

## Identification of eight *Panicum* species in Riverina region of NSW using DNA sequence analysis

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**Summary** Australia has over 30 *Panicum* spp. (panic grass) including several non-native species that cause crop and pasture loss due to competition. To develop appropriate management strategies for each species, it is critical to correctly identify panic grass species encountered. Currently, panic grass identification relies on microscopic examination of the inflorescence and spikelets, an approach that is only useful for flowering specimens and requires significant taxonomic expertise. To overcome this limitation, we applied both morphological and molecular techniques for identification of *Panicum* spp. in the Riverina region of New South Wales. We identified three molecular markers: one nuclear gene region (ITS) and two chloroplast gene regions (*matK* and *trnL* intron-*trnF*) capable of differentiating eight *Panicum* spp. Concatenation of sequences from ITS, *matK* and *trnL* intron-*trnF* gene regions provided clear separation of eight species collected regionally and identified a maximum intraspecific distance of 0.22% and minimum interspecific distance of 0.33%. Based on comparison with verified voucher specimens, *P. hillmanii* Chase was prevalent and constituted 78.9% of all samples collected and identified. DNA barcoding represents an accurate and potentially cost effective tool for distinguishing *Panicum* spp. at the species level regardless of growth stage. Molecular markers may also be useful for accurate demographic analysis of *Panicum* grass invasion in Australia and abroad.

**Keywords** *Panicum* spp., *Panicum hillmanii* Chase, DNA barcoding, *matK*, *trnL* intron-*trnF*, ITS, phylogenetic tree.

### INTRODUCTION

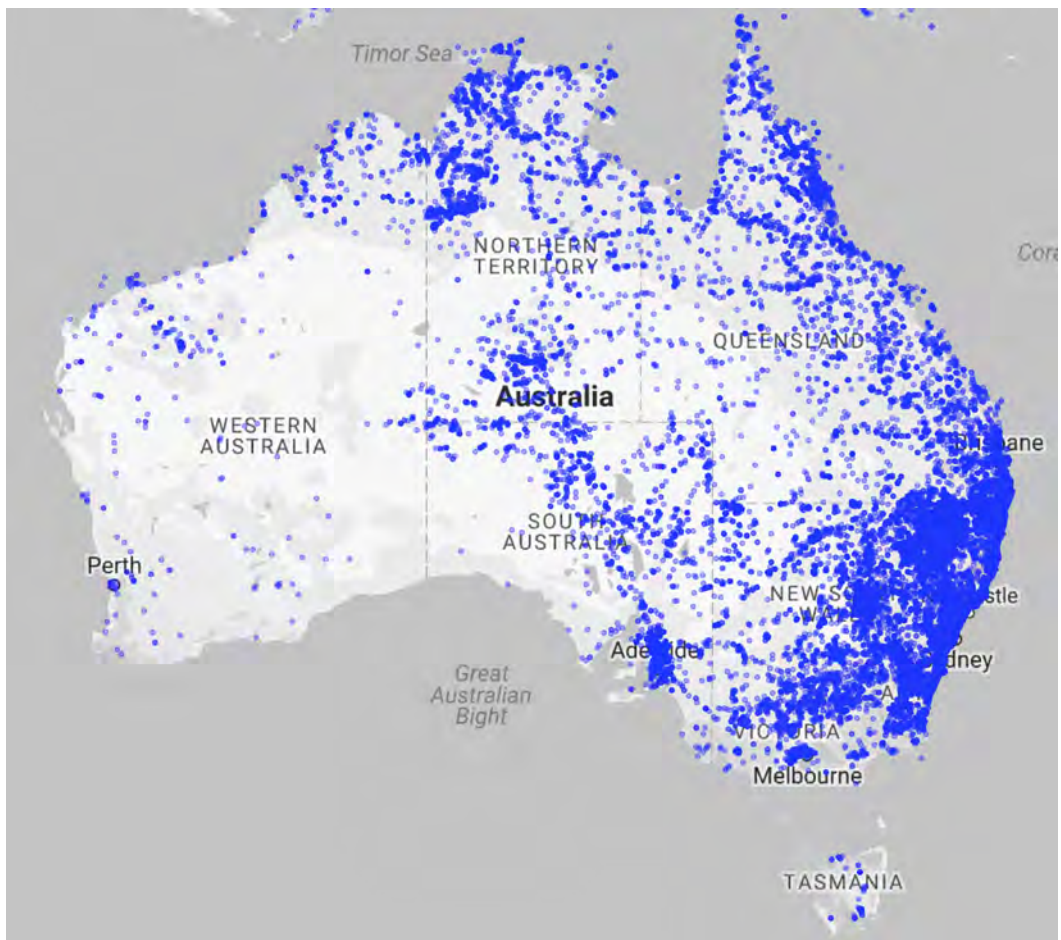
The genus *Panicum* is one of the largest genera in the Poaceae, and is globally distributed (Aliscioni *et al.* 2003). Depending on the taxonomic concepts

followed, up to 500 species are recognised (Byng 2014). Major photosynthetic types (C3, C4, and C3/C4 intermediate) reported in the Poaceae are represented in the genus (Zuloaga *et al.* 2010).

*Panicum* spp. are widely distributed in Australia (Figure 1) with 24 indigenous and 9 non-native species currently recognised (CHAH 2005 onwards). They are currently ranked as one of the most economically important weeds of summer fallows in Australia (Llewellyn *et al.* 2016). Their toxic profile in livestock is suggested to relate to the presence of saponins or sapogenins (Miles *et al.* 1992). However, variable steroidal saponin profiles may exist in different *Panicum* spp., and could be associated with their differential ability to cause hepatogenous or secondary photosensitisation in livestock (Quinn *et al.* 2014).

*Panicum* is a taxonomically challenging group due to the wide range of variation in both morphological and physiological features (Zuloaga *et al.* 2010). Subtle morphological differences required for species differentiation have not been well documented (Zuloaga *et al.* 2010). Existing identification keys to *Panicum* spp. in Australia are based primarily on reproductive features and therefore are only effective for plants in their reproductive phase (Coissac *et al.* 2016). Furthermore, keys for identification can be difficult to use with confidence and even experienced taxonomists find *Panicum* identification problematic at the species level. Therefore, establishment of robust genotyping methods are desirable for unequivocal plant species identification. Identification of *Panicum* at an individual plant level may also be critical for development of targeted management strategies for invasive and potentially toxic *Panicum* spp. (Quinn *et al.* 2014).

DNA barcoding, a simplified DNA-based species identification approach (Hebert *et al.* 2003), has been



**Figure 1.** Distribution of *Panicum* spp. in Australia based on herbarium specimens represented in major Australian herbaria. Collection data extracted from The Australasian Virtual Herbarium.

utilised as an important and complementary method to traditional morphological identification (Zhu *et al.* 2014). DNA barcoding uses standardised DNA regions ('barcodes') to identify different species (CBOL Plant Working Group 2009). However, the optimal suite of barcoding loci for *Panicum* spp. identification in Australia has not yet been established. Following on from Hollingsworth *et al.* 2011, one nuclear locus (ITS) and two chloroplast loci (*matK* and *trnL* intron-*trnF*) were evaluated in this study for their ability to differentiate eight *Panicum* spp. occurring in the Riverina region of New South Wales.

#### MATERIALS AND METHODS

In early 2017, fresh leaf samples and accompanying herbarium voucher specimens were collected from 90

*Panicum* plants dispersed across multiple sites within a 200 km radius range of Wagga Wagga, NSW (Figure 2). Voucher specimens were lodged at the Australian National Herbarium (ANH). Fresh leaf samples were stored in silica gel prior to extraction.

To supplement the field-collected materials, an additional 19 dried leaf samples, representing seven *Panicum* spp., were sampled from specimens within the ANH collection. A total of 36 samples, representing eight species found in Riverina, were selected for further analysis in this study (Table 1).

DNA extraction, PCR and sequencing were performed as described (Zhu *et al.* 2014) using primer pairs ITS5A-ITSR, 390F-1326R and *ucp\_c-ucp\_f* for ITS, *matK* and *trnL* intron-*trnF* regions, respectively. Geneious version 11.0.5 (Kearse *et al.* 2012) was used

to read, edit and align the sequences. Sequence alignments were analysed using MEGA7.0.26 (Kumar et al. 2016) to calculate intra- and interspecific distances with the Kimura 2-parameter (K2P) model. Sequences of all loci for each *Panicum* specimen were concatenated and phylogenetic relationships among species were inferred by MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) using default settings with GTR+R substitution model as suggested by JModelTest 2.1.10 (Darriba et al. 2012).

**Table 1.** *Panicum* specimens used for DNA extraction and sequencing.

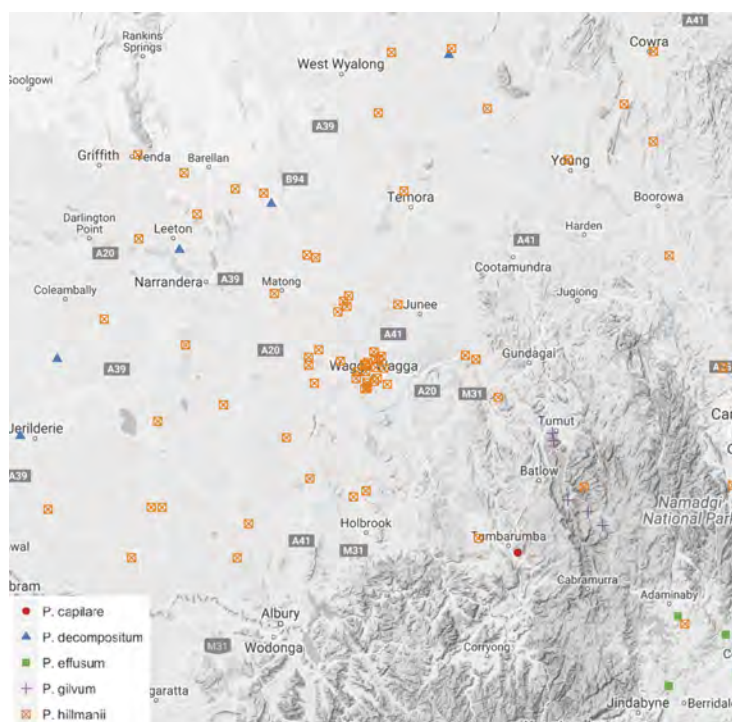
Species identification	Fresh leaf	Dried leaf (ANH)
<i>P. capillare</i> L.	1	2
<i>P. decompositum</i> R.Br.	3	5
<i>P. effusum</i> R.Br.	4	1
<i>P. gilvum</i> Launert	5	0
<i>P. hillmanii</i> Chase	4	2
<i>P. laevinode</i> Lindl.	0	3
<i>P. miliaceum</i> L.	0	3
<i>P. queenslandicum</i> Domin	0	3

## RESULTS AND DISCUSSION

The majority of field-collected specimens were identified as *P. hillmanii* (78.9%, 71/90) by comparison with verified voucher specimens at the ANH (Figure 2). Other species identified included *P. gilvum* (10.0%, 9/90), *P. decompositum* (5.6%, 5/90), *P. effusum* (4.4%, 4/90) and *P. capillare* (1.1%, 1/90). The predominance of *P. hillmanii*, a non-native species that was previously rarely reported in the Riverina region, suggests the species has spread considerably in recent years. Therefore, further investigation in regards to its invasion ecology should be conducted for the development of a more holistic management strategy.

Successful PCR amplification and sequencing was achieved in three selected gene loci (ITS, *matK* and *trnL* intron-*trnF*) for all specimens. Alignments were then truncated to 641, 730, and 750 bps for ITS, *matK* and *trnL* intron-*trnF*, respectively. In addition, concatenated data over all three gene loci were evaluated for phylogenetic analysis. Intraspecific and interspecific genetic distances were calculated for the eight species identified (Table 2).

The efficacy of DNA barcoding depends on the extent of intraspecific and interspecific divergence in a selected locus or combined loci, and the ideal situation



**Figure 2.** Location and identification of 90 field-collected *Panicum* spp. in a 200 km radius around Wagga Wagga, New South Wales.

**Table 2.** Intraspecific and interspecific K2P distances (%) of eight *Panicum* spp. (MinInterD: Minimum interspecific distance; MaxID: Maximum intraspecific distance; UAA: *trnL* intron-*trnF*; PC: *P. capillare*; PD: *P. decompositum*; PE: *P. effusum*; PG: *P. gilvum*; PH: *P. hillmanii* Chase; PL: *P. laevinode* Lindl; PM: *P. miliaceum*; PQ: *P. queenslandicum* Domin).

	ITS	<i>matK</i>	UAA	ITS- <i>matK</i> - UAA
MinInterD %	0.71	0.07	0	0.33
MaxID in PC%	0.33	0.09	0	0.22
MaxID in PD%	0.03	0.06	0	0.03
MaxID in PE%	0.34	0	0	0.15
MaxID in PG%	0	0	0	0
MaxID in PH%	0	0	0	0
MaxID in PL%	0.07	0.09	0	0.06
MaxID in PM%	0	0.09	0	0.03
MaxID in PQ%	0.14	0.14	0.27	0.16

is a lack of overlap between these two distance values (Aliabadian *et al.* 2009). For *Panicum* spp., ITS showed the highest minimum interspecific distance (0.71%), which was over 2-fold higher than the highest maximum intraspecific distance (*P. effusum*, 0.34%). This result confirmed that the ITS locus was used as an efficient barcode for separation of the eight *Panicum* spp. in this study. Conversely, the overlap between intraspecific and interspecific values of either the *matK* or the *trnL* intron-*trnF* regions suggests that use of these barcodes alone may prove problematic for differentiation of some *Panicum* spp.

In order to gain strong interspecies discriminatory power, the three loci were concatenated for phylogenetic analysis. The phylogenetic tree showed clear differentiation between the eight *Panicum* spp. evaluated (Figure 3). Investigation of the efficacy of this approach with other *Panicum* spp. will be undertaken in future studies using these loci.

When considering the phylogenetic tree generated, the primary division of interest with respect to the relationship between indigenous and non-native species is presented (Figure 3). Not surprisingly, all four indigenous species (*P. decompositum*, *P. effusum*, *P. laevinode* and *P. queenslandicum*), clustered together on clade 2, while the four non-native species (*P. capillare*, *P. gilvum*, *P. hillmanii* and *P. miliaceum*) clustered together on clade 1. This deviation suggests that indigenous Australian *Panicum* spp. maintain a unique genetic identity despite the opportunity for possible introgression or hybridisation events experienced during the naturalisation process by their non-native relatives (Hovick and Whitney 2014). However, a broader and geographically defined set of

global *Panicum* spp. specimens are required to further explore this observation.

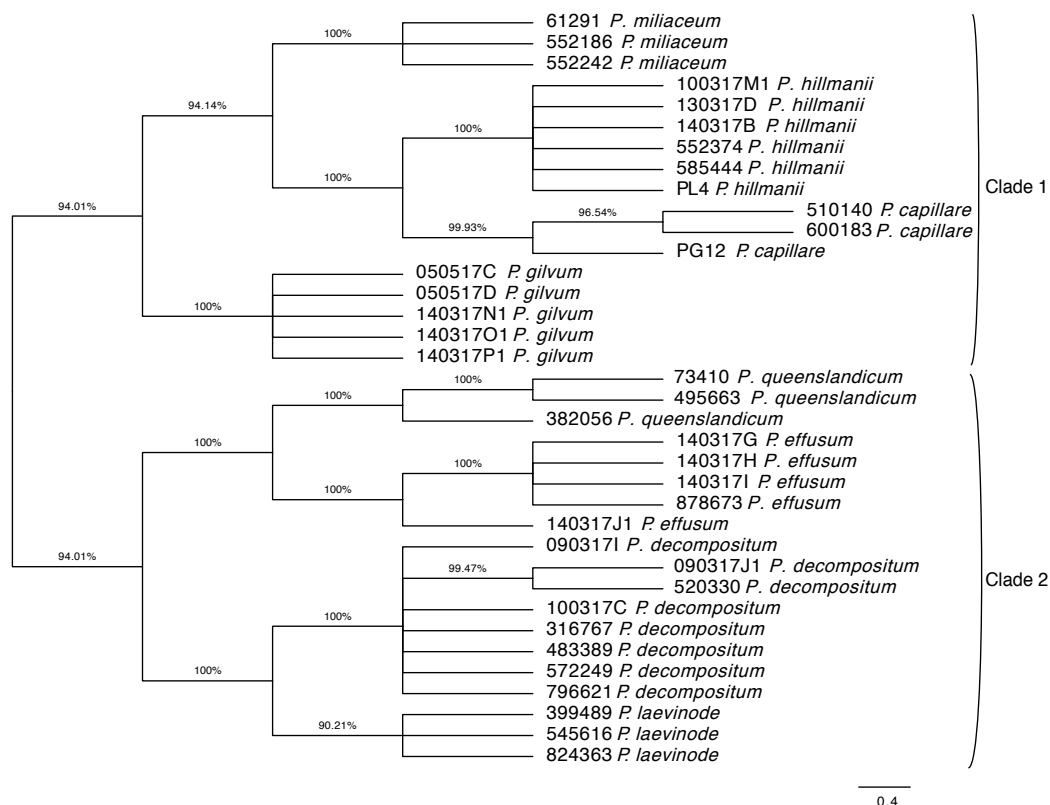
In conclusion, this study indicates that a genetic approach using three concatenated loci provided an accurate and potentially cost effective tool for distinguishing *Panicum* spp. at the species level, regardless of growth stage. Using this method, we confirmed that the majority of *Panicum* specimens collected across the Riverina in 2017 were *P. hillmanii*. This was somewhat unexpected since very few (6) herbarium collections had been made in NSW prior to our study, indicating that the species has been overlooked or its range has expanded relatively recently. Although voucher specimens keyed well to *P. hillmanii* based on morphology, further molecular analysis of samples from American herbaria will be undertaken to confirm whether the name is being correctly applied in Australia.

The genetic approach utilised in this study could have application in: 1) comprehensive surveillance studies of *Panicum* spp.; and 2) the development of more useful diagnostic tools, including loop-mediated isothermal amplification (LAMP), for rapid identification of *Panicum* spp. by stakeholders. Results may also provide improved options for invasive *Panicum* spp. control in Australian pastures and summer fallows.

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**Figure 3.** Bayesian phylogenetic relationships among eight *Panicum* spp inferred from the concatenation of three gene sequences. Clade posterior probability support >90% indicated at nodes.

## REFERENCES

- Aliabadian, M., Kaboli, M., Nijman, V. and Vences, M. (2009). Molecular identification of birds: performance of distance-based DNA barcoding in three genes to delimit parapatric species. *PLoS One* 4, e4119.
- Aliscioni, S.S., Giussani, L.M., Zuloaga, F.O. and Kellogg, E.A. (2003). A molecular phylogeny of *Panicum* (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. *American Journal of Botany* 90, 796-821.
- Byng, J.W. (2014). The flowering plants handbook: a practical guide to families and genera of the world (Plant Gateway, Hertford, United Kingdom).
- CBOL Plant Working Group (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* 106, 12794-97.
- CCHAH, Council of Heads of Australasian Herbaria (2005 onwards) Australian Plant Census. <https://biodiversity.org.au/nsi/services/APC> (accessed 10 April 2018).
- Coissac, E., Hollingsworth, P.M., Laverigne, S. and Taberlet, P. (2016). From barcodes to genomes: extending the concept of DNA barcoding. *Molecular Ecology* 25, 1423-28.
- Darriba, D., Taboada, G.L., Doallo, R. and Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270, 313-21.
- Hollingsworth, P.M., Graham, S.W. and Little, D.P. (2011). Choosing and using a plant DNA barcode. *PLoS One* 6, e19254.

- Hovick, S.M. and Whitney, K.D. (2014). Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. *Ecology Letters* 17, 1464-77.
- Huelsenbeck, J.P. and Ronquist, F. (2001). MR-BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17, 754-5.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28, 1647-9.
- Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33, 1870-4.
- Llewellyn, R.S., Ronning D., Ouzman J., Walker S., Mayfield A. and Clarke M. (2016). Impact of weeds on Australian grain production: the cost of weeds to Australian grain growers and the adoption of weed management and tillage practices Report for GRDC. CSIRO, Australia. [https://grdc.com.au/\\_\\_data/assets/pdf\\_file/0027/75843/grdc\\_weeds\\_review\\_r8.pdf.pdf](https://grdc.com.au/__data/assets/pdf_file/0027/75843/grdc_weeds_review_r8.pdf.pdf) (accessed 17 April 2018).
- Miles, C.O., Wilkins, A.L., Munday, S.C., Holland, P.T., Smith, B.L., Lancaster, M.J. and Enabling, P.P. (1992). Identification of the calcium salt of epismilagenin  $\beta$ -D-Glucuronide in the bile crystals of sheep affected by *Panicum diehotomiflorum* and *Panicum schinzii* toxicoses. *Journal of Agricultural and Food Chemistry* 40, 1606-9.
- Quinn, J.C., Kessell, A. and Weston, L.A. (2014). Secondary plant products causing photosensitization in grazing herbivores: Their structure, activity and regulation. *International Journal of Molecular Sciences* 15, 1441-65.
- Zhu, X., Meyer, L., Gopurenko, D. and Weston, P.A. (2014). Selection of DNA barcoding regions for identification and genetic analysis of two *Echium* invaders in Australia: *E. plantagineum* and *E. vulgare*. Proceedings of the 19<sup>th</sup> Australasian Weeds Conference, ed. M. Baker, pp. 396-400. (Weed Society of Tasmania, Hobart)
- Zuloaga, F.O., Scataglini, M.A. and Morrone, O. (2010). A phylogenetic evaluation of *Panicum* sects. Agrostioidea, Megista, Prionitina and Tenera (Panicoideae, Poaceae): Two new genera, Stephostachys and Sorengia. *Taxon* 59, 1535-46.