Eur Food Res Technol (2012) 234:853–861
DOI 10.1007/s00217-012-1688-9

ORIGINAL PAPER

Molecular diagnostics of Lemon Myrtle (*Backhousia citriodora* versus *Leptospermum citratum*)

Thomas Horn · Anna Barth · Michael Rühle · Annette Häser · Gabriele Jürges · Peter Nick

Received: 20 October 2011 / Revised: 1 February 2012 / Accepted: 8 February 2012 / Published online: 29 February 2012
© Springer-Verlag 2012

Abstract 'Lemon Myrtle' is becoming increasingly popular in Europe both for use in cuisine and phytotherapy. However, this common name covers two completely different species, *Backhousia citriodora* F. Muell. and *Leptospermum citratum* Challinor, Cheel & A.R.Penfold. These species differ with respect to secondary compounds and even can cause, if mixed up and applied in high dose, toxic effects. We describe how the two species can be discriminated microscopically making use of differences in the morphology of leaf pavement cells and the relative size of palisade parenchyma. Based on the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (rbcL) as molecular marker, the phylogenetic position of the two species within the Myrtaceae could be clarified. This sequence information was used to develop a simple assay to discriminate the two species even in dried and highly fragmented mixtures as typically occurring in commercial samples. This assay utilises the occurrence of single-nucleotide exchanges between those species that produce different fragments when the rbcL amplificates are restricted with *Sac* II.

Keywords *Backhousia citriodora* F.Muell · Lemon Myrtle · *Leptospermum citratum* Challinor, Cheel & A.R.Penfold · Molecular identification · Polymerase chain reaction (PCR) · Ribulose-bisphosphate carboxylase oxygenase large subunit (*rbcL*)

T. Horn · A. Barth · M. Rühle · A. Häser · G. Jürges · P. Nick (✉)
Molecular Cell Biology, Botanical Institute, Karlsruhe Institute of Technology, Kaiserstr. 2, 76128 Karlsruhe, Germany
e-mail: peter.nick@kit.edu

Introduction

As part of the trend for functional foods and dietary/health supplements numerous plant products derived from traditional herbal medicine of foreign cultures enter the European market. In the public perception, herbal products are considered to be 'natural' and therefore a priori harmless since they had been used traditionally. However, this perception ignores the fact that in most cases, there exists a specific cultural and medical tradition that safeguards against undesired side effects, adverse interactions, toxicity, and adulteration. When the use of these plants becomes isolated from this traditional context, health risks may result (reviewed in [1]). Many of these novel herbs are located somewhere at the interface between health and food supplements, and only few are classified as medicines and fall within the remit of the EU Traditional Herbal Medicinal Products Directive. Most herbal preparations that do not have a long historical record of use in the EU, or which do not have a defined medicinal use fall under the legislation of the Novel-Food-Regulation of the European Union [2]. Such food products require an explicit admission, before they can be commercialised. This includes that these products and their components can be unequivocally identified to exclude potential health risks. Thus, robust and reliable diagnostics of these plants and their parts in food preparations is a prerequisite for the control and surveillance of the Novel-Food-Regulation.

Food monitoring has the aim to safeguard consumers against deception and misinformation and is pivotal for consumer trust [3]. The non-standardised traditional nomenclature for novel herbs in combination with the prevalence of dried herbal mixtures poses special challenges to food monitoring. So far, microscopic diagnostics has been the most reliable way to test multi-component specimens such as those typical for herbal mixtures. There exists a

wealth of technical literature that describes and illustrates food plants commonly used in Europe, for example, to assist microscopical diagnostics [4]. However, for most of the introduced novel species, such information is completely lacking. This is not surprising—diagnostic assays of those plants have not been in the focus of the traditional medical systems that use those plants, but mainly deal with the medical and beneficial effects of these herbs.

The economic payoff that can be achieved by trendy herbal preparations, the limitations of supply for these exotic herb, and the difficulty to reliably address these ingredients in food diagnostics provide ideal conditions for the spread of surrogate or adulterated preparations. This problem warrants novel diagnostic methods that allow the identification of the individual components in these herbal mixtures. These methods have to be reliable, versatile, and cost-effective.

An example for the conflict between traditional nomenclature and the difficulty to warrant consumers against potential hazards is the novel trend herb 'Lemon Myrtle' derived from traditional medicine of the Australian Aborigines and presently popularised as 'Queen of the Lemon Herbs' as major ingredient of so called Bushfood Flavours. 'Lemon Myrtle' actually comprises two different species of the Myrtaceae: *Backhousia citriodora* F.Muell (common names 'Lemon Myrtle', 'Lemon Scented Myrtle', 'Lemon Scented Ironwood' [5]), and *Leptospermum citratum* (ex. J.F.Bailey & C.T.White) Challinor, Cheel & A.R.Penfold (the taxonomy of this genus has been revised several times, therefore there exist several traditional synonyms that are still in use: *Leptospermum petersonii* subsp. *petersonii*, *Leptospermum petersonii* F.M.Bailey, and *Leptospermum flavescens var. citratum* J.F.Bailey & C.T.White; common names 'Lemon Myrtle', 'Lemon Tea Tree', 'Lemon Scented Tea Tree' [6]). Both species originate from North West Australia (Queensland) are found as shrubs or small tree and are rich in aromatic oils.

The Australian Aborigines have used 'Lemon Myrtle' for both cooking and healing. The leaves are often used as dried flakes, or in the form of an encapsulated flavour essence for enhanced shelf-life and are used as flavours in shortbread, pasta, and macadamia and vegetable oils. However, the main use is teas, mostly in mixtures. In addition, 'Lemon Myrtle' is used as lemon flavour replacement in milk-based foods, such as cheesecake or ice-cream to avoid curdling associated with acidity of lemon fruits. In addition to its aromatic properties, extracts of 'Lemon Myrtle' possess a strong antimicrobial activity [7], are used as insect repellents [8], cure certain viral diseases such as *molluscum contagiosum* [9] and recently have acquired interest as efficient antifungal compound in the treatment of skin diseases [10] and food protection [11].

Leaves of 'Lemon Myrtle' are unusually rich in aromatic oils (typically up to 5%), with citral as dominating compound with additions of neral, geranial, myrcene, linalool, citronellal, cyclocitral and methyl-heptenone. However, rare chemotypes with a high content of L-citronellal exist as well, at least in *B. citriodora* [12]. Despite the generally positive effects of 'Lemon Myrtle', toxicity on mammalian cells [13], especially skin cells [14] have been reported, depending on concentration and composition of the extract. This underlines the need to control the use of 'Lemon Myrtle' and to discriminate at least between the two species commercialised as 'Lemon Myrtle'. To address not only the common tea mixtures, but also more processed forms of this product, we combined traditional microscopic analysis with molecular identification based on the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (rbcL). This marker has been recently proposed by an international consortium together with the matK marker as identifier for genetic bar coding of plants due to high coverage, sequencing accessibility and discriminative power [15]. Using this marker, we can clearly locate the position of the two species of 'Lemon Myrtle' within the Myrtaceae, and we can develop a simple assay to discriminate the two species even in dried and highly fragmented mixtures as typically occurring in commercial samples by combining PCR with a restriction digest.

Materials and methods

Plant material and samples

Since vegetative plant morphogenesis is variable depending on environmental factors, mainly light quantity and quality, the specimens were cultivated in parallel under identical conditions (substrate Floraton 3, day temperature 18–25 °C, night temperature 15 °C, illumination time 10 h) in the Botanical Garden of the Karlsruhe Institute of Technology. The specimens were purchased from a commercial source (Rühlemanns, Horstedt, Germany), and their identity verified by the morphology of flowers and leaves based on the taxonomic literature [16]. They are maintained as living specimens under the IPEN codes xx-0-UNKAR-2012-1844-Baccit-01 (*B. citriodora* F.Muell) and xx-0-UNKAR-2012-1845-Lepcit-01 (*L. citratum* (ex. J.F.Bailey & C.T.White) Challinor, Cheel & A.R.Penfold). Four commercial tea samples were used for validation of the assay and partially sorted and identified based on the microscopic criteria developed during the current study prior to molecular analysis. The composition of these samples is given in Table 2.

Extraction of genomic DNA

Fresh leaf material (preferably young leaves) was harvested from healthy plants. About 40 mg of the sample were

transferred into a reaction tube (2 ml, Eppendorf) together with one stainless steel bead (diameter 5 mm) and shock frozen in liquid nitrogen. The frozen sample was then ground three times for 15 s at 20 Hz (Tissuelyser, Qiagen, Hildesheim, Germany). After each individual grinding step, the sample was returned into liquid nitrogen to ensure that the powder did not thaw during the extraction. Genomic DNA was extracted using a modified extraction method based on cetyl trimethyl ammonium bromide (CTAB, [17]) using about 25 mg ground and frozen leaf tissue. The powder was complemented with 1-ml pre-warmed extraction buffer (3% w/v CTAB) containing 8 μl/ml mercaptoethanol and incubated for 30 min at 55 °C followed by a centrifugation to remove debris. Subsequently, the sample was digested with proteinase K (55 °C, 30 min), then mixed with 750 μl of chloroform/isoamylalcohol (24:1) and then spun down for 10 min (14,000 g, 25 °C), and the aqueous upper phase (containing the DNA) was transferred into a fresh reaction tube; the DNA was precipitated with 0.65 volumes of isopropanol, collected by centrifugation (10 min, 14,000 g), washed with 70% EtOH and dissolved in 50 μl ddH_2O. The concentration of the eluted DNA was determined photometrically (NanoDrop ND-100, peqlab). The $E260/E280$ of the extracted DNA was between 1.7 and 2.1. The quality of the DNA extracts was controlled by electrophoresis on a 1.5% agarose gel supplemented with 5% v/v of the fluorescent dye SYBR Safe (Invitrogen).

PCR-amplification and restriction digestion of rbcL

A partial sequence of the large subunit of the ribulose-bisphosphate carboxylase gene (rbcL), rbcLa, was amplified by PCR in a 10-μl reaction using 50 ng of genomic DNA as template and a reaction mix containing single-strength buffer (thermopol, NEB), 200 μM mixed dNTPs (NEB), 200 nM of each primer (rbcLa_F/rbcLa_R, Invitrogen), 0.4 units Taq polymerase (NEB) and 10 mg/ml bovine serum albumine (Sigma-Aldrich, Deisenhofen, Germany). The amplificates were separated by electrophoresis in a 1.5% agarose gel and their size verified using a 100-bp DNA ladder (NEB) after fluorescent staining with SYBR Safe (Invitrogen). The amplificates were extracted from the gel using the NucleoSpin® Extract II kit (Macherey–Nagel, Karlsruhe), following the protocol of the producer, and then sequenced (GATC Biotech, Konstanz). The sequences were verified by BLAST search and alignment with related rbcL-sequences (ClustalX, http://www.clustal.org) and are deposited in Genebank under the accession numbers JN676919 (*L. citratum*) and JN676920 (*B. citriodora*). To discriminate the two species, 6 μl of the rbcLa PCR were digested overnight at 37° C in a 25-μl reaction volume consisting of 2.5 μl 10× enzyme buffer (NEB, No. 4), 2.0 μl *Sac* II enzyme (NEB) and 14.5 μl bidestilled water. The digested amplificates were separated by electrophoresis in a 1.5% agarose gel along with a 100-bp DNA ladder as size marker (NEB).

Phylogenetic analysis of the rbcL sequence

The rbcL sequences were used as input for a BLAST search for Myrtaceae rbcL, the retrieved 16 sequences were automatically aligned using the ClustalX algorithm in MEGA 4.0 [18] and the evolutionary relationships were inferred by means of the neighbour-joining algorithm [19] with bootstrap values based on 500 replicates [20]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. All positions (both coding and non-coding) were included; gaps and missing data were eliminated from the dataset.

Light microscopy

Leaves and shoots of all specimens were documented macroscopically (Exilim Z750, Casio) and by stereo microscopy (M420, Leica; Bensheim) equipped with a digital camera (DFC 500, Leica; Bensheim) both in the fresh state and after drying. In addition, tangential hand sections from the adaxial and the abaxial surface of completely developed leaves were brightened with 60% chloral hydrate and then analysed under a light microscope (Axioskop, Zeiss; Jena) equipped with a digital image acquisition system (Axio-Cam, Zeiss; Jena). Cross-sections obtained after fixation for 50 min in paraformaldehyde (4% w/v) in 50 mM 1,4-piperazine-di-ethanesulfonic acid (PIPES) with 3 mM ethylene glycol-bis (β-aminomethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), adjusted to pH 6.8 with NaOH. After fixation, the specimens were washed three times for 10 min in fixation buffer without paraformaldehyde and subsequently dehydrated by a ethanolic series (50, 70, 90, 100, 100%; 30 min in each step). For embedding in LR White (Plano, Wetzlar, Germany), the specimens were led through a series with increasing proportions of LR White over EtOH on an orbital shaker (ratio 1:2 over night, 1:1 for 4 h, 2:1 over night, pure LR White over the day). Subsequently, the LR White embedded specimens were transferred into gelatine capsules used for electron microscopy and were then hardened over night in a drying oven. The embedded specimens were then sectioned on a microtome, collected on a slide and stained by toluidine blue [21], labelling non-lignified cell walls violet.

Results and discussion

Morphological and histological characteristics of *B. citriodora* and *L. citratum*

Plants of the two species can be clearly discriminated by habitus and morphology. Plants of *B. citriodora* show a

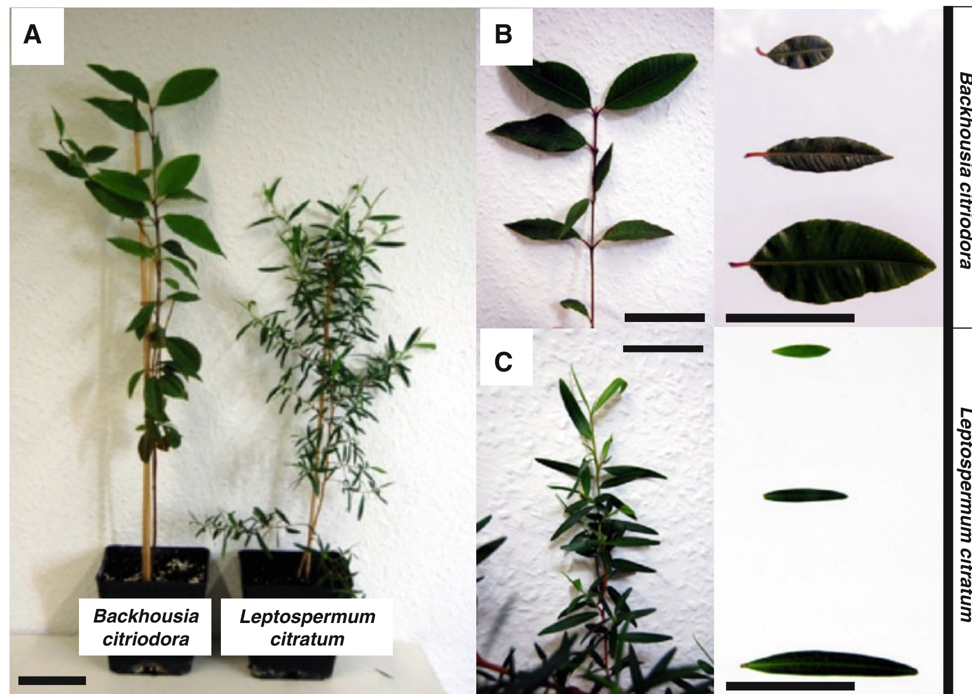

Fig. 1 Habitus of 'Lemon Myrtle'. **a** Young plant of *B. citriodora* and *L. citratum* in comparison. **b** Shoot tip and three leaves illustrating different stages of expansion for *B. citriodora*. **c** as **b** for *L. citratum*. Size bar 50 mm

clearer apical dominance, whereas in *L. citratum* side branches compete with the main axis producing a shrub-like appearance (Fig. 1a). Leaves of *B. citriodora* exhibit a broader lamina, with young leaves being slightly tinted by anthocyanins (Fig. 1b), whereas leaves of *L. citratum* are smaller and narrower with an eliptoidical shape and an intensely green colour (Fig. 1c).

Although it is easy to discern the two species, when whole plants are considered, the discrimination of commercial products based on these species is much more difficult. Both species share typical features of Myrtaceae such as numerous schizogenic oil cavities (Fig. 2c, d), idioblasts, filled with calcium oxalate druses and cells with single calcium oxalate crystals lining the vascular bundles (Table 1). Moreover, leaves of both species are covered with numerous unicellular, unbranched trichomes.

However, the two species can be discriminated from cross-sections of the shoots (Fig. 2c, d). In *B. citriodora*, the cross-section is round. In both cases, numerous oil cavities are embedded in the cortical parenchyma. In *B. citriodora*, the cortical parenchyma is separated into a external layer rich in chloroplasts, and an inner, chloroplast-depleted layer. The cortical parenchyma is subtended by a massive ring of phloëm fibers delineating phloëm, cambium and xylem, surrounding the extensive central pith parenchyma. In contrast, the cross-section of the shoot is triangular in *L. citratum*, and the central pith parenchyma is narrower in relation to the cortical parenchyma outside of the xylem ring.

The leaf of *B. citriodora* is bifacial with one tier of palisade parenchyma, subtended by a conventional spongy parenchyma with large intercellular spaces (Fig. 2a). In contrast, *L. citratum* is equifacial, with two tiers of palisade parenchyme at the upper, adaxial and one tier at the lower, abaxial side of the leaf, separated by one or two tiers of spongy parenchyma (Fig. 2b).

For the discrimination in processed samples, such as dried tea mixtures, the shape of epidermal pavement cells seems to be marker with the best discriminative power (Fig. 3a): In *B. citriodora*, only the lower, abaxial epidermis harbours guard cells. These are anemocytic. Pavement cells are generally puzzle-shaped in both sides of the leaves, polygonoid cells are observed only above vascular bundles. Unicellular, thick-walled trichomes are found in both leaf sides, whereby in the abaxial side, additionally, long hairs are found, in the adaxial side, trichomes appear slightly twisted (Fig. 3b). In *L. citratum*, pavement cells are always polygonoid and unlobed on both sides of the leaf (Fig. 3a). In addition, distinct cuticular folds are observed on both sides of the leaf that are absent in *B. citriodora*. Again, unicellular, thick-walled and slightly bent trichomes can be found on both leaf sides. However, here, the lower, abaxial face of the leaf the trichomes are less abundant as compared to the upper, adaxial face (Fig. 3b). Moreover, the long hairs met in *B. citriodora* are absent in *L. citratum*. A summary of the discriminative traits is given in Table 1.

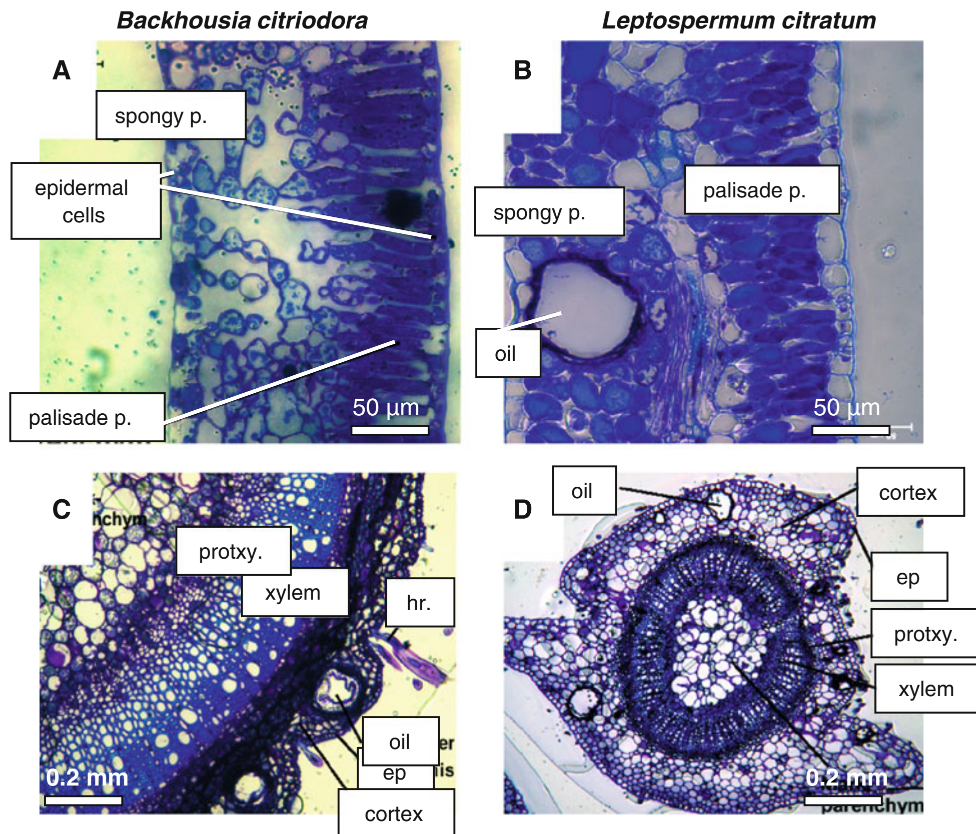

Fig. 2 Cross-sections through the aerial organs of 'Lemon Myrtle' after staining with Toluidine *Blue*. **a**, **b** Cross-section through the leaf blade in *B. citriodora* (**a**) and *L. citratum* (**b**). **c**, **d** Cross-section of the shoot in *B. citriodora* (**c**) and *L. citratum* (**d**). *Abbreviations*: *spongy p* spongy parenchyma, *palisade p* palisade parenchyma, *protx* protoxylem *ep* epidermis, *hr* hair

Table 1 Characteristic features observed in fully developed leaves of *B. citriodora* F.Muell and *L. citratum* Challinor, Cheel & A.R.Penfold

	Backhousia citriodora	*Leptospermum citratum*
Leaf type	Bifacial	Equifacial
E-ad	Puzzle-shaped pavement cells, polygonoid cells above vascular bundles	Polygonal pavement cells, distinct cuticular folds
E-ab	Like in E-ad	Like E-ad, with cuticular folds
Guard cells E-ad	None	Tetracytic, anemocytic
Guard cells E-ab	Anemocytic	Tetracytic, anemocytic
Trichomes E-ad	Unicellular, unbranched, weakly coiled	Unicellular, unbranched, weakly coiled trichomes that are aligned
Trichomes E-ab	Unicellular, unbranched, like in E-ad, in addition, more thick-walled and longer unicellular, unbranched, trichomes	Less than in E-ad
Mesophyll	Numerous schizogenic oil cavities Idioblasts, calcium oxalate druses Cell files filled with single calcium oxalate crystals lining vascular bundles	Numerous schizogenic oil cavities Idioblasts, calcium oxalate druses Cell files filled with single calcium oxalate crystals lining vascular bundles
Palisade cells/pavement cell	Mostly 6–8	Mostly 2–5

E-ad adaxial epidermis, E-ab abaxial epidermis

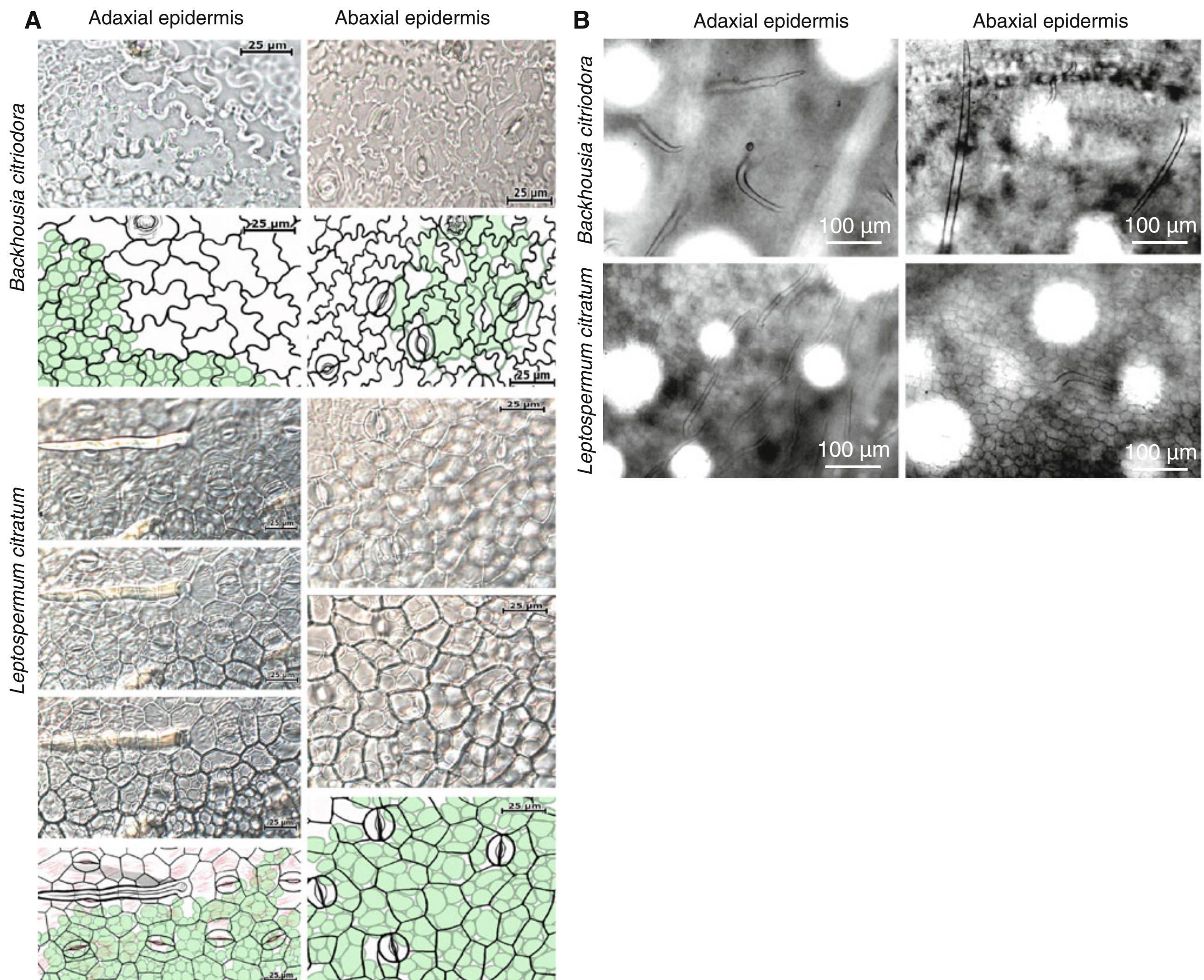

Fig. 3 **a** View on the adaxial, *upper* and the abaxial, *lower*, leaf epidermis of 'Lemon Myrtle' as visible by brightfield microscopy in tangential leaf sections. Different focal planes are shown for the abaxial epidermis to clarify the structure of guard cells and trichomes. The schematic drawings emphasise the relative size of epidermal pavement cells over the subtending cells of the palisade parenchyma (shaded in *green*). Note that puzzle-shaped pavement cells occur only in *B. citriodora*, whereas these cells are polygonal in *L. citratum.* **b** Trichomes at both leaf sides of the two 'Lemon Myrtle' accessions

Clarification of the phylogenetic position of the two species of 'Lemon Myrtle' based on the rbcL marker

The taxonomy of the genus *Leptospermum* has been revised several times [6] and is still obscured by the coexistence of different synonyms, not to speak about the ambiguous common names used by commercial providers. We therefore used a partial sequence of rbcL proposed as one of the central molecular markers for plant genetic bar coding [15] to determine the position of *L. citratum* with respect to *B. citriodora* and other Myrtaceae and to define sequence divergences that might be useful for molecular diagnostics. We successfully obtained and verified a rbcL sequence for both *L. citratum* (Genebank accession JN676919) and *B. citriodora* (Genebank accession JN676920). As to be expected, both sequences were highly similar (Fig. 4a). Available rbcL-sequences of the core Myrtaceae, *Olinia emarginata* and *Strasbourgeria robusta* as outgroup were used to determine the phylogenetic position of the two specimens of 'Lemon Myrtle' investigated in the current study. *B. citriodora* rbcL was located, along with a previously published sequence into the core Myrtaceae such as species of the genera *Eugenia, Myrtus, Eucalyptus, Myrcianthes* and *Metrosideros*. In contrast, *L. citratum* was found to be basal to the core Myrtaceae positioned distal to *O. emarginata* consistent with the reclassification of the genus *Leptospermum* into a specific subgenus Leptospermoideae.

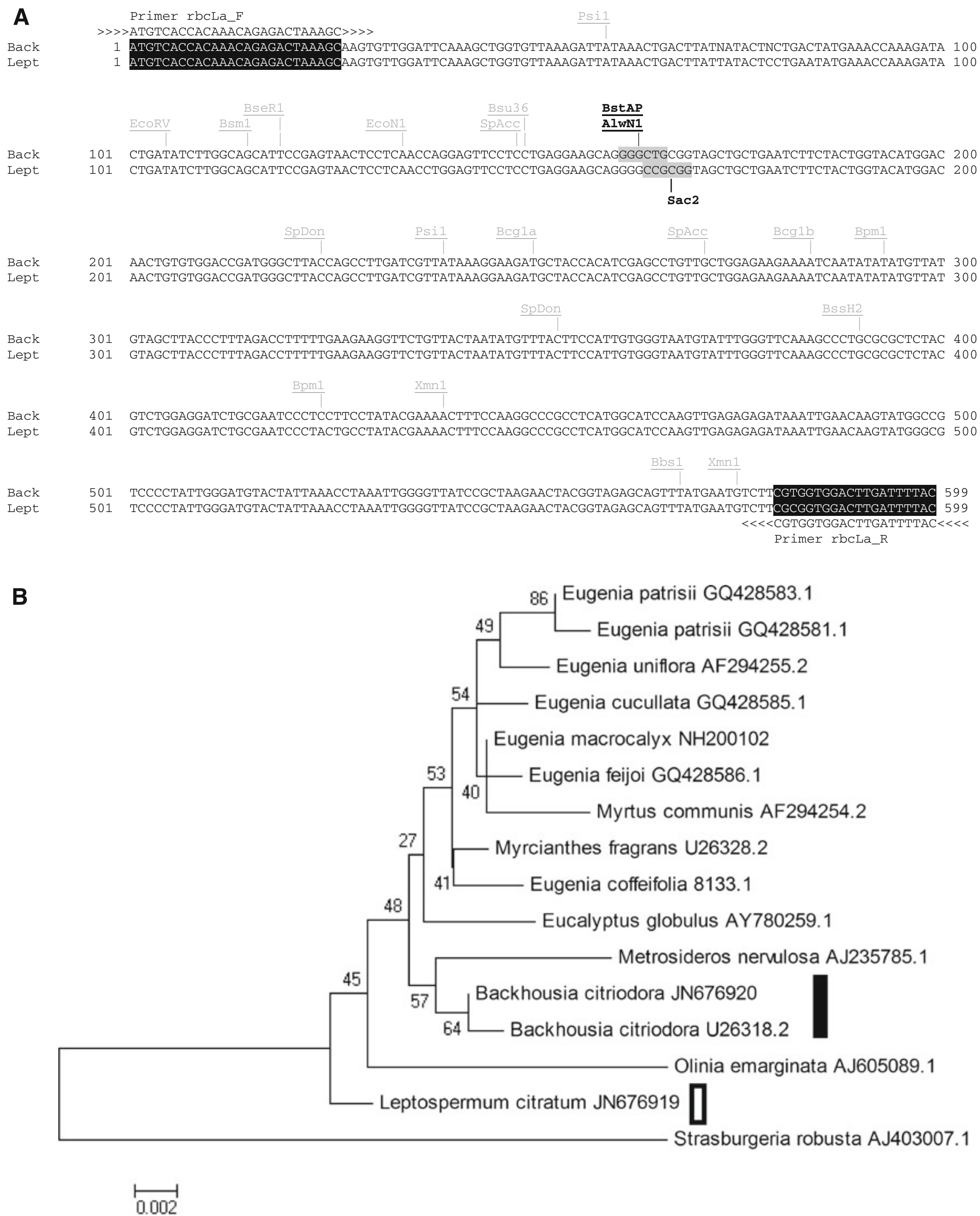

Fig. 4 Characteristics of the *rbcL* marker isolated from *B. citriodora* and *L. citratum*. **a** alignment with restriction sites. **b** NJ tree of 17 species of the Myrtaceae based on the *rbcL* marker. Bootstrap values are based on 500 replicates. *Boxes* highlight the position of *B. citriodora* and *L. citratum*

Molecular identification of 'Lemon Myrtle' based on restriction of the rbcL marker

Despite the high conservation of the rbcL marker between *B. citriodora* and *L. citratum*, a molecular differentiation should become possible by restriction analysis. We chose as target a specific *Sac* II restriction site differing between the two species of 'Lemon Myrtle'. The digest should yield two fragments of 458 and 141 bp in *L. citratum*, whereas in *B. citriodora*, the original 599 bp rbcL amplificate would remain complete (Fig. 5a). We tested this prediction using dried leaves from both species, amplified the rbcL marker

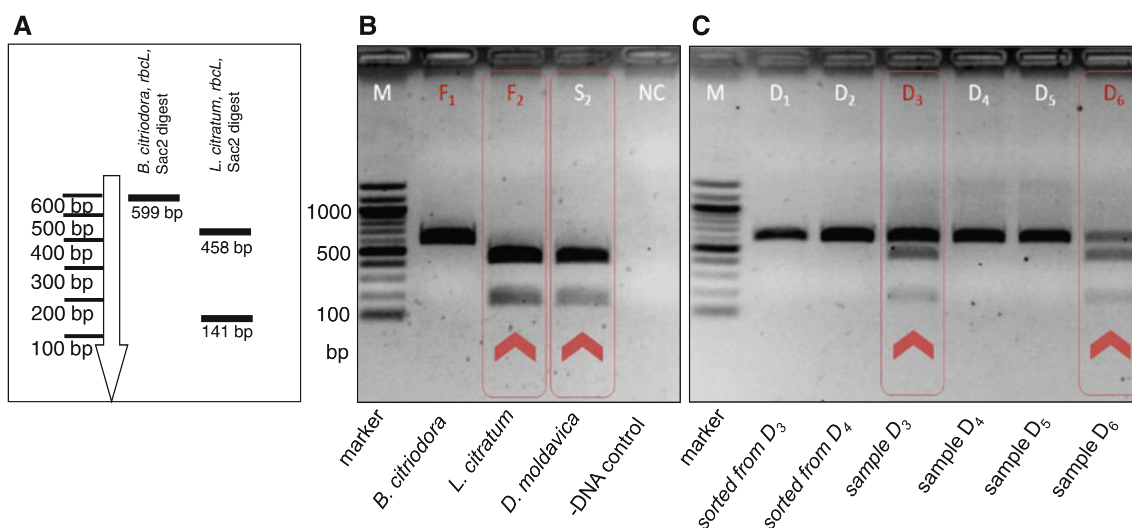

Fig. 5 Molecular diagnostics of 'Lemon Myrtle' based on restriction digest of the *rbcL* genomic fragment **a** Predicted fragment sizes for digest with *Sac* II in *B. citriodora* versus *L. citratum*. **b** Verification of the prediction using pure leaf samples of *B. citriodora*, *L. citratum* and the 'Lemon Myrtle' surrogate *D. moldavica*. M size marker (100-bp DNA ladder), DNA control test for the specificity of the PCR without added DNA template. *Arrows* indicate the two smaller fragments resulting from the restriction digest with Sac2. **c** Validation of the assay using commercial tea blends containing 'Lemon Myrtle'. Samples D_1 and D_2 have been sorted as **b**. Citriodora from the commercial products D_3 and D_4, respectively, using the microscopic discrimination given in Table 1. Commercial product D_3 contained also *Dracocephalum moldavicum*, commercial product D_4 did not. Commercial product D_5 contained *B. citriodora*, D_6 *L. citratum* as verified by microscopy

and restricted the amplificates by *Sac* II. The unrestricted rbcL amplificate was found to correspond to one band of around 600 bp in size for all species (data not shown). Upon *Sac* II restriction, there was no change for the *B. citriodora* amplificate, consistent with the prediction that, here, no *Sac* II recognition site is present (Fig. 5b). In contrast, restriction of *L. citratum* yielded a larger fragment of around 450 bp and a smaller fragment of around 150 bp, consistent with the prediction (Fig. 5a). A control, where the DNA template in the PCR mix was omitted, was included as negative control to check for potential contaminations. In addition to the two species of 'Lemon Myrtle', we tested *Dracocephalum moldavica*, which shares a similar citric flavour and is sometimes used in combination with 'Lemon Myrtle' (e.g. in commercial sample 3). Here, the restriction pattern resembled that observed for *L. citratum*.

We finally tested whether this assay can be administered to commercial samples where typically dried leaf fragments of 'Lemon Myrtle' are blended with other herbal compounds (Table 2). Sample D_3 contained 'Lemon Myrtle' as second compound followed by 'Moldavian Dragonhead' and produced a pattern with three bands (Fig. 5c) of 600 bp (corresponding to the full-length rbcL amplificate) and 450 + 150 bp (corresponding to the fragments expected for *L. citratum*). The morphological analysis gave clear evidence for *B. citriodora*, however (data not shown). To test, whether the two smaller bands are actually originating from *B. citriodora* or from the surrogate *D. moldavica*, the leaf fragments recognised as 'Lemon Myrtle' were sorted from the mixture and extracted and analysed separately. The resulting sample D_1 yielded only one band of 600 bp as to be expected for *B. citriodora*, suggesting that the smaller bands originated from the surrogate *D. moldavica*. Sample D_4 contained 'Lemon Myrtle' as one of the minor compounds and produced a band of 600 bp, which was consistent with the results of the morphological analysis clearly identifying *B. citriodora*. Again, these fragments were sorted and analysed separately yielding D_2 that produced a single band of 600 bp as expected. In sample D_5, 'Lemon Myrtle' (identified morphologically as *B. citriodora*) was a major component. Again, a single band of 600 bp was observed. In the last sample, D_6, 'Lemon Myrtle' the morphological features identified *L. citratum* as major component, consistent with the presence of the 450 and 150 bp bands. The upper 600-bp band, corresponding to the undigested amplificate, is most likely originating from the other components that do not harbour a corresponding Sac2 restriction site (data not shown).

Conclusions and outlook

As exemplary case study for many other cases, where plants used in traditional medical or culinary cultures are transferred as trend food supplements to the Western markets, we have analysed 'Lemon Myrtle', actually two distantly related species of the Myrtaceae derived from the

Table 2 Declared composition of commercial samples used in this study and rbcL fragments obtained after digest with Sac2

Sample	Declared composition	rbcL-Sac2 fragments (bp)
D_3	'Lemon Grass', 'Lemon Myrtle', 'Moldavian Dragonhead', 'Elderberry Flowers'	~600, ~450, ~150
D_4	'Ginger', 'Lemon Grass', 'Lemon Peel', 'Lemon Myrtle', 'Liquorhize'	~600
D_5	'Rooibos', 'Lemon Myrtle', 'Lemon Grass', 'Lemon Verbena', 'Cranberry', 'Orange Peel', 'Peppermint', 'Lemon Peel'	~600
D_6	'Orange Leaves', 'Ginger', 'Sweet Bramble Leaves', 'Lemon Myrtle', 'Natural Orange Oil'	~600, ~450, ~150

culture of the Australian Aborigenes. By a combination of classical microscopic analysis with molecular diagnostics using restriction length polymorphisms in the rbcL marker, we can discriminate between *B. citriodora* and *L. citratum* in dried mixtures as typically encountered in commercial samples.

Principally, this approach should be transferrable to more processed samples as well (such as 'Lemon Myrtle' flakes), where anatomical features are difficult to be assessed. However, if not supported by anatomical features, the approach suffers from limitations: in the presence of *D. moldavica* (which in some samples is added due to its similar lemon-type flavour), the restriction pattern hampers the discrimination between *B. citriodora* and *L. citratum*. Thus, in case that only the upper RAPD band is detected, this reports that neither *L. citratum* nor the surrogate *D. moldavica* are present. However, the presence of the smaller RAPD does not with certainty report the presence of *L. citratum*, because the same pattern could be caused by the surrogate *D. moldavica*. In order to extend the discriminative power of the assay to samples, where the anatomical features have been lost due to strong processing, we presently develop molecular and microscopic markers for a clear identification of *D. moldavica*. First data show that a strategy based on nested primers for the rbcL marker allows for superior discriminative power.

Acknowledgments We acknowledge Angelika Piernitzki and Joachim Daumann, Botanical Garden of the University, for excellent horticultural support during the project and Olivia Huber for skillful technical support during DNA extraction and analysis.

Conflict of interest The authors declare that they do not have any conflict of interest.

References

1. Ernst E (1998) Harmless herbs? A review of the recent literature. Am J Med 104:170–178
2. European Parliament and European Council (1997) Regulation on novel foods and novel food ingredients no. 258/97 of 27 January 1997. J Eur Comm L 43:1–5
3. Green JM, Draper AK, Dowler EA (2003) Short cuts to safety: risk and `rules of thumb' in accounts of food choice. Health Risk Soc 5:33–52
4. Hahn H, Michaelson I (1996) Mikroskopische Diagnostik pflanzlicher Nahrungs-, Genuss- und Futtermittel, einschliesslich Gewürze. Springer, Berlin
5. von Mueller FJH (1859) Fragmenta Phytographiae Australiae 1:78
6. Thompson J (1989) A revision of the genus *Leptospermum* (Myrtaceae). Telopea 3:301–448
7. Atkinson W, Brice H (1955) Antibacterial substances produced by flowering plants. Aust J Exp Biol 33:547–554
8. Greive KA, Staton JA, Miller PF, Peters BA, Oppenheim VMJ (2010) Development of *Melaleuca* oils as effective natural-based personal insect repellents. Aust J Entomol 49:40–48
9. Burke BE, Baillie JE, Olson RD (2004) Essential oil of Australian lemon myrtle (*Backhousia citriodora*) in the treatment of *molluscum contagiosum* in children. Biomed Pharmacother 58:245–247
10. Hood JR, Burton DM, Wilkinson JM, Cavanagh HMA (2010) The effect of *Leptospermum petersonii* essential oil on *Candida albicans* and *Aspergillus fumigatus*. Med Mycol 48:922–931
11. Lazar-Baker E, Hetherington S, Ku V, Newman S (2011) Evaluation of commercial essential oil samples on the growth of postharvest pathogen *Monilinia fructicola* (G. Winter) Honey. Lett Appl Microbiol 52:227–232
12. Doran JC, Brophy JJ, Lassak EV, House APN (2001) *Backhousia citriodora* F. Muell. Rediscovery and chemical characterization of the L-citronellal form and aspects of its breeding system. Flavour Fragr J 16:325–328
13. Hayes AJ, Markovic B (2002) Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. Food Chem Toxicol 40:535–543
14. Hayes AJ, Markovic B (2003) Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 2. Absorption and histopathology following application to human skin. Food Chem Toxicol 41:1409–1416
15. Consortium for the Barcode of Life (2009) A DNA barcode for land plants. Proc Natl Acad Sci USA 106:12794–12797
16. Walsh NG, Entwisle TJ (1996) Flora of Victoria, vol 3. Inkata Press, Melbourne
17. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem Bull 19:11–15
18. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
19. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
20. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
21. O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cell walls by Toluidine Blue O. Protoplasma 59:367–373