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Adulteration of herbal products: Bamboo tea authentication

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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

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ABSTRACT

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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 proof 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
used for construction, furniture and paper production. Domestically it is used as tool (e.g. farming, hunting, fishing, eating, weaving). The leaves of *Gelidocalamus latifolius* and *Indocalamus* species are used to wrap glutinous rice [Wu et al., 2006], those of broad-leaved species (e.g. *Sasa* species) are cut during the first 5 weeks, cleaned, dried, roasted and used for bamboo tea. For ages bamboo tea has been considered a delicious and healthy drink in the bamboo countries and is now spreading to other regions (e.g. Europe). It contains neither theine nor caffeine and is rich in protein, calcium, iron, magnesium and recommended for various pharmaceutical applications, particularly stomach pain [Liese 2015]. In Japan the leaves of *Sasa* plants (*S. palmata*, *S. senanensis* and rarely *S. yahikoensis* and *S. kurilensis*), which are called “Kuma-zasa”, have been used to treat burns or urinary hesitancy [Sasaki et al., 2007]. In China and Indonesia leaves of different species of *Bambusa*, *Phyllostachys*, *Fargesia* and *Indocalamus* are used for medicinal purposes [http://www.bamboocentral.org/pharmacopoeia.html].

According to Subhuti Dharmananda (http://www.itmonline.org/arts/bamboo.htm, Hsu et al. [1986], Zhen [1995]) the most frequently used leaves in Chinese herbal medicine are collected from the grass bamboo (*Lophatherum gracile*). It is also mentioned that the leaf of the black bamboo (*Phyllostachys nigra*) and of the grass bamboo is often confused both in China and the West [Jiao 2003]. Taxonomically, there are 1’641 bamboo species, 120 genera and 3 tribes [Soreng et al., 2015] making up the subfamily *Bambusoideae* (*Poaceae*). In appearance bamboos are either “woody” (lignified) or herbaceous. The first group can be divided into two distinct lineages - the temperate (*Arundinariae*) and tropical (*Bambuseae*) "woody" bamboos. Nested between the "woody" tribes are the herbaceous bamboos (*Olyreae*). Strictly speaking, the earlier mentioned grass bamboo (*Lophatherum gracile*) is not a bamboo. Instead it belongs to another *Poaceae* subfamily (*Panicoideae*), which also harbours members from the genus *Cymbopogon* (lemongrass), another common herbal tea ingredient.

**Identification of Herbal Product Components**

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

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

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

**Aim**

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

Reference Plants and Commercial Samples

Specimens of bamboo (Bambusoideae, table 1), lemongrass and Carnation (Cymbopogon and Dianthus, supplementary table 2) were acquired and cultivated in the botanical garden of the Karlsruhe Institute of Technology. Bambusoideae and Cymbopogon specimens were identified to genus level [Farrelly, 1984, Wu et al., 2006, 2007]. At least one specimen of each Dianthus species was identified to species level using morphological markers [Wu et al., 2001, Jäger et al., 2008]. Specimen details including 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).

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).

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

Morphological and Anatomical Evaluations

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

DNA based Evaluations

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

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

<table>
<thead>
<tr>
<th>Acc</th>
<th>Type</th>
<th>Leaf Component(s)</th>
<th>rbcLa</th>
<th>rbcLb</th>
<th>matK-KIM</th>
<th>ITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>FT</td>
<td>bamboo, lemongras</td>
<td>KU722894</td>
<td>KU722852</td>
<td>KU722866</td>
<td>KU722880</td>
</tr>
<tr>
<td>P2</td>
<td>FT</td>
<td>bamboo</td>
<td>KU722893</td>
<td>KU722851</td>
<td>KU722865</td>
<td>KU722879</td>
</tr>
<tr>
<td>P3</td>
<td>FT</td>
<td>bamboo</td>
<td>KU722891</td>
<td>KU722849</td>
<td>KU722863</td>
<td>KU722877</td>
</tr>
<tr>
<td>P4</td>
<td>SC</td>
<td>bamboo whole leaf</td>
<td>KU722892</td>
<td>KU722850</td>
<td>KU722864</td>
<td>KU722878</td>
</tr>
<tr>
<td>P5a</td>
<td>SC</td>
<td>bamboo</td>
<td>KX233507</td>
<td>KX233494</td>
<td>KX233503</td>
<td>-</td>
</tr>
<tr>
<td>P6</td>
<td>FT</td>
<td>bamboo</td>
<td>KX233506</td>
<td>KX233493</td>
<td>KX233502</td>
<td>-</td>
</tr>
<tr>
<td>P7</td>
<td>FT</td>
<td>bamboo, lemongras</td>
<td>KX233505</td>
<td>KX233492</td>
<td>KX233501</td>
<td>-</td>
</tr>
<tr>
<td>P8a</td>
<td>SC</td>
<td>bamboo</td>
<td>KX233508</td>
<td>KX233495</td>
<td>KX233504</td>
<td>-</td>
</tr>
</tbody>
</table>

*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.

Phylogenetic Diagnostics

To assess the diagnostic power of the used marker regions in a phylogenetic framework, we combined reference plant sequences with sequences of relevant taxonomic groups retrieved from Genbank (supplementary table 3 and 4). Sequence collections of each marker were aligned (coding regions: [Edgar 2004a,b], ITS: [Katoh 2002], [Katoh and Standley 2013] with L-INS-i), primer sites removed and trimmed using reference plant sequences. Subsequently each dataset was evaluated for its information content (alignment length, variable positions, parsimony information and singleton sites) and phylogenetic trees were computed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms implemented in MEGA6 [Tamura et al., 2013]. For UPGMA the evolutionary distances were computed using the p-distance method [Nei and Kumar, 2000] with all ambiguous positions removed for each sequence pair. The MP tree was obtained considering all sites using the Subtree-Pruning-Regrafting (SPR) algorithm [Nei and Kumar, 2000] with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The evolutionary history inferred by using the ML method was based on substitution models in combination with evolutionary rate differences among sites that had the lowest BIC (Bayesian Information Criterion) scores determined by analysing each dataset using MEGA6. Details are summarized in table 3. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining (NJ) method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. All trees were bootstrapped [Felsenstein 1985] using 500 replicates. Additionally, we computed UPGMA, MP and ML trees using concatenated datasets (rbcL = rbcLa and rbcLb; r+m = rbcLa, rbcLb and matK-KIM). The results were analysed by first collapsing branches corresponding to partitions reproduced in less than 50% bootstrap replicates and recording bootstrap support values for relevant monophyletic groups (sensu [Soreng et al., 2015]). All datasets and trees have been deposited.
in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S19113). For representation the dataset 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

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Model</th>
<th>Rates</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbcLa</td>
<td>K2</td>
<td>+G</td>
<td>5217</td>
</tr>
<tr>
<td>rbcLb</td>
<td>T92</td>
<td>+G</td>
<td>7177</td>
</tr>
<tr>
<td>rbcL</td>
<td>T92</td>
<td>+G</td>
<td>9380</td>
</tr>
<tr>
<td>matK-KIM</td>
<td>GTR</td>
<td>+G</td>
<td>10829</td>
</tr>
<tr>
<td>r+m</td>
<td>GTR</td>
<td>+G</td>
<td>19223</td>
</tr>
</tbody>
</table>

**Character based Diagnostics**

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

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

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

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

**RESULTS**

**Anatomical Evaluation**

Morphology as the study of forms visible to the unaided eye, in food diagnostics is complemented by anatomy, the study of cellular structures. For an intermediate between morphology and anatomy, in this study we used the term "macroscopic". The magnification used does not yet allow to observe cellular
structures in detail, but eases the study of their morphological manifestations. Both, microscopic and macroscopic anatomy are common techniques used in food diagnostics \cite{Hohmann and Gassner, 2007}.

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

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

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

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

**DNA based Evaluation**

All three cytoplasmic markers were retrieved with great success regarding PCR and sequencing results. ITS however turned out to be particularly problematic with bamboo samples. Preferential and co-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 (tesselation) 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.

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

General Assessments

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

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

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

Sequence data of adulterated (P1 - P4) and genuine (P5 - P8) bamboo products as well as all reference 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.

<table>
<thead>
<tr>
<th>Bamboo</th>
<th>Marker</th>
<th>Len</th>
<th>Con</th>
<th>Var</th>
<th>%</th>
<th>PaI</th>
<th>%</th>
<th>Sin</th>
<th>%</th>
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<td>761</td>
<td>53</td>
<td>6.5</td>
<td>26</td>
<td>49.1</td>
<td>27</td>
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<td>35</td>
<td>50.7</td>
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<td>49.3</td>
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<tr>
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<td>748</td>
<td>89</td>
<td>10.6</td>
<td>40</td>
<td>44.9</td>
<td>49</td>
<td>55.1</td>
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<tr>
<td>r+m</td>
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<td>1805</td>
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<td>8.0</td>
<td>75</td>
<td>47.5</td>
<td>83</td>
<td>52.5</td>
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<table>
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<th>Con</th>
<th>Var</th>
<th>%</th>
<th>PaI</th>
<th>%</th>
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<td>40.0</td>
<td>87</td>
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</table>

, e extended dataset

Phylogenetic Analysis

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

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

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

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

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

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

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

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

Phylogenetic Representation: Using the combined cytoplasmic dataset with sequences recovered 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.

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

**Diagnostic Analysis**

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

**Assessment of Diagnostic Potential:** The evaluation of bambusoid tribe classification using BLOG shows consistency among markers. Only the Arundinariae tribe shows 4% false negative classifications 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.

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

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

Comparing matK-KIM and ITS datasets for Dianthus (Figure 6B) shows the inability to distinguish D. chinensis and D. longicalyx based on matK-KIM data. Using ITS, information content increases enough to diagnose D. chinensis with a unique LOGic formula (pos181=g AND pos595=c) that also 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 (\(< 10 \times\)) 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 wavyness of the walls of upper and lower epidermal cells in some species is different, while in other species the wavyness 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)
also can contribute to a diagnostic evaluation but appear not to be exclusively distributed in one particular

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

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

DNA based Evaluations

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

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

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

Using the highly universal DNA Barcoding marker rbcLa, we introduced a PCR based diagnostic
solution for the detection of an adulterant of bamboo tea (carnation). Using 85 ITS sequences retrieved
from reference plants and Genbank in a character based DNA Barcoding approach, the classification pattern
of D. chinensis was also found in sequences obtained from adulterant samples P1 - P4. The diagnostic
solution also includes the differentiation of the two Poaceae tea components (bamboo and lemongrass).
To improve the significance (i.e. taxonomic depth) of the genetic test, other markers need to be evaluated.
While ITS has been used in bamboo [Cai et al., 2012], fungal contamination and ITS paralogs decrease the
applicability of this marker considerably. Other available DNA markers are for example GBSSI [Zhang
et al., 2012], Yang et al., 2008, 2010 [Peng et al., 2008] and COS [Li et al., 2008, Liu et al., 2013]. To
improve the robustness of the test, sampling within the temperate bamboo genera needs to be increased.
Furthermore, the genetic test could be improved by optimizing reaction conditions for the combined use
of more than one diagnostic primer.
What is Bamboo Tea? According to the NCBI Taxonomy the common name for the tribe Bambuseae is bamboo. This reflects an old systematic opinion [Zhang and Clark [2000] when Bambuseae still contained most Arundinariae species (e.g. Sasa and Phyllostachys). However, the most recent scientific usage of the term bamboo is found in [Soreng et al. [2015] where bamboo is the common name for the subfamily Bambusoideae (Poaceae). This group is characterized by high morphological diversity that appears not to be discretely associated with subordinate taxonomic entities. The reasons are believed to be related to morphological inter-gradation interpreted in various ways and the presence of hybrids that have been stabilized through clonal propagation [Tripplett and Clark [2010]. The taxonomic confusion within the group also is related to a peculiarity of the reproduction mode of bamboo. While most flowering plants are flowering regularly each year, bamboo is one of the groups where dramatically extended intervals exist - some as long as 120 years [Veller et al. [2015; Liese [2015].

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

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

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

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

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

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

Legal Scientific Framework: Article 2 of the European General Food Law Regulation [European Commission [2002] specifies “food” as any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. Tea products analysed in this study either consist of different “substances”, one of which is “bamboo leaf”, or only contain the latter. Consulting the List of Substances of the Competent Federal Government and Federal State Authorities (german version) for the category “plants and plant parts”, common names (e.g. apple, lemon and orange) used in ingredient lists of teas are found and mapped to the scientific name of the
corresponding plant the "substance" (e.g. fruit) is derived from. The common name bamboo can be mapped to two species of Dendrocalamus (D. asper and D. latiflorus) which are the source for bamboo sprouts. No other entries for bamboo are present. The English version of the mentioned list does not provide associations of common names with scientific names, representing one common unnecessary obstacle consumers and food business operators are confronted with. Since bamboo is an exotic group, we have to assume that corresponding substances used in products fall under the novel food legislation and might be listed in the novel food catalogue.

Foods or food ingredients which have not been used for human consumption to a significant degree in the European Union (EU) before 15 May 1997 are governed by the provisions of the Novel Food 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.

The same is most likely true for leaves of Dianthus species, particularly of the species D. chinensis 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.

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 proof 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.

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