diagnostic primer of different groups.

326 **Assessment of Diagnostic Potential:** The evaluation of bambusoid tribe classification using BLOG
 327 shows consistency among markers. Only the *Arundinarieae* tribe shows 4 % false negative classifications
 328 when using *rbcLa* data.

Figure 5. ARMS Diagnostics: Comparison of multiplex PCR results using *rbcLa* universal primers and diagnostic primers for Bamboo (B), lemongrass (L) and *Dianthus* (D) tea components. On the left are the results using DNA templates derived from products P1 to P8. Based on *rbcLa* sequence data fragment patterns were predicted (PFP). The *rbcLa* fragment with a size of around 600 bp represents the positive reaction control. Smaller fragments are called diagnostic fragment and indicate (+) the presence of a particular component (e.g. 306 bp fragment for lemongrass). On the right are representative results using DNA templates derived from reference plants. For the approximation of fragment size a 100 bp (NEB) size standard (M) was used.

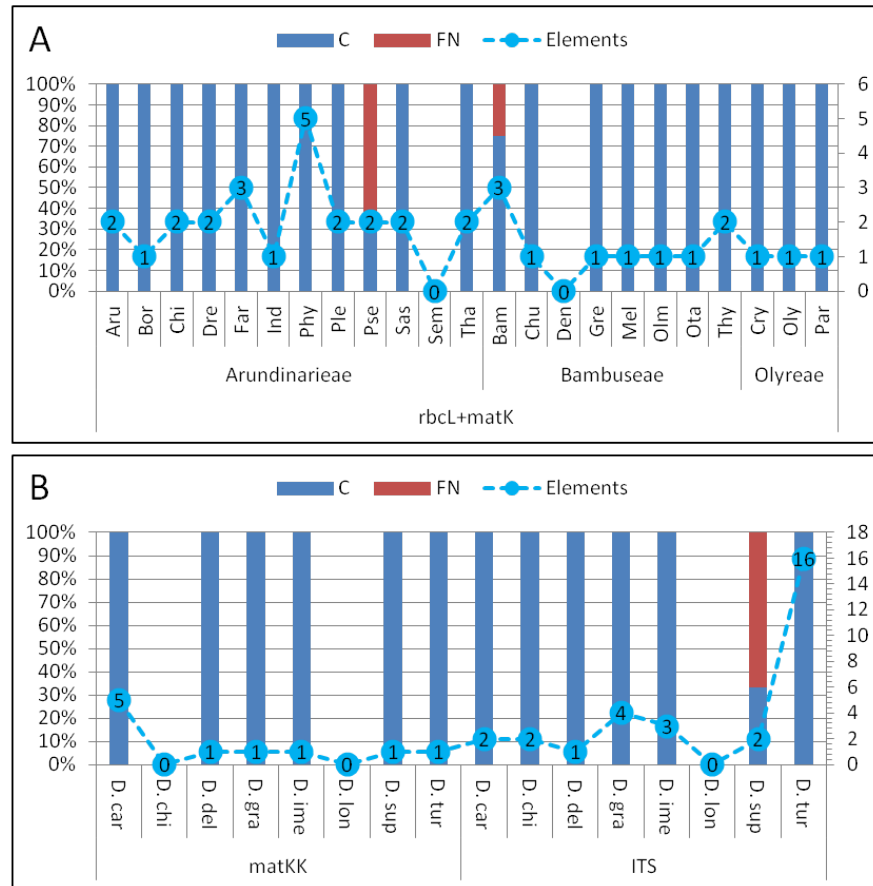


329 Comparing bambusoid genus classification (supplementary figure 1), the combined cytoplasmic
 330 dataset provides the highest diagnostic coverage of bambusoid genera. Only 14 of 23 bambusoid genera
 331 are at least partially diagnostically covered using single locus *rbcLa*. Using *rbcLb*, 20 of 23 genera are
 332 classified with 3 genera only partially (<50 %) covered. The combination of *rbcLa* and *rbcLb* reflects the
 333 result of *rbcLb* with full coverage of two of these genera (*Fargesia* and *Pseudosasa*) and an a slightly
 334 increased coverage of the third (*Phyllostachys*). Additionally, using provided LOGic formulas, the
 335 sequence of product sample P8 provides consistent characters (i.e. pos234=T AND pos490=T AND
 336 pos878=G) with that of *Pseudosasa*. The diagnostic value of the *matK-KIM* region is similar to that of
 337 *rbcLa* with 13 of 23 genera at least partially covered. The combined dataset of *rbcL* and *matK-KIM* only
 338 leaves two genera without diagnostic markers (i.e. *Semiarundinaria* and *Dendrocalamus*) and no false
 339 positives are detected (figure 6-A). Using provided LOGic formulas, sequences of product samples P5 -
 340 P7 provide consistent characters (i.e. pos12=T AND pos263=T AND pos701!=A AND pos738!=C AND
 341 pos1434=G) with that of *Phyllostachys*.

342 All markers, either as single or in combination, offer diagnostic solutions for the genera of *Olyreae*.
 343 While *Arundinarieae* genera are moderately covered using *rbcLa* data and are almost completely void
 344 of diagnostic solutions considering *matK-KIM* data, in *Bambuseae* the situation is reversed, *matK-KIM*
 345 being more informative. In regard of single locus diagnostics *rbcLb* is superior in the bambusoid group.

346 Comparing *matK-KIM* and ITS datasets for *Dianthus* (Figure 6-B) shows the inability to distinguish
 347 *D. chinensis* and *D. longicalyx* based on *matK-KIM* data. Using ITS, information content increases
 348 enough to diagnose *D. chinensis* with a unique LOGic formula (pos181=g AND pos595=c) that also
 349 applies to product samples P1 - P4.

Figure 6. Barcoding with LOGic formulas (BLOG) analysis of bamboo (A) using the combined cytoplasmic dataset and *Dianthus* (B) using matK-KIM and ITS (extended) single marker datasets. Results for species that only were present in the extended ITS dataset are not shown. The proportion (in %, primary y-axis) of coverage (C, blue) and false negatives (FN, red) using logic formulas is shown as bars. The number of elements (nucleotide positions) within the LOGic formula are represented in a line graph (secondary y-axis).



DISCUSSION

Anatomical Evaluations

Due to the absence of bambusoid leaf characteristics in samples P1 - P4, we can exclude a *Poaceae* and *Bambusoideae* origin of the leaves used in corresponding tea products. Stomata type and pattern of epidermal cells in comparison to reference plants from the genus *Dianthus* suggest the origin of leaves to be found within this group.

In contrast, observation of bambusoid leaf characteristics in samples P5 - P8 leads to the conclusion that genuine bamboo leaves have been used in corresponding tea products. Investigating the possibility to differentiate between bambusoid tribes, the most promising feature appears to be tessellation. The ability to observe this pattern without or only limited magnification ($\leq 10 \times$) in members of the *Arundinarieae* and the necessity of higher magnification ($\geq 40 \times$) in members of the *Bambuseae* can be used to separate both woody bamboo tribes [?]. Tessellation has also been observed with low magnification in samples P5 - P8. This suggests that the source species for bamboo tea leaves are likely to be from the *Arundinarieae* tribe.

Particular characteristics to differentiate between the bamboo genera were suggested by Wu [1962]. The waviness of the walls of upper and lower epidermal cells in some species is different, while in other species the waviness is constant. However, no quantification methodology nor any standard was suggested. Modifications of epidermal cells (i.e. uni- and bicellular hairs, spines, bristles and silica cells)

368 also can contribute to a diagnostic evaluation but appear not to be exclusively distributed in one particular
369 genus. Further studies are necessary to establish standards for potential diagnostic characters and to
370 evaluate their phenotypic plasticity.

371 One of the most challenging aspects of microscopic studies of dried bambusoid leaf samples are
372 abundant papillae, often overarching the stomates [Zhang and Clark, 2000], and achieving sufficient
373 clearing of the tissue samples.

374 Tesselation is also a useful diagnostic marker in separating bamboo from other *Poaceae* groups (e.g.
375 lemongrass). Additional anatomical markers for this purpose are fusoid cells [Motomura et al., 2004, ?,
376 ?] and invaginated arm cells in the chlorenchyma [Zhang and Clark, 2000]. Both cell types, however,
377 only can be observed in cross sections. Due to the processed nature (i.e. drying) of product samples, a
378 more laborious sample preparation method is required (embedding) and results are likely to be biased by
379 artefacts introduced by the drying process (e.g. collapsed parenchymatic cells). Based on our analyses,
380 we compiled an anatomic diagnostics key for the differentiation of bamboo, lemongrass and carnation
381 (supplementary table 5).

382 DNA based Evaluations

383 Morphological traits used to determine the genus of bamboo specimens were shown to be highly congruent
384 with plastid RFLP data and the plastid genome has been extensively evaluated for its phylogenetic and
385 phylogenomic potential to elucidate relationships that have been intractable [Watanabe et al., 1994]. The
386 analysis of six bamboo chloroplast genomes, however, revealed low levels of variation in *Bambusoideae*
387 and difficulties in resolving diversification among temperate woody clades (*Arundinarieae*) even with
388 complete chloroplast genome sequences Zhang et al. [2011].

389 With DNA Barcoding, ideally, one is able to determine the species of a specimen by comparing
390 sequence information of a standardized DNA region to a database of species barcodes. Since there is
391 no single universal locus in plants available with whom one could determine the identity of specimens
392 with high success rates, using more than one locus is the most promising choice. Beside the official
393 plant barcode markers (*rbcL* and *matK*) other complementary markers can be used. Lack of taxonomic
394 universality (*ycf1*) and sequencing universality (*psbA-trnH*) as well as co-amplification of fungal DNA
395 or interference of paralogs in downstream analysis (ITS) are common issues associated with alternative
396 markers. While *psbA-trnH* has been shown to outperform *rbcL* and *matK* in some cases [Kress and
397 Erickson, 2007, Wong et al., 2013], in temperate bamboos has much lower divergence rates and showed
398 even less discrimination power than *rbcL* [Cai et al., 2012].

399 Combining *rbcL* and *matK* barcoding marker data in a phylogenetic analysis, we were able to limit the
400 possible taxonomic origin of bamboo leaves used in tea products to the bambusoid tribe *Arundinarieae* and
401 3 of 4 product samples could be further traced to a *Phyllostachys* clade. Using the combined cytoplasmic
402 dataset in a character based DNA Barcoding approach (i.e. BLOG) further improved our results and we
403 were able to connect characteristic patterns (LOGic formulas) of two bambusoid genera (i.e. *Phyllostachys*
404 and *Pseudosasa*) to the genuine bamboo product samples (P5 - P7 and P8 respectively). In general our
405 evaluation of diagnostic potential demonstrated the diagnostic value of *rbcL* and *matK* on the generic
406 level in bamboos and provides solutions to diagnose most (19 of 23) of the bamboo genera for which *rbcL*
407 and *matK* sequence information is currently available in Genbank.

408 Using the highly universal DNA Barcoding marker *rbcLa*, we introduced a PCR based diagnostic
409 solution for the detection of an adulterant of bamboo tea (carnation). Using 85 ITS sequences retrieved
410 from reference plants and Genbank in a character based DNA Barcoding approach, the classification pattern
411 of *D. chinensis* was also found in sequences obtained from adulterant samples P1 - P4. The diagnostic
412 solution also includes the differentiation of the two *Poaceae* tea components (bamboo and lemongrass).
413 To improve the significance (i.e. taxonomic depth) of the genetic test, other markers need to be evaluated.
414 While ITS has been used in bamboo [Cai et al., 2012], fungal contamination and ITS paralogs decrease the
415 applicability of this marker considerably. Other available DNA markers are for example GBSSI [Zhang
416 et al., 2012, Yang et al., 2008, 2010, Peng et al., 2008] and COS [Li et al., 2008, Liu et al., 2013]. To
417 improve the robustness of the test, sampling within the temperate bamboo genera needs to be increased.
418 Furthermore, the genetic test could be improved by optimizing reaction conditions for the combined use
419 of more than one diagnostic primer.

420 Conclusion

421 **What is Bamboo Tea?** According to the NCBI Taxonomy the common name for the tribe *Bambuseae* is
422 bamboo. This reflects an old systematic opinion [Zhang and Clark, 2000] when *Bambuseae* still contained
423 most *Arundinarieae* species (e.g. *Sasa* and *Phyllostachys*). However, the most recent scientific usage of
424 the term bamboo is found in Soreng et al. [2015] where bamboo is the common name for the subfamily
425 *Bambusoideae* (*Poaceae*). This group is characterized by high morphological diversity that appears not
426 to be discretely associated with subordinate taxonomic entities. The reasons are believed to be related
427 to morphological inter-gradation interpreted in various ways and the presence of hybrids that have been
428 stabilized through clonal propagation [Triplett and Clark, 2010]. The taxonomic confusion within the
429 group also is related to a peculiarity of the reproduction mode of bamboo. While most flowering plants
430 are flowering regularly each year, bamboo is one of the groups where dramatically extended intervals
431 exist - some as long as 120 years [Veller et al., 2015, Liese, 2015].

432 Although DNA based approaches to classification of bamboos are characterized by limited information
433 of genetic markers, the subfamily has been well established and the temperate woody clade (*Arundi-*
434 *narieae*) was resolved to an acceptable degree, delivering additional information about associations of
435 particular genera and biogeographic hypotheses [Triplett and Clark, 2010]. All commercial samples of
436 genuine bamboo tea analysed in the present study could be placed within the *Arundinarieae* tribe using
437 macroscopic leaf characteristics. Furthermore, they could be traced to internal groups by phylogenetic
438 methodology (*Phyllostachys* clade) and a character based DNA Barcoding approach (*Phyllostachys* and
439 *Pseudosasa* genera).

440 **Carnation = Bamboo tea?** From an evolutionary perspective, bamboo and carnation are fairly
441 different groups of plants with more than a hundred million years of independent development between
442 them [Chaw et al., 2004]. How is it possible to confuse such distinct groups?

443 Scientific names exist because they allow us to communicate precisely. However, it is also com-
444 mon for humans to label things by its appearance instead of its true identity. So it is not surprising
445 to find a simple explanation for a potentially severe adulteration of teas supposedly containing bam-
446 boo leaves: A product description (retrieved in July 2014 from [http://www.happyluckys.com/
447 bamboo-tea-carnation](http://www.happyluckys.com/bamboo-tea-carnation)) of so called Bamboo Tea Carnation is advertised by the following sen-
448 tence:

449 "There are well over a hundred varieties of bamboo growing in China. This is not one of them,
450 actually belonging to the genus of Carnations (*Dianthus*), but the young shoots closely resemble bamboo
451 in appearance..."

452 Communication using the term bamboo in conjunction with tea obviously is ambiguous and may have
453 caused the declaration error on corresponding products. Since these products had been on the market for
454 at least 1.5 years before they were discontinued, we must ask what consequences this may have had for
455 consumers?

456 Several species of carnation are mentioned in an ethno-medicinal context [Chandra and Rawat, 2015].
457 Particularly in traditional Chinese medicine two species - *D. chinensis* and *D. superbus* - are widely used
458 as Dianthi herba for the treatment of diuresis and strangury [Committee, 2010]. Chemical constituents are
459 saponins [Oshima et al., 1984, Hong-Yu et al., 1994], flavonoids, sterol, glycosides and cyclopeptides [Han
460 et al., 2015, 2014, Hsieh et al., 2004]. Studies on bioactivity have shown various effects. Cyclopeptides
461 for example showed anti-bacterial, anti-fungal, estrogen-like, uterotonic, haemolytic and cardio-toxic
462 effects. The uterotonic effect is the reason why Qu mai (Dianthi herba) should not be prescribed to
463 pregnant women [Wu, 2005]. By selling bamboo tea that actually contains *Dianthus* species, consumers
464 are betrayed. Additionally, if the *Dianthus* species is known to have an effect on the dynamics of the uterus,
465 pregnant women are put in harms way. Our data strongly suggests that leaves found in adulterated bamboo
466 tea are from *D. chinensis* and measures to prevent this kind of misdirections have to be implemented
467 immediately.

468 **Legal Scientific Framework:** Article 2 of the European General Food Law Regulation [Euro-
469 pean Commission, 2002] specifies "food" as any substance or product, whether processed, partially
470 processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. Tea products
471 analysed in this study either consist of different "substances", one of which is "bamboo leaf", or only
472 contain the latter. Consulting the List of Substances of the Competent Federal Government and Federal
473 State Authorities (german version) for the category "plants and plant parts", common names (e.g. apple,
474 lemon and orange) used in ingredient lists of teas are found and mapped to the scientific name of the

475 corresponding plant the "substance" (e.g. fruit) is derived from. The common name bamboo can be
476 mapped to two species of *Dendrocalamus* (*D. asper* and *D. latiflorus*) which are the source for bamboo
477 sprouts. No other entries for bamboo are present. The english version of the mentioned list does not
478 provide associations of common names with scientific names, representing one common unnecessary
479 obstacle consumers and food business operators are confronted with. Since bamboo is an exotic group,
480 we have to assume that corresponding substances used in products fall under the novel food legislation
481 and might be listed in the novel food catalogue.

482 Foods or food ingredients which have not been used for human consumption to a significant degree
483 in the European Union (EU) before 15 May 1997 are governed by the provisions of the Novel Food
484 Regulation (NFR) [European Commission, 1997]. The Novel Food Catalogue (NFC) (http://ec.europa.eu/food/safety/novel_food/catalogue/index_en.htm) lists products of animal and plant origin that are subject to the NFR or are being evaluated in that regard. The information is based on data provided by the EU Member States. It is stated to be a non-exhaustive list and should serve as orientation on whether a product will need an authorisation under the NFR. Analysing the content of the NFC, there are currently (Jun.2016) 6 species of 4 genera mentioned: *Bambusa oldhamii* (listed with the synonym: *Sinocalamus oldhamii*), *Dendrocalamus latiflorus*, *D. asper*, *Gigantochloa albociliata*, *G. levis* and *Phyllostachys edulis*. The immature shoot of these species is used as food substance and according to the NFC none of them are subject to the NFR. Additionally there exists an entry for *Bambusa* species with a status indicating that history of use as a food of bamboo leaves is not known to any Member State and thus, bamboo leaves, if they were to be used as a food might be subject to the NFR and require a safety assessment before they may be placed on the market. According to this statement, based on current scientific data, the leaves of over 1600 species of the *Bambusoideae* (*Poaceae*), if used as "substance" in tea, put corresponding products in violation of the NFR.

498 The same is most likely true for leaves of *Dianthus* species, particularly of the species *D. chinensis*
499 which we found in tea products in place of genuine bamboo leaves. Due to their application in traditional Chinese medicine and contraindications for pregnant women, the admissibility as food has to be questioned.

502 Concluding, the use of the term bamboo for product components has several disadvantages. Firstly, a false impression of identity is promoted. Although the corresponding taxonomic entity has been shown to be monophyletic and offers unique characteristics, the contained morphological diversity deserves recognition beyond the subfamily rank. Secondly, the systematically broad range of the term may be perceived as ignorance and promote intentional adulteration or may lead to additional accidental confusions caused by lack of clarity. Any scientific approach for the safety assessment of botanicals and botanical preparations needs precision in regard of the corresponding taxonomy. Using a too broad approach always will prove to be negligent and impede precise diagnostics. Experience tells us, that we cannot identify all natural units with little effort. To be able to differentiate on a level where genetic markers show coherence between the unit and its inherited chemical profiles - which ultimately is the empirical dimension used to assess safety - systematic knowledge is of primary importance.

513 ACKNOWLEDGMENTS

514 We applied the SDC approach for the sequence of authors [Tschardt et al., 2007]. We acknowledge
515 support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of Karlsruhe Institute of
516 Technology. We also thank the garden staff of the Karlsruhe Institute of Technology for taking good care
517 of the reference plants and the trainees of the botanical institute for their contributions to this work. Lastly
518 we acknowledge the contributions of Esther Huber with her bachelor thesis [Huber, 2014] about bamboo
519 and its use as food.

520 REFERENCES

- 521 Alvarez, J. M., Rocha, J. F., and Machado, S. R. (2008). Bulliform cells in *Loudetiopsis chrysothrix*
522 (Nees) Conert and *Tristachya leiostachya* Nees (Poaceae): Structure in relation to function. *Brazilian*
523 *Archives of Biology and Technology*, 51(1):113–119.
- 524 Beal, W. J. (1886). The Bulliform or Hygroscopic Cells of Grasses and Sedges Compared. *Botanical*
525 *Gazette*, 11(12):321–326.

- 526 Bertolazzi, P., Felici, G., and Weitschek, E. (2009). Learning to classify species with barcodes. *BMC*
527 *bioinformatics*, 10 Suppl 1:S7.
- 528 Cai, Z. M., Zhang, Y. X., Zhang, L. N., Gao, L. M., and Li, D. Z. (2012). Testing four candidate barcoding
529 markers in temperate woody bamboos (Poaceae: Bambusoideae). *Journal of Systematics and Evolution*,
530 50(6):527–539.
- 531 CBOL Plant Working Group (2009). A DNA barcode for land plants. *Proceedings of the National*
532 *Academy of Sciences*, 106(31):12794–12797.
- 533 Chandra, S. and Rawat, D. (2015). Medicinal plants of the family Caryophyllaceae: a review of ethno-
534 medicinal uses and pharmacological properties. *Integrative Medicine Research*, 4(3):123–131.
- 535 Chaw, S. M., Chang, C. C., Chen, H. L., and Li, W. H. (2004). Dating the monocot-dicot divergence
536 and the origin of core eudicots using whole chloroplast genomes. *Journal of Molecular Evolution*,
537 58(4):424–441.
- 538 Committee, C. P. (2010). Chinese pharmacopoeia. *China Medica Science Press: Beijing, China*.
- 539 Dnyaneshwar, W., Preeti, C., Kalpana, J., and Bhushan, P. (2006). Development and application of
540 RAPD-SCAR marker for identification of *Phyllanthus emblica* LINN. *Biological & pharmaceutical*
541 *bulletin*, 29(November):2313–2316.
- 542 Dong, W., Cheng, T., Li, C., Xu, C., Long, P., Chen, C., and Zhou, S. (2014). Discriminating plants
543 using the DNA barcode rbcLb: An appraisal based on a large data set. *Molecular Ecology Resources*,
544 14(2):336–343.
- 545 Edgar, R. C. (2004a). MUSCLE: a multiple sequence alignment method with reduced time and space
546 complexity. *BMC bioinformatics*, 5(1):113.
- 547 Edgar, R. C. (2004b). MUSCLE: multiple sequence alignment with high accuracy and high throughput.
548 *Nucleic acids research*, 32(5):1792–7.
- 549 European Commission, T. (1997). Regulation (EC) No 258/97 of the European Parliament and of the
550 Council of 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal of the*
551 *European Communities*, L43(14/02/97):1–6.
- 552 European Commission, T. (2002). Regulation (EC) No 178/2002 of the European Parliament and
553 the Council of 28 January 2002 laying down the general principles and requirements of food law,
554 establishing the European Food Safety Authority and laying down procedures in matters of food safety.
555 *Official Journal of the European Communities*, L31(01/02/2002):1–24.
- 556 Farrelly, D. (1984). *The book of bamboo: a comprehensive guide to this remarkable plant, its uses, and*
557 *its history*. Sierra Club Books.
- 558 Federici, S., Galimberti, A., Bartolucci, F., Bruni, I., De mattia, F., Cortis, P., and Labra, M. (2013).
559 DNA barcoding to analyse taxonomically complex groups in plants: The case of *Thymus* (Lamiaceae).
560 *Botanical Journal of the Linnean Society*, 171:687–699.
- 561 Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*,
562 39(4):783–791.
- 563 Han, J., Huang, M., Wang, Z., Zheng, Y., Zeng, G., He, W., and Tan, N. (2015). Cyclopentapeptides
564 from *Dianthus chinensis*. *Journal of peptide science : an official publication of the European Peptide*
565 *Society*, 21(7):550–3.
- 566 Han, J., Wang, Z., Zheng, Y.-Q., Zeng, G.-Z., He, W.-J., and Tan, N.-H. (2014). New dicyclopeptides
567 from *Dianthus chinensis*. *Yao xue xue bao = Acta pharmaceutica Sinica*, 49(5):656–60.
- 568 Hebert, P. D. N., Cywinska, A., Ball, S. L., and DeWaard, J. R. (2003). Biological identifications through
569 DNA barcodes. *Proceedings. Biological sciences / The Royal Society*, 270(February 2003):313–321.
- 570 Hohmann, B. and Gassner, G. (2007). *Mikroskopische Untersuchung pflanzlicher Lebensmittel und*
571 *Futtermittel : der Gassner*. Behr's Verlag, Hamburg, 6. Aufl. edition.
- 572 Hong-Yu, L., Koike, K., and Ohmoto, T. (1994). Triterpenoid saponins from *Dianthus chinensis*.
573 *Phytochemistry*, 35(3):751–756.
- 574 Horn, T., Barth, A., Rühle, M., Häser, A., Jürges, G., and Nick, P. (2012). Molecular diagnostics of
575 Lemon Myrtle (*Backhousia citriodora* versus *Leptospermum citratum*). *European Food Research and*
576 *Technology*, 234(5):853–861.
- 577 Horn, T., Völker, J., Rühle, M., Häser, A., Jürges, G., and Nick, P. (2013). Genetic authentication by
578 RFLP versus ARMS? The case of Moldavian dragonhead (*Dracocephalum moldavica* L.). *European*
579 *Food Research and Technology*, 238(1):93–104.
- 580 Hsieh, P.-W., Chang, F.-R., Wu, C.-C., Wu, K.-Y., Li, C.-M., Chen, S.-L., and Wu, Y.-C. (2004). New

- 581 cytotoxic cyclic peptides and dianthramide from *Dianthus superbus*. *Journal of natural products*,
582 67(9):1522–7.
- 583 Hsu, H., Chen, Y., Sheu, S., and Hsu, C. (1986). *Oriental material medica - a concise guide*. Oriental
584 Healing Arts Institute.
- 585 Huber, E. (2014). Molekulare Authentifizierung von Bambus als Novel Food. Technical report.
- 586 Huh, M. K. and Bang, K. H. (2006). Identification of *Atractylodes japonica* and *A. macrocephala* by
587 RAPD analysis and SCAR markers. *Silvae Genetica*, 55(1983):101–105.
- 588 Jäger, E., Ebel, F., Hanelt, P., and Müller, G. (2008). *Rothmaler Band 5. Exkursionsflora von Deutschland*.
589 *Krautige Zier- und Nutzpflanzen*. Spektrum Akademischer Verlag.
- 590 Jiao, S.-D. (2003). *Ten Lectures on the Use of Medicinals from the Personal Experience of Jiao Shu De*.
591 Paradigm Publications.
- 592 Katoh, K. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier
593 transform. *Nucleic Acids Research*, 30(14):3059–3066.
- 594 Katoh, K. and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7:
595 improvements in performance and usability. *Molecular biology and evolution*, 30(4):772–80.
- 596 Kress, W. J. and Erickson, D. L. (2007). A Two-Locus Global DNA Barcode for Land Plants: The Coding
597 *rbcl* Gene Complements the Non-Coding *trnH-psbA* Spacer Region. *PLoS ONE*, 2(6):e508.
- 598 Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., and Bermingham, E.
599 (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama.
600 *Proceedings of the National Academy of Sciences of the United States of America*, 106(44):18621–
601 18626.
- 602 Lee, M. Y., Doh, E. J., Park, C. H., Kim, Y. H., Kim, E. S., Ko, B. S., and Oh, S.-E. (2006). Development
603 of SCAR marker for discrimination of *Artemisia princeps* and *A. argyi* from other *Artemisia* herbs.
604 *Biological & pharmaceutical bulletin*, 29(April):629–633.
- 605 Li, M., Wunder, J., Bissoli, G., Scarponi, E., Gazzani, S., Barbaro, E., Saedler, H., and Varotto, C. (2008).
606 Development of COS genes as universally amplifiable markers for phylogenetic reconstructions of
607 closely related plant species. *Cladistics*, 24(5):727–745.
- 608 Li, X., Ding, X., Chu, B., Ding, C., Gu, S., Qian, L., Wang, Y., and Zhou, Q. (2007). Molecular
609 authentication of *Alisma orientale* by PCR-RFLP and ARMS. *Planta Medica*, 73:67–70.
- 610 Liese, W. (2015). *Bamboo: The Plant and its Uses*. Tropical Forestry. Springer, Cham.
- 611 Liu, H., Guo, X., Wu, J., Chen, G. B., and Ying, Y. (2013). Development of universal genetic markers
612 based on single-copy orthologous (COSII) genes in Poaceae. *Plant Cell Reports*, 32(3):379–388.
- 613 Marieschi, M., Torelli, A., Poli, F., Bianchi, A., and Bruni, R. (2010). Quality control of commercial
614 Mediterranean oregano: Development of SCAR markers for the detection of the adulterants *Cistus*
615 *incanus* L., *Rubus caesius* L. and *Rhus coriaria* L. *Food Control*, 21(7):998–1003.
- 616 Motomura, H., Fujii, T., and Suzuki, M. (2004). Silica deposition in relation to ageing of leaf tissues in
617 *Sasa veitchii* (Carrière) Rehd. (Poaceae: Bambusoideae). *Annals of Botany*, 93(3):235–248.
- 618 Nei, M. and Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press.
- 619 Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., and Ragupathy, S. (2013). DNA
620 barcoding detects contamination and substitution in North American herbal products. *BMC medicine*,
621 11(1):222.
- 622 Newton, C. R., Graham, a., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C., and
623 Markham, a. F. (1989). Analysis of any point mutation in DNA. The amplification refractory mutation
624 system (ARMS). *Nucleic acids research*, 17(7):2503–2516.
- 625 Oshima, Y., Ohsawa, T., and Hikino, H. (1984). Structures of Dianosides G, H and I, Triterpenoid
626 Saponins of *Dianthus superbus* var. *longicalycinus* Herbs1. *Planta medica*, 50(3):254–258.
- 627 Peng, S., Yang, H. Q., and Li, D. Z. (2008). Highly heterogeneous generic delimitation within the
628 temperate bamboo clade (Poaceae: Bambusoideae): Evidence from GBSSI and ITS sequences. *Taxon*,
629 57(3):799–810.
- 630 Rambaut, A. (2014). Tree Figure Drawing Tool.
- 631 Rohweder, O., Schlumpf, R., and Krattinger, K. (1971). Anmerkungen zum diacytischen Spaltöff-
632 nungstyp und zur taxonomischen Bedeutung der Spaltöffnungen im allgemeinen. *Mitteilungen aus*
633 *dem Botanischen Museum der Universität Zürich*, 5(255):275–285.
- 634 Roy, S., Tyagi, A., Shukla, V., Kumar, A., Singh, U. M., Chaudhary, L. B., Datt, B., Bag, S. K., Singh,
635 P. K., Nair, N. K., Husain, T., and Tuli, R. (2010). Universal plant DNA barcode loci may not work in

- 636 complex groups: A case study with Indian berberis species. *PLoS ONE*, 5(10):e13674.
- 637 Sasaki, Y., Komatsu, K., Takido, M., Takeshita, K., Kashiwagi, H., and Nagumo, S. (2007). Genetic
638 profiling of Sasa species by analysis of chloroplast intron between rbcL and ORF106 and partial
639 ORF106 regions. *Biological & pharmaceutical bulletin*, 30(8):1511–1515.
- 640 Seberg, O. and Petersen, G. (2009). How many loci does it take to DNA barcode a crocus? *PLoS ONE*,
641 4(2):2–7.
- 642 Soltis, P. S., Soltis, D. E., and Smiley, C. J. (1992). An rbcL sequence from a Miocene Taxodium
643 (bald cypress). *Proceedings of the National Academy of Sciences of the United States of America*,
644 89(January):449–451.
- 645 Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., Filgueiras,
646 T. S., Davis, J. I., and Morrone, O. (2015). A worldwide phylogenetic classification of the Poaceae
647 (Gramineae). *Journal of Systematics and Evolution*, 53(2):117–137.
- 648 Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermet, T., Corthier, G.,
649 Brochmann, C., and Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron
650 for plant DNA barcoding. *Nucleic Acids Research*, 35(3):e14.
- 651 Tamura, K., Stecher, G., Peterson, D., Filipiński, A., and Kumar, S. (2013). MEGA6: Molecular Evolution-
652 ary Genetics Analysis version 6.0. *Molecular biology and evolution*, 30(12):2725–9.
- 653 Torelli, A., Marieschi, M., and Bruni, R. (2014). Authentication of saffron (*Crocus sativus* L.) in different
654 processed, retail products by means of SCAR markers. *Food Control*, 36(1):126–131.
- 655 Triplett, J. K. and Clark, L. G. (2010). Phylogeny of the Temperate Bamboos (Poaceae: Bambusoideae:
656 Bambuseae) with an Emphasis on Arundinaria and Allies. *Systematic Botany*, 35(1):102–120.
- 657 Tschardt, T., Hochberg, M. E., Rand, T. A., Resh, V. H., and Krauss, J. (2007). Author sequence and
658 credit for contributions in multi-authored publications. *PLoS biology*, 5(1):e18.
- 659 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. (2012).
660 Primer3 - new capabilities and interfaces. *Nucleic acids research*, 40(15):e115.
- 661 Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., and Leunissen, J. A. M. (2007).
662 Primer3Plus - an enhanced web interface to Primer3. *Nucleic acids research*, 35(Web Server issue):71–
663 4.
- 664 Veller, C., Nowak, M. A., and Davis, C. C. (2015). Extended flowering intervals of bamboos evolved by
665 discrete multiplication. *Ecology Letters*, 18(7):653–659.
- 666 Vieira, R. C., Gomes, D. M. S., Sarahyba, L. S., and Arruda, R. C. O. (2002). Leaf anatomy of three
667 herbaceous bamboo species. *Brazilian Journal of Biology*, 62(4b):907–922.
- 668 Wang, C. Z., Li, P., Ding, J. Y., Peng, X., and Yuan, C. S. (2007). Simultaneous identification of *Bulbus*
669 *Fritillariae cirrhosae* using PCR-RFLP analysis. *Phytomedicine*, 14:628–632.
- 670 Wang, H., Sun, H., Kwon, W. S., Jin, H., and Yang, D. C. (2010). A PCR-based SNP marker for specific
671 authentication of Korean ginseng (*panax ginseng*) cultivar "Chunpoong". *Molecular Biology Reports*,
672 37:1053–1057.
- 673 Wang, J., Ha, W.-Y., Ngan, F.-N., But, P. P.-H., and Shaw, P.-C. (2001). Application of Sequence
674 Characterized Amplified Region (SCAR) Analysis to Authenticate *Panax* Species and Their Adulterants.
675 *Planta medica*, 67:781–783.
- 676 Ward, J., Gilmore, S. R., Robertson, J., and Peakall, R. (2009). A grass molecular identification system
677 for forensic botany: A critical evaluation of the strengths and limitations. *Journal of Forensic Sciences*,
678 54(6):1254–1260.
- 679 Watanabe, M., Ito, M., and Kurita, S. (1994). Chloroplast DNA phylogeny of Asian Bamboos (Bambu-
680 soideae, Poaceae) and its systematic implication. *Journal of Plant Research*, 107(3):253–261.
- 681 Weitschek, E., Van Velzen, R., Felici, G., and Bertolazzi, P. (2013). BLOG 2.0: A software system
682 for character-based species classification with DNA Barcode sequences. What it does, how to use it.
683 *Molecular Ecology Resources*, 13:1043–1046.
- 684 White, T. J., Bruns, S., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal
685 ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*,
686 pages 315–322. Academic Press, Inc.
- 687 Wong, K.-L., But, P. P.-H., and Shaw, P.-C. (2013). Evaluation of seven DNA barcodes for differentiating
688 closely related medicinal *Gentiana* species and their adulterants. *Chinese medicine*, 8(1):16.
- 689 Wu, C. (1962). The classification of Bambuseae based on leaf anatomy. *Bot. Bull. Acad. Sinica.(NS)*,
690 3:83–108.

- 691 Wu, J. N. (2005). *An Illustrated Chinese Materia Medica*. Oxford University Press.
- 692 Wu, Z., Raven, P., and Hong, D. (2001). Flora of China. Vol. 6: Caryophyllaceae through Lardizabalaceae.
693 *Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.*
- 694 Wu, Z., Raven, P., and Hong, D. (2006). Flora of China. Vol. 22: Poaceae. *Science Press, Beijing, and*
695 *Missouri Botanical Garden Press, St. Louis.*
- 696 Wu, Z., Raven, P., and Hong, D. (2007). Flora of China. Vol. 12: Hippocastanaceae through Theaceae.
697 *Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.*
- 698 Yang, D.-Y., Fushimi, H., Cai, S.-Q., and Komatsu, K. (2004). Polymerase chain reaction-restriction
699 fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system (ARMS)
700 analyses of medicinally used Rheum species and their application for identification of Rhei Rhizoma.
701 *Biological & pharmaceutical bulletin*, 27(5):661–669.
- 702 Yang, H.-Q., Yang, J.-B., Peng, Z.-H., Gao, J., Yang, Y.-M., Peng, S., and Li, D.-Z. (2008). A molecular
703 phylogenetic and fruit evolutionary analysis of the major groups of the paleotropical woody bamboos
704 (Gramineae: Bambusoideae) based on nuclear ITS, GBSSI gene and plastid trnL-F DNA sequences.
705 *Molecular phylogenetics and evolution*, 48(3):809–24.
- 706 Yang, J. B., Yang, H. Q., Li, D. Z., Wong, K. M., and Yang, Y. M. (2010). Phylogeny of Bambusa and its
707 allies (Poaceae: Bambusoideae) inferred from nuclear GBSSI gene and plastid psbA-trnH, rpl32-trnL
708 and rps16 intron DNA sequences. *Taxon*, 59(4):1102–1110.
- 709 Zhang, W. and Clark, L. (2000). Phylogeny and classification of the Bambusoideae (Poaceae). *Grasses:*
710 *systematics and evolution*.
- 711 Zhang, W., Wendel, J. F., and Clark, L. G. (1997). Bamboozled again! Inadvertent isolation of fungal
712 rDNA sequences from bamboos (Poaceae: Bambusoideae). *Molecular phylogenetics and evolution*,
713 8(2):205–17.
- 714 Zhang, Y.-J., Ma, P.-F., and Li, D.-Z. (2011). High-throughput sequencing of six bamboo chloroplast
715 genomes: phylogenetic implications for temperate woody bamboos (Poaceae: Bambusoideae). *PloS*
716 *one*, 6(5):e20596.
- 717 Zhang, Y.-X., Zeng, C.-X., and Li, D.-Z. (2012). Complex evolution in Arundinarieae (Poaceae: Bambu-
718 soideae): incongruence between plastid and nuclear GBSSI gene phylogenies. *Molecular phylogenetics*
719 *and evolution*, 63(3):777–97.
- 720 Zhen, Z. (1995). *Advanced Textbook on Traditional Chinese Medicine and Pharmacology: History, Basic*
721 *Theory, Diagnostics*. New World Press.