Molecular identification of *Solanum elaeagnifolium* in Australia using DNA barcoding, a solution for better management

Xiaocheng Zhu[1,2], David Gopurenko[1,2], Laurence A.R. Haegi[3] and Hanwen Wu[1,2]
[1] Department of Primary Industries, DPI Biosecurity and Food Safety, Wagga Wagga, New South Wales 2650, Australia
[2] Graham Centre for Agricultural Innovation (Charles Sturt University and NSW Department of Primary Industries), Charles Sturt University, Wagga Wagga, New South Wales 2678, Australia
[3] State Herbarium of South Australia, GPO Box 1047, Adelaide, South Australia 5001, Australia
(xiaocheng.zhu@dpi.nsw.gov.au)

Summary The problematic weed silverleaf nightshade (*Solanum elaeagnifolium* Cav.) was introduced to Australia during the early 20th century from North America and has since emerged as a Weed of National Significance. This species is almost impossible to eradicate after establishment, with its management in areas of new emergence therefore much aided by prompt identification and early action. Identification of the weed is often confounded because of the weed's remarkable similarity (especially pre-floral stages) to a variety of native *Solanum* species. DNA barcoding for improved identification may therefore improve its management. Sequences of the nuclear *waxy* gene and two chloroplast genes (*matK* and *trnL-trnF*) were compared among *S. elaeagnifolium* (N = 12) and 10 native species (*S. brownii*, *S. centrale*, *S. coactiliferum*, *S. esuriale*, *S. jucundum*, *S. lasiophyllum*, *S. lithophyllum*, *S. petrophilum*, *S. quadriloculatum* and *S. sturtianum*) with which the weed is commonly confused. A further five species including *S. campanulatum*, *S. chenopodinum*, *S. cleistogamum*, *S. nummularium* and *S. succosum,* were also analysed to provide a wide cross-section of Australian native species. Clear and well supported genetic separations of all species were evident at each of the three gene regions. The maximum observed genetic distance in *S. elaeagnifolium* was 0.41%, and the minimum distance to its nearest genetic neighbour species was 1.12%. Our study provides a reliable genetic tool for *S. elaeagnifolium* identification irrespective of developmental stage, assisting efficient management aimed at reducing the risk of further spread of this weed to new areas.

Keywords Granule-bound starch synthase 1, silverleaf nightshade, Australian native *Solanum*, WoNS.

INTRODUCTION

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a deep-rooted, summer-growing perennial. It is listed as Weed of National Significance, infesting over 350,000 ha in Australia (Stanton *et al.* 2009). It competes for soil water and nutrients and causes up to 77% yield loss in cereal (Stanton *et al.* 2009). Due to the deep root system and its high re-generation ability, this species is almost impossible to eradicate after establishment (Stanton *et al.* 2009).

Prompt identification and early action is required for effective management especially in the areas of new incursions. However, although *S. elaeagnifolium* originated in North America, it is remarkably similar to several Australian native species especially at pre-floral stage. Like the vast majority of Australian native *Solanum*, silverleaf nightshade belongs to the *Leptostemonum* or 'spiny solanum' clade in *Solanum*. Distinguishing these native species from *S. elaeagnifolium* is currently based on subtle morphological features requiring specialised knowledge. Field identifications are therefore often unreliable (Zhu *et al.* 2013). Misidentification of *S. elaeagnifolium* as *S. esuriale* Lindl. in South Australia (1918) contributed to the delay of co-ordinated control until 1958 (Hosking *et al.* 2000). Similarly, native *Solanum* species are also commonly misidentified as *S. elaeagnifolium*, resulting in erroneous species distribution records, and unnecessary control of native biodiversity. Our previous study demonstrated how use of microsatellite (SSR) markers could differentiate *S. elaeagnifolium* but was restricted to only one native species, *S. esuriale* (Zhu *et al.* 2011).

DNA barcoding is a sequence-based method widely used to assist alpha-taxonomic identifications (Gopurenko *et al.* 2013). Phylogenetic relationships among *Solanum* species have been reported using various nuclear and chloroplast genes (Aubriot *et al.* 2016, Levin *et al.* 2006, Zhang *et al.* 2013), and such genes are potentially also useful for DNA barcoding purposes here. However, these studies generally used single replicates of examined species and some did not include both *S. elaeagnifolium* and similar Australian native species. Limited replicate sampling can underestimate intraspecific genetic diversity and lead to erroneous DNA barcode identifications (Levin *et al.* 2005, Zhang *et al.* 2013). This study aims to provide

a reliable tool to distinguish *S. elaeagnifolium* from 15 Australian native *Solanum* species irrespective of growth stage.

MATERIALS AND METHODS

Solanum elaeagnifolium (N = 12) and vouchered samples of 15 Australian native spiny *Solanum* species were obtained from recent field collections, and specimens available at the State Herbarium of South Australia, the National Herbarium of New South Wales and the Queensland Herbarium. These included ten species morphologically similar to *S. elaeagnifolium* (*S. brownie* Dunal., *S. central* J.M.Black, *S. coactiliferum* J.M.Black, *S. esuriale* Lindl., *S. jucundum* A.R.Bean, *S. lasiophyllum* Poir., *S. lithophilum* F.Muell., *S. petrophilum* F.Muell., *S. quadriloculatum* F.Muell. and *S. sturtianum* A.R.Bean (Table 1) and a further five species from diverse groups within the 'spiny solanums' (*S. campanulatum* R.Br., *S. chenopodinum* F.Muell., *S. cleistogamum* Symon, *S. nummularium* S.Moore and *S. succosum* A.R.Bean & Albr.) for comparative purposes. Replicates were included for all species except *S. centrale*.

DNA extraction, PCR and sequencing were performed as described (Gopurenko *et al.* 2013), but modified for plant DNA barcoding (Zhu *et al.* 2017). Chloroplast DNA genes (*matK* and *trnL-trnF*) were PCR amplified using primer pairs 390F/1326R (Cuenoud *et al.* 2002), and ucp_c/ucp_f (Taberlet *et al.* 1991), respectively. The nuclear *waxy* gene (exons 2–7) was targeted using primers 181F & 1171R designed by Walsh and Hoot (2001) as reported in Levin *et al.* (2005). To improve amplification of *waxy* we modified primer 181F as ("waxyF") 5' CGGGTAATGACAATATCCCC 3'. Gene sequences were concatenated using FABOX (Villesen 2007). Inter- and intra- specific pairwise genetic distances (unweighted) were calculated using MEGA7 (Kumar *et al.* 2016). Optimal nucleotide-substitution models for six unlinked partitions of the sequence alignment (*matK* codons 1, 2 and 3; *trnL-trnF*; *waxy* introns; *waxy* exons) were identified using Jmodeltest 2.0 (Darriba *et al.* 2012) and implemented in a Bayesian phylogenetic analysis run for 80 million generations using MrBayes 3.2.4 (Ronquist and Huelsenbeck 2003).

RESULTS AND DISCUSSION

High quality sequences were obtained for all samples at all three gene regions. Alignments were truncated to 771, 1042 and 950 bp for *matK*, *trnL-trnF* and *waxy*, respectively. There were 168 parsimoniously informative sites and 66 singletons. Most species have a very low within-species diversity, ranging from 0 to 0.29 %, with exception of *S. elaeagnifolium, S. nummularium* and *S. jucundum* where within species diversity was higher at 0.41, 0.44 and 0.45 %, respectively.

Table 1. Species compared in this study. N: number of samples sequenced; D_{ma} (%): maximum % intraspecific genetic distance; D_{mi} (%): minimum % interspecific genetic distance between *S. elaeagnifolium* and other species.

Species	N	D_{ma} (%)	D_{mi} (%)
S. brownii	3	0.18	1.28
S. campanulatum	2	0.18	1.35
S. centrale	1	NA	1.40
S. chenopodinum	2	0.18	1.61
S. cleistogamum	4	0.25	1.12
S. coactiliferum	2	0.07	1.23
S. elaeagnifolium	12	0.41	NA
S. esuriale	4	0.11	1.55
S. jucundum	4	0.45	1.38
S. lasiophyllum	2	0.00	1.42
S. lithophyllum	2	0.04	1.23
S. nummularium	2	0.44	1.53
S. petrophilum	2	0.04	1.43
S. quadriloculatum	4	0.15	1.39
S. sturtianum	2	0.11	1.38
S. succosum	2	0.29	1.19

Although remarkably similar in morphological traits, *S. elaeagnifolium* is genetically very different from all tested Australian native species (Table 1). The maximum genetic distance observed in *S. elaeagnifolium* (0.41%, Table 1) was less than half the distance (1.12%) observed to *S. cleistogamum,* its nearest neighbour (Table 1, Figure 1). The large genetic distance between *S. elaeagnifolium* and Australian native *Solanum* is consistent with a previous study which highlighted limited allelic overlap between *S. elaeagnifolium* and *S. esuriale* using SSR markers (Zhu *et al.* 2011). Four, four and nine alleles specific to *S. elaeagnifolium* were identified at *matK*, *trnL-trnF* and *waxy* genes, respectively. These genetic markers could potentially be used in development of a Loop mediated isothermal amplification (LAMP) assay for targeted molecular diagnostics and rapid field identification of the species (Gopurenko *et al.* 2017).

The phylogenetic tree clearly separated all species at high posterior probabilities (Figure 1). *Solanum elaeagnifolium* formed a distinct clade from other species. This is consistent with previous molecular

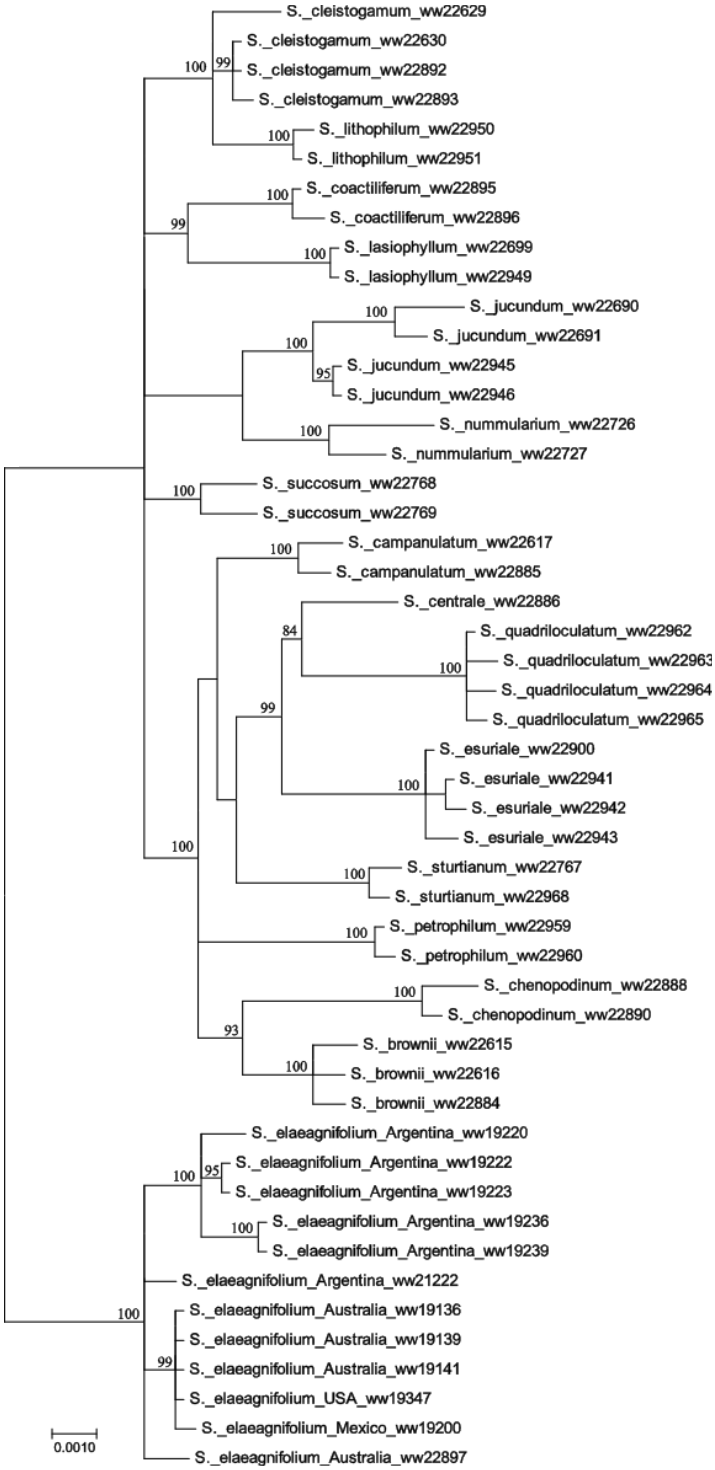

Figure 1. Bayesian phylogenetic relationships among *S. elaeagnifolium* and 15 Australian native *Solanum* species. Clade posterior probability support >80% indicated at nodes.

phylogenetic studies of 'spiny solanums' (Levin *et al.* 2006, Vorontsova *et al.* 2013) and extends this outcome to a wider range of Australian species. A more comprehensive molecular phylogenetic study currently underway by the authors is expected to throw more light on the relationships among Australian native 'spiny solanums' and *S. elaeagnifolium*.

In conclusion, DNA barcoding using three genes clearly distinguished *S. elaeagnifolium* from 10 morphologically very similar native *Solanum* species and five other closely related *Solanum* species. All three genes have alleles specific for *S. elaeagnifolium;* these could potentially be used in molecular genetic diagnostics for identification of the species. This in turn could improve management of SLN especially at the early stage of the invasion and avoid the unnecessary control of morphologically similar native species.

ACKNOWLEDGMENTS

We acknowledge the Biosecurity SA, PIRSA and Meat and Livestock Australia for funding support. Dr Franco Chiarini (Argentina), Dr Miguel Mellado (Mexico), Edwin Morris (United States of America), Dr John Heap and organisations including the State Herbarium of South Australia, National Herbarium of New South Wales and the Queensland Herbarium are thanked for access to specimens used in analysis.

REFERENCES

Aubriot, X., Singh, P. and Knapp, S. (2016). Tropical Asian species show that the Old World clade of 'Spiny Solanums' (*Solanum* subgenus *Leptostemonum* pro parte: Solanaceae) is not monophyletic. *Botanical Journal of the Linnean Society* 181, 199-223.

Cuenoud, P., Savolainen, V., Chatrou, L.W., Powell, M., Grayer, R.J. and Chase, M.W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *American Journal of Botany* 89, 132-44.

Darriba, D., Taboada, G.L., Doallo, R. and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772-72.

Gopurenko, D., Fletcher, M.J., Löcker, H. and Mitchell, A. (2013). Morphological and DNA barcode species identifications of leafhoppers, planthoppers and treehoppers (Hemiptera: Auchenorrhyncha) at Barrow Island. *In* 'Records of the Western Australian Museum, supplement 83: the terrestrial invertebrate fauna of Barrow Island', eds N.R. Gunawardene, J.D. Majer, C.K. Taylor and M.S. Harvey, pp. 253-85. (Western Australian Museum Publications).

Gopurenko, D., Wang, A. and Wu, H. (2017). Developing a portable DNA-based platform, for rapid on-site identification of Chilean needle grass and serrated tussock. Proceedings of the 19th NSW Biennial Weeds Conference, pp. 145-51. (Weed Science Society of NSW, Armidale)

Hosking, J.R., Sainty, G.R. and Jacobs, S.W.L. (2000). Certainty and uncertaintly in plant identification. *The New Mexico Botanist* 15, 1-4.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33, 1870-74.

Levin, R.A., Nicole, R.M. and Bohs, L. (2006). Phylogenetic relationships among the 'Spiny Solanums' (*Solanum* Subgenus *Leptostemonum*, Solanaceae). *American Journal of Botany* 93, 157-69.

Levin, R.A., Watson, K. and Bohs, L. (2005). A four-gene study of evolutionary relationships in *Solanum* section *Acanthophora*. *American Journal of Botany* 92, 603-12.

Ronquist, F. and Huelsenbeck, J. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-4.

Stanton, R., Heap, J.W., Carter, R.J. and Wu, H. (2009). *Solanum elaeagnifolium*. *In* 'The biology of Australian weeds', volume 3, ed. F.D. Panetta, pp. 274-93. (R.G. and F.J. Richardson, Melbourne).

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17, 1105-9.

Villesen, P. (2007). FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* 7, 965-68.

Vorontsova, M.S., Stern, S., Bohs, L. and Knapp, S. (2013). African spiny *Solanum* (subgenus *Leptostemonum*, Solanaceae): a thorny phylogenetic tangle. *Botanical Journal of the Linnean Society* 173, 176-93.

Walsh, B.M. and Hoot, S.B. (2001). Phylogenetic relationships of capsicum (Solanaceae) Using DNA sequences from two noncoding regions: the chloroplast atpB-rbcL spacer region and nuclear waxy introns. *International Journal of Plant Sciences* 162, 1409-18.

Zhang, W., Fan, X., Zhu, S., Zhao, H. and Fu, L. (2013). Species-specific identification from incomplete sampling: applying DNA barcodes to monitoring invasive *Solanum* plants. *Plos One* 8, e55927.

Zhu, X., Weston, P.A., Skoneczny, D., Gopurenko, D., Meyer, L., Lepschi, B.J., Callaway, R.M., Gurr, G.M. and Weston, L.A. (2017). Ecology and genetics affect relative invasion success of two

Echium species in southern Australia. *Scientific Reports* 7, srep42792.

Zhu, X.C., Burrows, G., Wu, H., Raman, H., Stanton, R. and Lemerle, D. (2011). Identification of silverleaf nightshade using microsatellite markers and microstructure. Proceedings of the 23rd Asian-Pacific Weed Science Society Conference, pp. 604-9. (Asian-Pacific Weed Science Society, Cairns).

Zhu, X.C., Wu, H.W., Stanton, R., Burrows, G.E., Lemerle, D. and Raman, H. (2013). Morphological variation of *Solanum elaeagnifolium* in south-eastern Australia. *Weed Research* 53, 337-43.