DNA Barcode Authentication of Saw Palmetto Herbal Dietary Supplements

Damon P. Little¹ & Marc L. Jeanson²

¹Lewis B. and Dorothy Cullman Program for Molecular Systematics, The New York Botanical Garden, 2900 Southern Boulevard, Bronx, New York 10458, United States of America, ²Museum National d'Histoire Naturelle, Département Systématique et Evolution, UMR 602 Herbiers plantes vasculaires, Case postale 39, F-75231 Paris cedex 05, France.

Herbal dietary supplements made from saw palmetto (Serenoa repens; Arecales) fruit are commonly consumed to ameliorate benign prostate hyperplasia. A novel DNA mini–barcode assay to accurately identify (specificity = 1.00 (95% confidence interval = 0.74–1.00); sensitivity = 1.00 (95% confidence interval = 0.66–1.00); n = 31) saw palmetto dietary supplements was designed from a DNA barcode reference library created for this purpose. The mini–barcodes were used to estimate the frequency of mislabeled saw palmetto herbal dietary supplements on the market in the United States of America. Of the 37 supplements examined, amplifiable DNA could be extracted from 34 (92%). Mini–barcode analysis of these supplements demonstrated that 29 (85%) contain saw palmetto and that 2 (6%) supplements contain related species that cannot be legally sold as herbal dietary supplements in the United States of America. The identity of 3 (9%) supplements could not be conclusively determined.

Serenoa repens (W.Bartram) Small–commonly known as saw palmetto–is a palm (Arecales) indigenous to the southeastern United States of America (Alabama, Florida, Georgia, Louisiana, Mississippi, and South Carolina)¹. The closest living relative of S. repens⁴–⁶, Acoelorrhaphe wrightii (Grisebach & H.Wendland) H.Wendland ex Beccari, occurs in United States of America (southern Florida), Bahamas, Cuba, southeastern México (Campeche, Chiapas, Tabasco, Veracruz, Yucatán, and Quintana Roo), Belize, Guatemala, Honduras, Nicaragua, Colombia (Isla de Providencia), and Costa Rica⁵–⁶. Although morphological and molecular data strongly support the close relationship between S. repens and A. wrightii, until recently their relationship to the other species of tribe Livistoneae could not be resolved²–⁴. New data suggest that S. repens and A. wrightii are sister to subtribe Livistiniae (Johannesteijsmannia, Lanonia, Licuala, Livistona, Pholidocarpus, Pritchardiopsis, and Saribus) and that the Acoelorrhaphe/Serenoa/Livistiniae clade is in turn sister to Brahea and subtribe Rhapidinae (Chamaerops, Guihata, Maxburretia, Raphidophyllum, Rhaps, and Trachycarpus)⁶.

The fruit (drupe) of S. repens are ellipsoid, about 2 cm in length, 1 cm wide, smooth, blue–black when mature (green to yellow–orange when immature)⁷–⁹. The fruits are eaten by an assortment of wild animals, livestock, and people⁷–⁹. When labeled as saw palmetto, S. repens can be legally sold in the United States of America as an herbal dietary supplement¹⁰. In 2011, it was the third most popular supplement in sales totaling more than US$ 18 million¹¹. Although the fruits of S. repens are reported to be useful in the treatment of 51 different medical ailments⁸, the fruits are most frequently taken to ameliorate benign prostate hyperplasia⁷–⁹,¹². Extracts of S. repens fruit inhibit the conversion of testosterone to dihydrotestosterone by 5a-reductases¹³–¹⁶. Benign prostate hyperplasia is associated with elevated concentrations of dihydrotestosterone¹⁰. Clinical studies report few adverse events from S. repens consumption (mostly mild)¹⁷,¹⁸, but treatment outcomes vary greatly–on average little success has been reported¹⁹.

Wild S. repens grows abundantly on as many as 450,000 hectares² of costal sand dunes, mesic hammocks, pine flatwoods, and sand–pine scrub¹⁷–⁹. Each hectare annually produces an average of 200 kg of fruit (range = 100–1,500 kg/hectare)². The magnitude of annual fruit harvest is unknown, but estimates are as high as 6,800,000 kg². Almost all of the fruit is harvested from wild plants² and approximately half is picked by independent wild-crafters⁶. Fruit is often harvested when immature: a final product with a minimum of 10% mature (blue–black) and 60% partially ripened (yellow–orange) fruit is commercially acceptable⁸.

DNA barcode researchers collectively aim to produce a global public reference library of standardized, high–quality, vouched DNA sequences that can be used to identify specimens. The protein coding plastid genes matK and rbcL have been sanctioned by the Consortium for the Barcode of Life for use in plant DNA barcoding²⁰,²¹. By using standard genomic regions, data and protocols can be shared thus maximizing scarce research funds.
We aim to (i) generate and test a DNA barcode reference library for *S. repens*, (ii) devise a barcode assay capable of unambiguously identifying *S. repens*, and (iii) estimate the frequency of mislabeled saw palmetto herbal dietary supplements on the market in the United States of America.

**Results**

For this study, 27 *matK* and 37 *rbcL* barcode sequences where generated from 37 morphologically identifiable specimens (Table 1; GenBank KF746442–KF746505). Median sequence quality (B50) of the newly generated sequences was 0.891 (IQR = 0.829–0.928) for *matK* and 0.909 (IQR = 0.756–0.939) for *rbcL*. Trimmed and edited *matK* sequences were 840 bp in length for *A. wrightii* and 837 bp in length for all other species examined (*A. wrightii* has a lysine AAAG inserted at nucleotide position 306). All Trimmed and edited *rbcL* sequences are 607 bp in length.

When the newly generated sequences were analyzed in concert with publicly available sequences (Table 2), no unambiguous *matK* sequence variation was detected within *S. repens* (n = 12) or *A. wrightii* (n = 15). Variation was detected at two *rbcL* nucleotide positions in *S. repens* (n = 18): GenBank sequence AJ621936 had a ‘C’ at nucleotide position 60 whereas all other sequences examined had an ‘A’ at that nucleotide position and GenBank sequence M88185 had a ‘C’ at nucleotide position 234 whereas all other sequences examined had a ‘T’. Both nucleotide substitutions are predicted to result in amino acid substitutions. Neither nucleotide substitution has been detected in more than one individual. No *rbcL* sequence variation was detected in *A. wrightii* (n = 17).

*Serenoa repens* and *A. wrightii* can be consistently distinguished from *Brahea*, Livistoneae, and Raphidinae by a combination of *matK* nucleotide positions 802 and 818 (Fig. 1). *Serenoa repens*, *A. wrightii*, and *Pholidocarpus majadum* Becc. (tribe Livistoneae) have a ‘G’ at nucleotide position 818 whereas all other examined species have an ‘A’. *Pholidocarpus majadum* has an ‘A’ at nucleotide position 802 and thus can be differentiated from *S. repens* and *A. wrightii* which have a ‘T’ at that nucleotide position. *Serenoa repens* and *A. wrightii* can be differentiated from one another by a three–base insertion in *A. wrightii* at *matK* nucleotide position 306. *Serenoa repens* and *A. wrightii* can also be differentiated from one another by *rbcL* nucleotide positions 292 (*S. repens* has a ‘C’, *A. wrightii* has a ‘T’) and 398 (*S. repens* has an ‘A’, *A. wrightii* has a ‘C’, Fig. 1).

Preliminary attempts to PCR amplify full–length barcode markers from saw palmetto herbal supplements were unsuccessful. Fragmented DNA was determined to be the primary cause of PCR failure—the barcode regions are larger than the average fragment size in DNA extracts of saw palmetto herbal supplements. To overcome DNA fragmentation, novel mini–barcode PCR primers were designed to amplify positions diagnostic of *S. repens* while limiting the amplicon size to 200 bp or less (Fig. 1). Unfortunately, there are no regions less than 200 bp within *matK* or *rbcL* that can consistently distinguish *S. repens* from the other species examined. A novel *matK* mini–barcode was designed to span nucleotide positions 802 and 818 and can thus distinguish *S. repens* and *A. wrightii* from all of the other species examined. A novel *rbcL* mini–barcode was designed to span nucleotide positions 292 and 398 and can thus distinguish *S. repens* from *A. wrightii* (Fig. 1). In combination these novel mini–barcodes can distinguish *S. repens* from all of the other species examined.

PCR amplification with the novel mini–barcode primer sets worked well on the 31 morphologically identifiable validation samples as well as saw palmetto herbal supplements. Median sequence quality (B50) of validation mini–barcode sequences was 0.633 (IQR = 0.455–0.732) for *matK* and 0.530 (IQR = 0.386–0.689) for *rbcL*. All validation samples were correctly identified using the combination of *matK* and *rbcL* mini–barcodes (*n* = 13 *S. repens*; *n* = 18 *A. wrightii*; specificity = 1.00 (95% confidence interval = 0.74–1.00); sensitivity = 1.00 (95% confidence interval = 0.66–1.00)).

Of the 37 saw palmetto herbal supplements examined, amplifiable DNA could be extracted from 34 (92%). At least one mini–barcode could be PCR amplified and sequenced from all 34 samples. Both *matK* and *rbcL* mini–barcodes could be PCR amplified and sequenced from 30 of the samples (81%). Mini–barcode analysis conclusively demonstrates that 29 (85%) saw palmetto herbal supplements contain *S. repens* (Fig. 1, supplement type A). The identity of 3 (9%) supplements could not be definitively determined due to failure of the *rbcL* mini–barcode to amplify and sequence (Fig. 1, supplement type B). These supplements could be composed of *S. repens*, they could contain *A. wrightii*, or a mixture of *S. repens* and *A. wrightii*. Two (6%) supplements contain related species that cannot be legally sold as herbal dietary supplements in the United States of America—a—one supplement (Fig. 1, supplement type D: it is a species of *Brahea*, *Chamaecrops*, *Guaihaia*, *Johannesteijsmannia*, *Lanania*, *Lxicula*, *Livistona*, *Maxbaretia*, *Rhapidophyllum*, *Rhapis*, *Sarbius*, or *Trachycarpus*).

**Discussion**

All newly generated *matK* and *rbcL* reference sequences exceed the quality requirements of the BARCODE data standard (version 2.3).

Intraspecific sequence variation was detected at two *rbcL* nucleotide positions in previously published23,25 *S. repens* sequences. Such barcode variation is uncommon in plants—particularly in *rbcL*. From the available data, we cannot determine if the variation is real or the result of sequencing error. If genuine, both of these nucleotide substitutions would result in amino acid substitutions. The fact that these variable sites have not been found in more than one individual strongly suggests that the variation is artificial. The *rbcL* mini–barcode does not include these, possibly variable, nucleotide positions and thus these nucleotide positions had no influence on the resulting species identifications (Fig. 1).

Our inability to PCR amplify full–length barcodes from saw palmetto herbal supplements was not unexpected: the processing of plant materials frequently results in highly fragmented DNA, particularly if the samples are heated42–51. Failure of PCR amplification from degraded DNA samples is frequently reported when amplicons greater than 200 bp42–51, thus one cannot expect full–length barcodes to reliably amplify from processed materials given that the median full–length *matK* barcode is 889 bp (IQR = 880–889)21 and *rbcL* is uniformly 654 bp21. Mini–barcodes were thus designed to ensure PCR amplification from degraded samples.

Amplifiable DNA could not be extracted from three saw palmetto herbal supplements. This is possible that amplifiable DNA belonging to *S. repens* (or closely related species) was absent from the herbal supplements because (i) the supplements did not contain any *S. repens* (or closely related species); (ii) alternatively the herbal supplements contained *S. repens* (or closely related species), but the material was processed in such a way that all amplifiable *S. repens* DNA was destroyed; or (iii) amplifiable DNA was present, but PCR inhibitory compounds were co–purified with the DNA. The successful PCR amplification and sequencing of only the *matK* mini–barcode from four saw palmetto herbal supplements cannot be conclusively explained without assuming that region containing the *rbcL* mini–barcode is more sensitive to DNA degradation than the region containing the *matK* mini–barcode.

The validation experiment conclusively demonstrates that it is possible to distinguish between *S. repens* and closely related species using a combination of *matK* and *rbcL* mini–barcodes (specificity = 1.00; sensitivity = 1.00). Samples can be unambiguously identified provided that both mini–barcodes can be PCR amplified and sequenced. Without the *matK* mini–barcode, it is not possible to distinguish among *S. repens*, *Brahea* and most Raphidinae (Fig. 1). Without the *rbcL* mini–barcode, it is not possible to distinguish between *S. repens* and *A. wrightii* (Fig. 1).
| Species | Provenance | Voucher specimen | Sample type |
|---------|------------|------------------|-------------|
| Acoelorrhaphe wrightii (Grisebach & H.Wendland) H.Wendland ex Beccari | Belize (Cayo) | Atha et al. 957 [NY] | matK and rbcL reference |
| Cuba (Isla de la Juventud) | Ratter 5191 [NY] | validation |
| Cuba (La Habana) | Britton et al. 13344 [NY] | validation |
| Cuba (La Habana) | Shafer 223 [NY] | matK and rbcL reference; validation |
| Cuba (Mayabeque) | Ekman 908 [NY] | matK and rbcL reference; validation |
| Cuba (Mayabeque) | Leon 14264 [NY] | validation |
| Cuba (Pinar del Río) | Britton et al. 9614 [NY] | matK and rbcL reference; validation |
| Cuba (Pinar del Río) | Shafer 10620 [NY] | matK and rbcL reference; validation |
| Cuba (Pinar del Río) | Shafer 426 [NY] | validation |
| Cuba (Pinar del Río) | Van Hermann 594 [NY] | validation |
| Cuba (unknown) | Avarco 4208 [NY] | validation |
| Cuba (Villa Clara) | Britton et al. 10269 [NY] | validation |
| Cuba (Villa Clara) | Combs 465 [NY] | rbcL reference; validation |
| Guatemala (Peten') | Contreras 4012 [NY] | rbcL reference; validation |
| Guatemala (Peten') | Contreras 5362 [NY] | rbcL reference; validation |
| Guatemala (Sololá) | Lenz 2010 [NY] | matK and rbcL reference; validation |
| Honduras (Colón) | Saunders 413 [NY] | matK and rbcL reference; validation |
| Mexico (Campeche) | Gutierrez 5120 [NY] | matK and rbcL reference; validation |
| Mexico (Quintana Roo) | Sanders and Frame 1719 [NY] | matK and rbcL reference; validation |
| Mexico (Veracruz) | Nee 32437 [NY] | matK and rbcL reference; validation |
| Nicaragua (Región Autónoma del Atlántico Norte) | Reveal 7365 [NY] | matK and rbcL reference; validation |
| Nicaragua (Región Autónoma del Atlántico Norte) | Stevens 8558 [NY] | validation |
| Brahea aculeata (Brandegee) H.E.Moore | United States of America (Florida) | Cooley 9332 [NY] | matK and rbcL reference |
| Brahea dulcis Mart. | Mexico (Sonora) | Felger 5023 [NY] | matK and rbcL reference |
| Chamaerops humilis L. | Guatemala (Huehuetenango) | Castillo et al. 2699 [NY] | matK and rbcL reference |
| Licuala tansachana Hodel | Morocco (Tanger-Tetouan) | Lauria 2006-402 [NY] | matK and rbcL reference |
| Livistona humilis R.Br. | Thailand (Naraathiwat) | Hodel 1601 [NY] | rbcL reference |
| Rhipidophyllum hystrix (Frazer ex Thouin) | Australia (Northern Territory) | Mumir 5590 [NY] | matK and rbcL reference |
| H.Wendl. & Drude | United States of America (Mississippi) | Bryson 22098 [NY] | matK and rbcL reference |
| Serenoa repens (W.Bartram) Small | United States of America (Florida) | Atha et al. 2530 [NY] | matK and rbcL reference; validation |
| United States of America (Florida) | Coker s.n. 1939 December 26 [NY] | validation |
| United States of America (Florida) | Cooley 2540 [NY] | matK and rbcL reference |
| United States of America (Florida) | Cooley 2655 [NY] | rbcL reference; validation |
| United States of America (Florida) | Curtiss 6195 [NY] | matK and rbcL reference; validation |
| United States of America (Florida) | Fortsch et al. 17 [NY] | validation |
| United States of America (Florida) | Hess et al. 8487 [NY] | rbcL reference; validation |
| United States of America (Florida) | Hill 13337 [NY] | rbcL reference; validation |
| United States of America (Florida) | Hill 1337 [NY] | rbcL reference; validation |
| United States of America (Florida) | Laconet et al. 861 [NY] | rbcL reference; validation |
| United States of America (Florida) | Moldenke 331 [NY] | rbcL reference; validation |
| United States of America (Florida) | Nash 644 [NY] | rbcL reference; validation |
| United States of America (Florida) | Nelson 17035 [NY] | rbcL reference; validation |
| United States of America (Florida) | Nolan 31 [NY] | matK and rbcL reference; validation |
| United States of America (Florida) | Small 2111 [NY] | validation |
| United States of America (Florida) | Small 2267 [NY] | validation |
| United States of America (Florida) | Standley 152 [NY] | rbcL reference; validation |
| United States of America (Georgia) | Harper 1817 [NY] | validation |
| United States of America (South Carolina) | Radford 11512 [NY] | matK and rbcL reference |
| unknown | anonymous [Museum of Wesleyan University, Barratt herbarium] s.n. [NY] | rbcL reference |
| Trachycarpus fortunei (Hook.) H.Wendl. | Cultivated | Hill 22260 [NY] | reference |
Two saw palmetto herbal supplements (6%), in our sample, were unambiguously mislabeled. One of these supplements contained *A. wrightii* (Fig. 1). Given the relative rarity of *A. wrightii* within the native geographic range of *S. repens* and the distinct macro–morphological differences (*S. repens* is an acaulous to short stemmed palm whereas *A. wrightii* grows in clusters of tall slender stems), it is difficult to imagine such a misidentification occurring at the point of harvest. It seems most likely that fruits—which appear similar in both species—were misidentified post harvest. We cannot explain the other mislabeled saw palmetto herbal supplement.

| Subtribe | Genus          | matK          | rbcl          |
|----------|----------------|---------------|---------------|
|          | *Brahea*       | AM114580      | HQ720245      |
|          |                | HQ720246      | HQ720247      |
|          |                | HQ720248      | HQ720249      |
|          |                | HQ720250      |               |
|          | *Rhapidinae*   | AM114568      | HQ720251      |
|          | *Chamaerops*   | HQ720307      |               |
|          |                | AM114569      | HQ720273      |
|          |                | HQ720274      | HQ720275      |
|          | *Rhapidinae*   | AM114557      | HQ720323      |
|          | *Guhaia*       | AM114572      | HQ720297      |
|          |                | HQ720311      |               |
|          | *Rhapidinae*   | AM114573      | HQ720308      |
|          | *Rhapidinae*   | HQ720310      |               |
|          | *Rhapidinae*   | HQ720311      |               |
|          | *Rhapidinae*   | HQ720315      |               |
|          | *Trachycarpus* | AM114570      | HQ720312      |
|          |                | HQ720313      |               |
|          | *Livistoninae* | AM114574      | HQ720276      |
|          | *Lanonia*      | HQ720279      | HQ720281      |
|          |                | HQ720282      |               |
|          | *Livistoninae* | AM114575      | HQ720156      |
|          | *Licula*       | HQ720158      | HQ720161      |
|          |                | HQ720167      | HQ720171      |
|          |                | HQ720172      |               |
|          |                | HQ720176      | HQ720179      |
|          |                | HQ720180      | HQ720182      |
|          |                | HQ720183      | HQ720185      |
|          |                | HQ720187      | HQ720285      |
|          |                | HQ720286      | HQ720287      |
|          |                | HQ720288      | HQ720290      |
|          |                | HQ720291      | HQ720292      |
|          | *Livistoninae* | AM114574      | HQ720190      |
|          | *Livistona*    | HQ720191      | HQ720306      |
|          |                | HQ720330      | HQ720331      |
|          |                | HQ720333      | HQ720335      |
|          |                | HQ720336      | HQ720337      |
|          |                | HQ720339      | HQ720340      |
|          |                | HQ720341      |               |
|          | *Livistoninae* | AM114577      | HQ720294      |
|          | *Pholidocarpus*| AM114578      | AM110196      |
|          | *Livistona*    | AM110196      |               |
|          | *Pritchardiopsis*| AM114578    |               |
|          | *Saribus*      | AM110196      |               |

Two saw palmetto herbal supplements (6%), in our sample, were unambiguously mislabeled. One of these supplements contained *A. wrightii* (Fig. 1). Given the relative rarity of *A. wrightii* within the native geographic range of *S. repens* and the distinct macro–morphological differences (*S. repens* is an acaulous to short stemmed palm whereas *A. wrightii* grows in clusters of tall slender stems), it is difficult to imagine such a misidentification occurring at the point of harvest. It seems most likely that fruits—which appear similar in both species—were misidentified post harvest. We cannot explain the other mislabeled saw palmetto herbal supplement.

Figure 1 | Variable nucleotide positions for mini–barcode sequences. Diagnostic positions that, in combination, unambiguously differentiate *Serenoa repens* from its close relatives are highlighted. Nucleotide positions are numbered in reference to Britton et al. 9614 (NY). Periods indicate nucleotides identical to *Brahea*. Question marks indicate unsequenced positions. The four sequence types (A, B, C, and D) found in herbal supplements are reported.
Variation in the chemical composition of S. repens fruit and fruit extracts is commonly cited to explain the mixed treatment outcomes observed in clinical studies. An alternate explanation is species misidentification. Between 4% and 15% of the samples we examined were misidentified. If our sample is representative, misidentification may account for a substantial portion of the variation observed in clinical studies. To ensure that misidentified materials are not inadvertently used, clinical researchers should authenticate all saw palmetto herbal supplements using the DNA barcode methodology described here.

Methods

Plant material. Reference and validation samples were morphologically identified by the authors (Table 1). Validation samples were arbitrarily selected from the set of morphologically identified samples. Herbal supplements were purchased in retail stores or online. The herbal supplements consisted of dry, cut, and sifted plant materials (gelatin capsules or compression tablets).

DNA extraction. Samples (10 mg) of dried leaf tissue or herbal supplements were disrupted in a 1.6 mL tube using two stainless steel ball bearings (3 mm) and a TissueLyser (Qagen) at 30 Hz (2 × 1.5 min). Samples were incubated for 18 h at 42°C with 40 rpm horizontal shaking in 600 μL extraction buffer (8 mM NaCl, 16 mM sucrose, 5.8 mM EDTA, 0.5% [w/v] sodium dodecyl sulphate, 12.4 mM Tris [pH 9.1], and 200 μg/mL proteinase K). After incubation, 200 μL of 3 M potassium acetate (pH 4.7) was added to each sample. Following 10 min of incubation at 0°C, samples were centrifuged at 14,000 g for 5 min. 600 μL of each sample’s aqueous phase was mixed with 900 μL purified DNA.

DNA amplification and sequencing. Markers were amplified in 15 μL Polymerase Chain Reactions (PCR). Each reaction contained 1.5 μL PCR buffer (200 mM tris [pH 8.8], 100 mM KCl, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 1% [v/v] Triton X-100, 50% [v/v] sucrose, 0.25% [w/v] creatine red), 0.2 μM dNTPs, 18 mM betaine (rbcL mini-barcode only), 0.5 (rbcL only) or 1.0 μM/L of each amplification primer (Table 3), 0.25 units of Taq polymerase, 0.025 mg/mL bovine serum albumin, and 0.5 μL purified DNA. The matK reaction mixtures were incubated for 150 sec at 95°C, cycled 10 times (30 sec at 95°C, 30 sec at 58°C, 30 sec at 72°C), and 30 sec at 66°C, and incubated 10 min at 72°C. The rbcL reaction mixtures were incubated for 150 sec at 95°C, cycled 35 times (30 sec at 95°C, 30 sec at 58°C, 30 sec at 72°C), and incubated 10 min at 72°C. The matK mini–barcode and rbcL mini–barcode reaction mixtures were incubated for 150 sec at 95°C, cycled 35 times (30 sec at 95°C, 30 sec at 66°C), and incubated 10 min at 66°C. PCR products were treated with ExoSAP-IT (USB) and bidirectionally sequenced on a 3730 automated sequencer (Life Technologies) using the amplification primers and BigDye v3.1 (Life Technologies; High–Throughput Genomics Unit, University of Washington).

Data analysis. Raw chromatograms were processed with KB (version 1.4; Life Technologies) and contigs were created and edited with Sequencher (version 4.10; Gene Codes). Sequence quality was evaluated using B (version 1.2) with the quality threshold (q) set to 30. Publicly available reference sequences where analyzed along with the sequences generated for this study (Tables 1 and 2). Diagnostic nucleotide positions were located in multiple sequence alignments constructed with MUSCLE (version 3.8). Novel mini–barcode primers spanning diagnostic positions were designed with PRIMER3 (version 1.1). Sequences from validation samples and herbal supplements were taxonomically identified using BRONX (version 2.0).
30. Bruni, I. et al. DNA barcoding as an effective tool in improving a digital plant identification system: a case study for the area of Mt. Valerio, Trieste (NE Italy). *PLOS One* 7, e43256 (2012).

31. Miller, S. E., Hrcek, J., Novotny, V., Weiblen, G. D. & Hebert, P. D. N. DNA barcodes of caterpillars (Lepidoptera) from Papua New Guinea. *Proc. Entomol. Soc. Wash.* 115, 107–109 (2013).

32. Hanner, R. Proposed Standards for BARCODE Records in INSDC (BRLs) (Database Working Group, Consortium for the Barcode of Life, 2009). [Retrieved from http://barcodeing.si.edu/PDF/DWG_data_standards-Final.pdf. Accessed 5 January 2013].

33. Newmaster, S. G., Fazekas, A. J., Steeves, R. A. D. & Janovec, J. Testing candidate plant barcode regions in the Myrtaceae. *Mol. Ecol. Resour.* 8, 480–490 (2008).

34. Du, Z.-Y., Qimike, A., Yang, C.-F., Chen, J.-M. & Wang, Q.-F. Testing four barcoding markers for species identification of Potamogetonaceae. *J. Syst. Evol.* 49, 246–251 (2011).

35. Nicôle, S. et al. Biodiversity studies in *Phaseolus* species by DNA barcoding. *Genome* 54, 529–545 (2011).

36. Ren, H., Lu, L., Wang, H. & Li, D.-Z. DNA barcoding of *Nicotiana*, *S*. *Ann. Bot. Fenn.* 49, 319–330 (2012).

37. Shi, L.-C. and Tao, Z. –Y., Qimike, A., Yang, C.-F., Chen, J.-M. & Wang, Q.-F. Testing the potential of proposed DNA barcodes for species identification of Zingiberaeae. *J. Syst. Evol.* 49, 261–266 (2011).

38. De Vere, N. et al. DNA barcoding the native flowering plants and conifers of Wales. *PLOS One* 7, e37945 (2012).

39. Yang, H. –Q., Dong, Y. –R., Gu, Z. –J., Liang, N. & Yang, J. –B. A preliminary assessment of *matK*, *rbcL* and *trnH-psbA* as DNA barcodes for *Calamus* (Arecaceae) species in China with a note on ITS. *Ann. Bot. Fenn.* 49, 319–330 (2012).

40. Aubriot, X., Lowry, P. P., Cruaud, C., Couloux, A. & Haevermans, T. DNA barcoding in a biodiversity hot spot: potential value for the identification of *Malagasy Euphorbia* listed in CITES Appendices I and II. *Mol. Ecol. Resour.* 13, 57–65 (2013).

41. Federici, S. et al. DNA barcoding to analyse taxonomically complex groups in plants: the case of *Thymus* (Lamiaceae). *Bot. J. Linn. Soc.* 171, 687–699 (2013).

42. Hellebrand, M., Nagy, M. & Mörsel, J. –T. Determination of DNA traces in rapseseed oil. *Z. Fär Leh. –forschung* 206, 237–242 (1998).

43. Meyer, R. Development and application of DNA analytical methods for the detection of GMOs in food. *Food Control* 10, 391–399 (1999).

44. Busconi, M. et al. DNA extraction from olive oil and its use in the identification of the production cultivar. *Food Chem.* 83, 127–134 (2003).

45. Tilley, M. PCR amplification of wheat sequences from DNA extracted during milling and baking. *Cereal Chem.* 81, 44–47 (2004).

46. Murray, S. R., Butler, R. C., Hardacre, A. K. & Timmerman–Vaughan, G. M. Use of quantitative real-time PCR to estimate maize endogenous DNA degradation after cooking and extrusion or in food products. *J. Agric. Food Chem.* 55, 2231–2239 (2007).

47. Gryson, N., Messens, K. & Dewettinck, K. PCR detection of soy ingredients in bread. *Eur. Food Res. Technol.* 227, 345–351 (2008).

48. Oguchi, T. et al. Investigation of residual DNAs in sugar from sugar beet (*Beta vulgaris* L.). *Food Hgy. Saf. Sci. (Shokuhin Eiseigaku Zasshi)* 50, 41–46 (2009).

49. Staats, M. et al. DNA damage in plant herbarium tissue. *PLOS One* 6, e28448 (2011).

50. Baker, D. A., Stevenson, D. W. & Little, D. P. DNA barcode identification of black cohosh herbal dietary supplements. *J. Aouc Int.* 95, 1023–1032 (2012).

51. Fernandes, T. J. R., Oliveira, M. B. P. P. & Mafra, I. Tracing transgenic maize as affected by breadmaking process and raw material for the production of a traditional maize bread, broa. *Food Chem.* 138, 687–692 (2013).

52. Habib, F. K. & Wyllie, M. G. Not all brands are created equal: a comparison of selected components of different brands of *Serenoa repens* extract. *Prostate Cancer Prostatic Dis.* 7, 195–200 (2004).

53. Scaglione, F., Lucini, V., Pannacci, M., Dugnani, S. & Leone, C. Comparison of the potency of 10 different brands of *Serenoa repens* extracts. *Eur. Rev. Med. Pharmacol. Sci.* 16, 569–574 (2012).

54. Alexander, P. J., Rajanikanth, G., Bacon, C. D. & Bailey, C. D. Recovery of plant DNA using a reciprocating saw and silica–based columns. *Mol. Ecol. Notes* 7, 5–9 (2007).

55. Elphinstone, M. S., Hinten, G. N., Anderson, M. J. & Nock, C. J. An inexpensive and high–throughput procedure to extract and purify total genomic DNA for population studies. *Mol. Ecol. Notes* 3, 317–320 (2003).

56. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797 (2004).

57. Koressaar, T. & Remm, M. Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23, 1289–1291 (2007).

58. Little, D. P. DNA barcode sequence identification incorporating taxonomic hierarchy and within taxon variability. *PLOS One* 6, e20552 (2011).