DNA barcoding for identification of exotic grass species present in eastern Australia

Aisuo Wang1,2, David Gopurenko1,2, Hanwen Wu1, Rex Stanton1,2 and Brendan J. Lepschi3
1Department of Primary Industries, Wagga Wagga Agricultural Institute, Private Mail Bag, Wagga Wagga, NSW 2650, Australia
2Graham Centre for Agricultural Innovation, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
3Australian National Herbarium, Centre for Plant Biodiversity Research, GPO Box 1600, Canberra, ACT 2601, Australia
(aisuo.wang@dpi.nsw.gov.au)

Summary Grass identification traditionally relies on morphological examination of floral material, and often requires specialist knowledge of morphological characteristics diagnostic for the various genera and species. DNA barcoding may provide a rapid genetic screening tool to identify selected noxious grass weeds at the vegetative growth stage. We tested the utility of 18 chloroplast and nuclear genes as potential DNA barcodes for species identifications of introduced grasses present in Eastern Australia. Grasses examined (N = 417) included Nassella neesiana (Trin. & Rupr.) Barkworth (Chilean needle grass), Nassella trichotoma (Nees) Hack. ex Arechav. (serrated tussock), Eragrostis curvula (Schrad.) Nees (African love grass) and 26 other weed species collected from New South Wales (NSW), Australian Capital Territory (ACT) and other parts of Australia. Our preliminary results revealed three chloroplast genes, matK, ndhK and petL, which exhibit potentials as DNA barcodes for distinguishing and identifying weeds species of interest.

Keywords DNA barcoding, weeds, Nassella neesiana, Nassella trichotoma, Eragrostis curvula.

INTRODUCTION
Invasive grass weeds can cause severe losses to farmers by reducing productivity of grazing land and livestock, and increasing weed control costs. Accurate identification of invasive species, at all stages of growth, allows for efficient control and management of potential infestations.

Grass identification traditionally relies on examination of floral material, and often requires specialist knowledge of the morphological characteristics exhibited in the grasses. The misidentification of weeds might lead to either control measures needlessly being imposed on desirable native species, or lack of prompt control of introduced species.

DNA barcoding (Hebert et al. 2003), an expedient sequence based means of identifying species across all stages of development and even from trace amounts of tissue (Pradosh and Sankar 2013), will be tested here to develop libraries of diagnostic sequences for identifying the key noxious grasses and other morphologically similar grasses present in eastern Australia. Once developed, these DNA barcodes could be used to rapidly screen query specimens collected in the field for identification purposes. This will enable targeting control activities to noxious weed populations in a timely manner, thereby reducing unnecessary spraying and removal of visually similar, more desirable grasses.

In this paper some preliminary results are reported.

MATERIALS AND METHODS
A total of 417 samples of 29 species were included in this study. Material includes live plants sampled from the field in NSW and the ACT (N = 333) as well as herbarium specimens from across mainland Australia, sampled from the collections of the Australian National Herbarium in Canberra (N = 84). Particular emphasis was given to three major weed species, Nassella neesiana (N = 85), Nassella trichotoma (N = 62) and Eragrostis curvula (N = 73). Each specimen was allocated a unique specimen ID for DNA analyses.

DNA was extracted from leaf tissue of each specimen (<1 mg) using a Corbett Research 1820 X-tractor Gene robotic system. Established DNA extraction protocols were followed (Gopurenko et al. 2013).

In order to identify the most suitable markers for DNA barcoding of these weed species, we screened 18 chloroplast and nuclear genes targets (atpF, cemA, G3pdh, infA, ITS, matK, ndhK, petA, petL, psbK, rbcL, rpl16, rps14, tRNA-Leu, tRNA-Ser, tRNA-Thr, trnH and YCF6) to determine levels of PCR fidelity across genera and species (Table 1) and identify intra/ interspecific sequence differences among taxa. All PCR procedures followed Gopurenko et al. (2013) with the exception of primers used in PCR.

Bidirectional sequencing was conducted at the Australian Genome Research Facility for successful PCR products. All ABI trace sequences were quality
checked and assembled using Lasergene SeqMan Pro
ver. 8.1.0(3) (DNASTAR Inc., Maddison, WI, USA). Assembled sequences were exported to BioEdit (Hall
1999) and aligned using ClustalW (Larkin et al. 2007). Sequence alignments were imported into MEGA 6.0
(Tamura et al. 2013) and analysed as genetic distance
trees using the neighbour-joining (NJ) method. Pair-
wise distances among sequences were adjusted as per
the Kimura two-parameter model, and statistical sup-
port for all nodes in trees were estimated by bootstrap
replication (N = 1000 replicates).

RESULTS AND DISCUSSION
Our preliminary results identified three promising
chloroplast genes: matK, ndhK and petL, which PCR
amplified successfully across species, and provided
levels of sequence polymorphisms useful to this
study. These gene targets separate taxa to genus level
and in most cases to species level (Figure 1, Figure 2,
Figure 3). There are, however, clear differences in
the ability of each gene to act as reliable stand-alone DNA
barcode for species identification. For example, N.
neesiana and N. trichotoma are marginally separated
in the NJ tree inferred from matK (Figure 1) and
well separated in the petL tree (Figure 3); however
these two species are poorly separated at the ndhK
tree (Figure 2). In contrast, the ndhK tree provided
clear separation of the Digitaria species [Digitaria
divaricatissima (R.Br.) Hughes and Digitaria brownii
(Roem. & Schult.) Hughes] but not at the petL gene
(Figure 2, Figure 3).

These results suggest that it is unlikely DNA bar-
coding for reliable identification of these taxa is fea-
sible based on a single ubiquitous gene target; rather,
specific genes (and/or combinations of these genes) are
likely to be needed to genetically distinguish between
closely related species within particular genera.

Although we have identified several targets from
the chloroplast genome, which will be useful as DNA
codes at many of the species surveyed, we have not
identified similar informative nuclear genes despite
our screening over several of the commonly targeted
nuclear DNA barcode gene regions. It would be highly
advantageous to identify useful nuclear DNA barcode

Table 1. Summary of specimen sampling and molecular analyses.

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of species</th>
<th>No. of specimens</th>
<th>No. of genes surveyed</th>
<th>Genes surveyed</th>
<th>Partially sequenced genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austrostipa</td>
<td>2</td>
<td>12</td>
<td>2</td>
<td>ndhK, petL</td>
<td></td>
</tr>
<tr>
<td>Chloris</td>
<td>6</td>
<td>50</td>
<td>9</td>
<td>atpF, cemA, infA, ITS, ndhK, petL, rbcL, rps14, tRNA-Ser</td>
<td>atpF, infA, rps14, ndhK, cemA, petL</td>
</tr>
<tr>
<td>Digitaria</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>ndhK, petL</td>
<td>ndhK, petL</td>
</tr>
<tr>
<td>Enteropogon</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>atpF, cemA, infA, ITS, ndhK, petL, rbcL, rps14, tRNA-Ser</td>
<td>atpF, infA, rps14, ndhK, cemA, petL</td>
</tr>
<tr>
<td>Eragrostis</td>
<td>2</td>
<td>77</td>
<td>9</td>
<td>atpF, cemA, infA, ITS, ndhK, petL, rbcL, rps14, tRNA-Ser</td>
<td>atpF, infA, rps14, ndhK, cemA, petL</td>
</tr>
<tr>
<td>Hyparrhenia</td>
<td>1</td>
<td>14</td>
<td>2</td>
<td>ndhK, petL</td>
<td>ndhK, petL</td>
</tr>
</tbody>
</table>
regions, as these will provide species delimitations independent of the chloroplast genome and more importantly provide a comparative means to identify instances of gene flow and or hybridization between taxonomically defined morpho-species. To this end, more nuclear gene targets need to be screened for their utility as DNA barcode markers.

As this study is still at its early stage, the current results are promising. Further study to increase both the taxonomic breadth and the geographic range of species examined, will critically identify levels of intra and inter-specific genetic distances at the assayed genes and determine their reliability as DNA barcodes for accurate species identifications.

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