Plant Systematics and Evolution (2025) 311:29
https://doi.org/10.1007/s00606-025-01959-w

RESEARCH

DNA barcode assessment of *Xanthium* section *Xanthium* cockle burrs in Australia

David Gopurenko[1] · Xiaocheng Zhu[1] · R. M. S. P. Rathnayake[1] · Hanwen Wu[1] · Bruce A. Auld[2] · James Hereward[3] · Heather Smith[4] · Stephen B. Johnson[4] · Karen A. Kirkby[5] · Graham W. Charles[5]

Received: 31 January 2025 / Accepted: 15 July 2025 / Published online: 11 August 2025
© Crown 2025

Abstract

Xanthium cockle burrs are invasive weeds in many countries, affecting local ecosystems and agriculture. All *Xanthium* in Australia are naturalised weeds from multiple overseas sources, but their taxonomic identities are contentious. In particular, species identities of four burr varieties or "complexes" under *Xanthium* section *Xanthium* in Australia are unclear. These complexes, commonly referred to as "Noogoora", "Californian", "Hunter", and "South American" burr, are regarded as either varieties of a single species (*Xanthium strumarium*) or four separate species in the section. To genetically determine the species identities of these complexes in Australia, we compared their chloroplast DNA barcodes to reference sequences of vouchered *Xanthium* species reported by Tomasello (2018). We barcoded 173 plants from 26 sites consisting of single complex burrs and 175 plants from sites containing mixed burr complexes. Single complex sites contained either of two DNA barcode clades. One clade consisting of Noogoora and Californian burrs matched reference sequences of *X. chinense*. The second clade consisting of Hunter and South American burrs matched *X. orientale*. Genetic exchange between these two species in Australia was partially evident at some sites containing sympatric complexes or putative hybrids. Reference *X. strumarium* sequences were unmatched to the DNA barcodes of specimens sampled in Australia, and subsequently, we advise against future claims to a historical presence of this species in Australia. Our findings are significant for taxonomic treatment of *Xanthium* species in Australia and for consideration of source-specific biological controls of these weeds.

Keywords Chloroplast DNA · Naturalised weed · Noogoora burr · *Xanthium strumarium*

Handling Editor: Yun-Peng Zhao.

✉ David Gopurenko
david.gopurenko@dpi.nsw.gov.au

Xiaocheng Zhu
xiaocheng.zhu@dpi.nsw.gov.au

R. M. S. P. Rathnayake
sujeewa.rathnayake@dpi.nsw.gov.au

Hanwen Wu
hanwen.wu@dpi.nsw.gov.au

Bruce A. Auld
bruceaauld@gmail.com

James Hereward
j.hereward@uq.edu.au

Heather Smith
heather.smith@dpi.nsw.gov.au

Stephen B. Johnson
stephen.johnson@dpi.nsw.gov.au

Karen A. Kirkby
karen.kirkby@dpi.nsw.gov.au

Graham W. Charles
graham.charles@dpi.nsw.gov.au

1 NSW Department of Primary Industries and Regional Development (DPIRD), Wagga Wagga Agricultural Institute, Wagga Wagga, NSW 2650, Australia

2 Bruce A. Auld & Associates, "Samara", Borenore, NSW 2800, Australia

3 School of the Environment, The University of Queensland, St Lucia, Brisbane, Qld 4072, Australia

4 NSW DPIRD, Orange Agricultural Institute, Orange, NSW 2800, Australia

5 NSW DPIRD, Australian Cotton Research Institute, Narrabri, NSW 2390, Australia

Introduction

The genus *Xanthium* L. (Asteraceae: Heliantheae: Ambrosiinae) comprises herbaceous plants characterised by the presence of spiny fruiting body, or "cocklebur", capitula (each containing two seeds). These burrs can be dispersed over long distances when entrained in water courses or by entanglement in animal fur (Lechowicz 1984; Hocking and Liddle 1986). The genus is native to areas in Eurasia and the Americas (Millspaugh and Sherff 1919; Widder 1923), but is now naturalised as an introduced weed in many parts of the world. The plant has uses in traditional medicine (Mitich 1987; Khan et al. 2020) and purportedly as a dye (Hicks 1971). It is otherwise an invasive weed which can outcompete endemic and agricultural plants (Oveisi et al. 2013; Qureshi et al. 2019). The plant is highly toxic to domestic animals and some livestock (Cole et al. 1989; Frohne and Pfänder 2005), and entanglement of its burrs affects the quality and processing of wool and fibres (Hocking and Liddle 1986).

Xanthium spp. display considerable phenotypic variation throughout their Old and New World distributions. The taxonomic classification of *Xanthium* species has been contentious. Morphology-based species descriptions in the genus range from the initial report by Linnaeus (1753) of two species (*X. strumarium* L. and *X. spinosum* L.) to more than 20 species variously reported by Widder (1923) and others (Millspaugh and Sherff 1919; Hicks 1971). Influential taxonomic revisions of *Xanthium* are summarised in McMillan (1975), Hocking and Liddle (1986) and more recently by Tomasello (2018), who reported a coalescent-based genetic analysis of 17 vouchered *Xanthium* species. Tomasello (2018) recognised five *Xanthium* species, largely corresponding to the infrageneric sections and series listed by Widder (1923). These included *Xanthium* section *Acanthoxanthium* DC., consisting of two species (*X. spinosum* and *X. ambrosioides* Hook. & Arn.), and *X.* sect. *Xanthium* (=*X.* sect. *Euxanthium* DC.) consisting of three species (*X. strumarium*, *X. orientale*, and *X. chinense*). Recent *Xanthium* works focused on palynotaxonomy (Coutinho et al. 2021), integrative taxonomy (Noedoost and Vaezi 2021), and keyed species listings (Keil and Baldwin 2023) have adopted the taxonomic revisions reported by Tomasello (2018).

In Australia, *Xanthium* are present in all states and territories as naturalised weeds that were introduced from various sources during the last 100 years (McMillan 1973b, 1975). The two *X.* sect. *Acanthoxanthium* species in Australia are readily identifiable by a suite of fixed features and are colloquially named as "Bathurst burr" and "Argentine cocklebur". In contrast, *X.* sect. *Xanthium* in Australia is represented by anywhere between one and four species depending on the taxonomic stance of the reporting authority (McMillan 1975; Moran and Marshall 1978; Hocking and Liddle 1986). McMillan (1973b) referred all *X.* section *Xanthium* in Australia to a provisional *X. strumarium* complex (proposed by Löve and Dansereau (1959)) inclusive of four morpho-physiological complexes resembling *X.* section *Xanthium* species in the Americas. McMillan (1975) subsequently recognised these four morphological complexes as the species *X. chinense*, *X. italicum* Mor., *X. californicum* Greene, and *X. cavanillesii* Schouw. Hocking and Liddle (1986) later recognised a partially different array of four *X. strumarium* complex species in Australia listed as (including colloquial names): *X. occidentale* Bertol. ("Noogoora burr"), *X. italicum* Mor. ("Hunter burr"), *X. orientale* ("Californian Burr"), and *X. cavanillesii* ("South American burr"). The nomenclatures reported by McMillan (1975) and Hocking and Liddle (1986) are summarised in Table 1. The nomenclature reported by Hocking and Liddle (1986) was generally adopted in much of the subsequent reporting of the four burr complexes in Australia, though some reporting bodies conservatively refer only to *X. strumarium* (sensu lato), and in some instances colloquially only to "Noogoora burr complex".

An improved understanding of *Xanthium* sect. *Xanthium* species diversity present in Australia is essential for managing these introduced weeds. For example, implementation of species-centric chemical or biological control programmes to limit cockle burr populations in Australia may prove partially ineffective if a section of the introduced burr

Table 1 Four morphological complexes of *Xanthium strumarium* L. (sensu lato) burr in Australia. Their putative species identities reported by McMillan (1975) and by Hocking and Liddle (1986). The number (*N*) of DNA barcoded Australian specimens obtained from single complex populations and their genetic match to sequence accessions of species recognised by Tomasello (2018) is outlined

Burr complex*	*N*	McMillan (1975)	Hocking and Liddle (1986)	Tomasello (2018)
Noogoora	50	*X. chinense* Mill	*X. occidentale* Bertol	*X. chinense*
Californian	77	*X. californicum* Greene	*X. orientale* L	*X. chinense*
Hunter	27	*X. italicum* Mor	*X. italicum*	*X. orientale*
South American	19	*X. cavanilessii* Schouw	*X. cavanilessii*	*X. orientale*

*Burr complex as reported by Hocking and Liddle (1986); previously McMillan (1975) colloquially named complexes (top down) as: "Chinense", "Pennsylvanicum", "Italicum", and "Cavanilessii"

diversity in Australia has inherent evolved resistance or non-susceptibility to such controls (Morin 2009). DNA barcoding (Hebert et al. 2003) may be useful for distinguishing and identifying different *Xanthium* species in Australia. This genetic approach provided accurate species identifications of other invasive weeds in Australia (Shaik et al. 2016; Zhu et al. 2018) and may be similarly useful for resolving the species identities of *X.* sect. *Xanthium* in Australia. Reference genetic sequences of vouchered *Xanthium* species recognised by Tomasello (2018) are available for comparison to the four morphological complexes of burr in Australia. Here, we broadly sampled the four morphological complexes of *X.* sect. *Xanthium* in Australia and used DNA barcoding to genetically identify each complex to species based on their match to *Xanthium* species recognised by Tomasello (2018).

Materials and methods

Mature *Xanthium* sect. *Xanthium* plants ($N=348$) were sampled from various localities in Australia (Online Resource 1) by NSW DPIRD staff and other contributors (see Acknowledgements). Specimens were identified to burr complex (Table 1) following morpho-diagnostic descriptions of the complexes in Australia as reported by Hocking and Liddle (1986).

Specimens were curated at the Orange Agricultural Institute. These collections included 173 specimens sampled from 26 sites each consisting of a single burr complex. Six additional sites consisted of multiple burr complexes or putative hybrids between complexes, and 175 specimens were sampled. Also, four *X. spinosum* specimens were sampled and used as outgroups in genetic analyses. Specimens were individually recorded to alphanumeric identifiers for cataloguing of their sample details and their identified morphological complex (Online Resource 1). Dried leaf samples of seedlings from field collected fruits which germinated in a glasshouse at Orange Agricultural Institute, were sent to Wagga Wagga Agricultural Institute for genetic analysis.

DNA extractions were performed using GenElute™ 96 Well Tissue Genomic DNA Purification Kit (Sigma-Aldrich) following recommended protocols, including overnight specimen lysis of unground dry leaf tissue (2 mm^2). Twenty-three samples from the four burr complexes were taken from single complex sites and used in pilot DNA barcode tests to assess the utility of nine loci for identifying *Xanthium* species in Australia (Online Resource 2). The pilot included the four loci used for genetic identification of vouchered *Xanthium* species (Tomasello and Heubl 2017; Tomasello 2018) and five other loci broadly informative for plant DNA barcoding (Wang et al. 2017; Zhu et al. 2018). Subsequently, two chloroplast loci, *rps*16-*trn*Q and *psb*A-*trn*H (Table 2) were chosen for broader DNA barcoding of the sampled specimens, and exemplar haplotype sequences of the complexes in Australia were released to GenBank under accessions PQ669692–PQ669705.

Polymerase chain reactions (PCRs) at final volumes of 15 µL were prepared in a Corbett CAS-1200 liquid handling workstation. PCRs used 1 µL of DNA extract in 15 µL reactions; in some instances, extracts were diluted 1:10 with laboratory grade ddH_2O to improve amplifications. PCRs included Thermo Fischer Scientific reagents: 1X buffer, 2.8 mM MgCl2, 0.4 units of Platinum® Taq polymerase (Invitrogen), 200 µM dNTPs, and 2 µM each of forward and reverse oligo-nucleotide primers. Reactions were run in an Eppendorf Mastercycler set with a universal thermal profile for all primer combinations: 95 °C for 2 min; 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s; 72 °C for 5 min; storage at 4 °C. PCR products stained with SYBR™ Safe (Invitrogen) were visualised on a BioRad UV transilluminator after electrophoresis through a 1.5% agarose gel in 1% TAE buffer. Stained PCR products were qualitatively checked for expected fragment size against E-Gel 100-bp ladder size marker (Invitrogen). PCR products were bi-directionally Sanger sequenced at the Australian Genome Research Facility (AGRF; Brisbane node). AB1 chromatogram sequences were quality-checked, aligned to primer truncated reference sequences, and saved as FASTA format alignments using SeqMan Pro ver. 8.1.0(3) (DNASTAR, Inc., Maddison, WI, USA). Additional edits to FASTA alignments were accomplished using BioEdit ver. 7.0.5.3 (Hall 1999) and FABOX (Villesen 2007).

Table 2 Primers used to PCR two plastid loci. Directions of forward (_F) and reverse (_R) primers denoted by suffix at primer names. Loci reported in Tomasello and Heubl (2017) and used in Tomasello (2018) for taxonomic revisions of *Xanthium*

Locus	Primer name	Sequence 5'−3'	Source
*psb*A-*trnH*			
	trnH(GUG)_F	ACTGCCTTGATCCACTTGGC	(Hamilton 1999)
	psbA_R	CGAAGCTCCATCTACAAATGG	(Hamilton 1999)
*rps*16-*trn*Q			
	trnQ_F	CCCGCTATTCGGAGGTTCGA	(Calviño and Downie 2007)
	rps16-1r_R	ATCGTGTCCTTCAAGTCGCA	(Calviño and Downie 2007)

DNA barcode analyses of all Australian specimens included GenBank sequence accessions (KY215737—KY215804) of vouchered Northern hemisphere specimens used in recent support of a revised species taxonomy of the *Xanthium* genus (Tomasello 2018). Sequence haplotype variation among loci and specimens was examined using MEGA version X (Kumar et al. 2018). Pairwise genetic distances (unweighted) among sequences were calculated using MEGA. Unidentified nucleotide sites and indels at loci were excluded from the pairwise comparisons. Genetic distance relationships among sequences were inferred and visualised using neighbour joining (NJ) tree analysis. Bootstrap replication (10,000 replicates) determined the level of support for nodes in NJ trees.

Results

Pilot PCR trials of nine loci identified two informative and reliable loci (*rps*16-*trn*Q and *psb*A-*trn*H) for our broader DNA barcoding of *Xanthium* samples (Online Resource 2). The seven other loci were either uninformative for genetic separation of the four complexes in Australia, or they failed to adequately PCR amplify from most (> 70%) specimens. We note the nuclear encoded ITS and *D35* loci reported (Tomasello 2018) jointly with other loci for genetic identification of *Xanthium* species, lacked any variation for distinguishing the four burr complexes in Australia. The plastid DNA *rps*16-*trn*Q intergenic spacer locus was optimal as a DNA barcode in our study. PCR products 954–958 bp were reliably amplified from 348 *X.* sect. *Xanthium* specimens and their sequences resolved as four haplotypes (Online Resource 1). These haplotypes contained two informative nucleotide sites for distance-based separation of the four burr complexes as two genetic groups (Fig. 1 and Online Resource 3).

Neighbour joining analysis indicated the *rps*16-*trn*Q locus was effective as a stand-alone DNA barcode for *in -silico* identification of voucher reference sequences reported to *X.* sect. *Xanthium* species by Tomasello (2018). Of the specimens sampled from single complex sample sites, one genetic clade consisting entirely of Noogoora burr and Californian burr was genetically matched (99.99–100% similarity) to Old and New World voucher accessions of *X. chinense* recognised by Tomasello (2018). The second genetic clade contained all Hunter burr and South American burr sequences, and these matched (100%) to accessions of *X. orientale* recognised by Tomasello (2018). Additional sequences of four

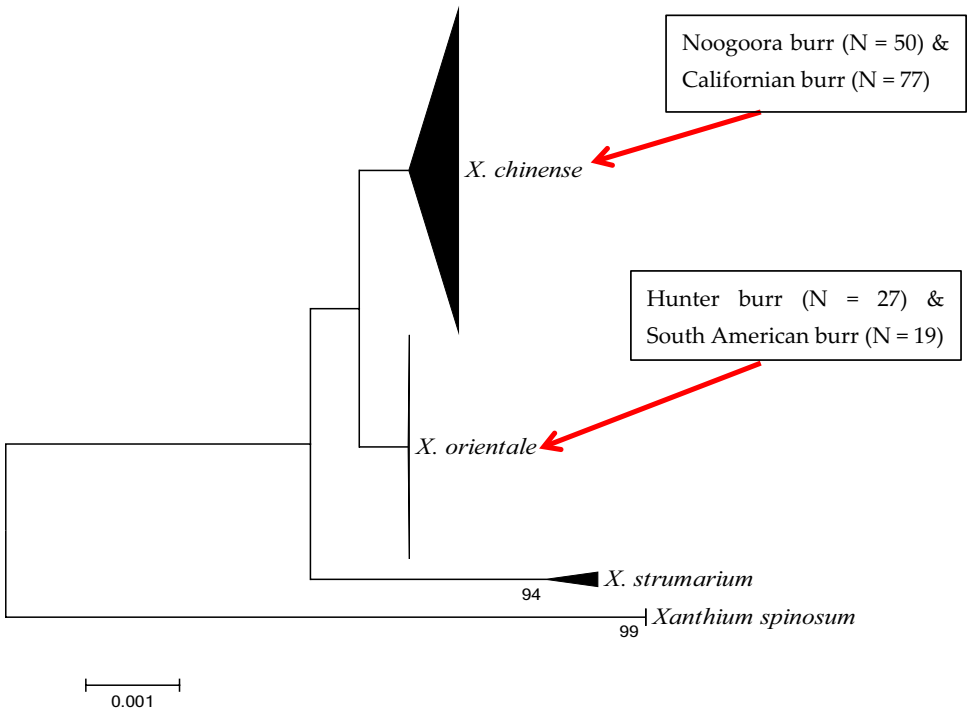


Fig. 1 Neighbour joining tree of pairwise *rps*16-*trn*Q sequence distances among sampled burrs and references of *Xanthium* species. Enclosed boxes indicate burr complexes (Noogoora, Californian, Hunter and South American) sampled (N) from single complex populations in Australia and their genetic match (red arrow) to references of *X. chinense* Mill. and *X. orientale* L. recognised by Tomasello (2018). References of *X. strumarium* L. are unmatched to Australian specimens. Outgroup *X. spinosum* L includes four sampled Bathurst burr. Terminal clades collapsed to indicate depth of intraspecific diversity, scale bar equals 0.1% sequence difference. Clade supports at nodes (> 70%) as shown. Un-collapsed version of this distance tree inclusive of all sampled specimens is seen in Online Resource 3

Bathurst burr (*X. spinosum*) specimens sampled in Australia were genetically matched to an outgroup clade with other reported accessions of the species.

A 2nd plastid locus (*psb*A-*trn*H intergenic spacer) was amplified from 107 *X.* sect. *Xanthium* specimens (representing > 72% of 150 tested specimens) to obtain sequences 408–424 bp long. The sequences resolved as two haplotypes (Online Resource 1) that differed by a single nucleotide. These two haplotypes separated the four complexes sampled from single complex sites as two genetic clades, consistent with the separation of specimens evidenced at the *rps*16-*trn*Q locus. The two *psb*A-*trn*H haplotypes were matched to reference accessions of both *X. chinense* and *X. orientale*, and neighbour joining analysis of the locus (Online Resource 4) indicated *X. chinense* was paraphyletic with respect to *X. orientale*. References of the two species could not be totally distinguished at this locus, and it was impossible to separately assign their identities to the two *psb*A-*trn*H clades found among Australian burr complexes. An additional sequence of a Bathurst burr specimen was correctly assigned to accessions of *X. spinosum*.

Three NSW sites contained sympatric plants of two or more non-intergraded complexes (Table 3). DNA barcodes from Hunter burr and Noogoora burr plants co-present at Bullawa creek ($N=46$) and Griffith ($N=12$) all conformed with the genetic segregation of the two complexes to *X. orientale* and *X. chinense*, respectively as evidenced at *rps*16-*trn*Q. This was consistent with that observed at single complex sites where all plants of a complex were genetically identified to a single *Xanthium* species. In contrast, DNA barcodes from Hunter burr and Noogoora burr plants co-present at Wee Waa ($N=47$) did not all conform with the genetic segregation of the complexes to *X. orientale* and *X. chinense*. Some plants at the site exhibited a DNA barcode of the co-occurring *Xanthium* species. A single Californian burr at the Wee Waa site, had a *X. chinense* DNA barcode consistent with that seen at sites consisting only of this species.

Three NSW sites, where each had morphological intergrades between burrs (and by inference putative hybrids), varied in genetic outcome (Table 3). The intergrades between Californian and South American burrs at Ashley ($N=8$) had *rps*16-*trn*Q DNA barcodes of either *X. orientale* or *X. chinense*. In contrast, the intergrades between Hunter burr and other complexes at Gunnedah ($N=22$) were all fixed for DNA barcodes of *X. orientale* typically seen at sites consisting only of Hunter or South American burr. Similarly, plants at Baan Baa identified as Noogoora burr ($N=25$) or intergrades between Californian and Hunter burr ($N=12$) were all fixed for *X. chinense* barcodes typically seen at single complex sites consisting of Californian or Noogoora burr. Two burrs at Jerry's Plains ($N=2$) had *X. chinense* barcodes, in an otherwise homogenous sample of Hunter burrs which all exhibited *X. orientale* barcodes. A retrospective morphological examination of these two aberrant specimens identified them as intergrades of Californian and Hunter burrs, and similar to the intergrades identified at Baan Baa. Similarly, one burr at Boundary Bend with a *X. orientale* barcode in an otherwise homogenous sample of Californian burrs was retrospectively identified as an intergrade of Californian/South American burrs.

None of the *rps*16-*trn*Q or *psb*A-*trn*H sequences from Australian burr specimens was matched to Northern hemisphere reference accessions of *X. strumarium* L. (sensu stricto) recognised by Tomasello (2018) (Fig. 1., Online Resource 3 and 4).

Table 3 Presence or absence of genetic hybrids detected at sites containing morphological intergrades between *Xanthium* complexes, or at sites containing sympatric complexes but no intergrades. Presence of hybrids reported as "fixed" for a single-species DNA barcode among all plants, or "admixed" for reciprocal sharing of species DNA barcodes among plants. Absence of hybrids reported as "no genetic exchange". Quality of soils at sample sites (high or low) as indicated

Intergrades	Sympatric complexes no intergrades
Baan Baa^{H}: fixed	Bullawa^{L}: no genetic exchange
Gunnedah^{H}: fixed	Griffith^{L}: no genetic exchange
Ashley^{H}: admixed	WeeWaa^{H}: admixed

H: high−quality floodplain soil

L: low−quality sandy creek bed soil

Discussion

A global perspective of *Xanthium* taxonomy and the naturalised complexes in Australia

Considerable uncertainty exists concerning species identities of *Xanthium* section *Xanthium* in Australia. This is reflective of the overall taxonomic uncertainty concerning the genus following the first formal description of *Xanthium* species (*X. strumarium* and *X. spinosum*) by Linnaeus (1753) over 270 years ago. Widder (1923) recognised two species under *Xanthium* section *Acanthoxanthium* and more than 18 other species placed under *X.* sect. *Xanthium* (formerly *X.* sect. *Euxanthium*). In contrast, Löve and Dansereau (1959) recognised just two species in the genus and reduced all *X.* section *Xanthium* taxa reported from the New World to a polymorphic *X. strumarium* complex. This widely adopted classification was used to conservatively assign four informal morpho-complexes of *X.* sect. *Xanthium* taxa found in Australia

under the general description of *X. strumarium* (sensu lato) (McMillan 1973b, 1975; Moran et al. 1981; Hocking and Liddle 1986). The four complexes occupy markedly different sized areas in Australia, and this may be due to differences in their reproductive strategies and/or photoperiodic requirements for flowering (Moran et al. 1981). Further species delimitation of the four informal complexes in Australia has been contentious. This taxonomic uncertainty is in part compounded by subtlety and lability of primary morphological features used to distinguish the four complexes to their putative species designations. The most recent delimitation of global *Xanthium* species proposed by Tomasello (2018) provides a comparative genetic and taxonomic framework to investigate the four morphological complexes of burr observed in Australia. Tomasello (2018) coalescent-based delimitation of species in *Xanthium*, using multi-locus sequences from museum vouchered Old and New World specimens, supported recognition of five morphologically keyed species sorted between *X.* sect. *Acanthoxanthium* [species: *X. ambrosioides* and *X. spinosum*] and *X.* section *Xanthium* [species: *X. chinense*, *X. orientale* and *X. strumarium*]. Preliminary genomic sequencing of over 730 loci from *Xanthium* reference specimens reported by Tomasello and Manzo (2023) is further supportive of the species recognised by Tomasello (2018).

Our DNA barcoding of plastid genes sampled from single complex sites strongly supports the presence in Australia of two of the three species of *Xanthium* section *Xanthium* recognised by Tomasello (2018). Accordingly, the colloquially named "Noogoora" and "Californian" burr complexes in Australia reported by Hocking and Liddle (1986) are genetically identical and match to accessions of *X. chinense*, whereas the "Hunter" and "South American" burrs match to *X. orientale*, as recognised by Tomasello (2018). Including the two readily identifiable *X.* sect. *Acanthoxanthium* species not targeted in our sample (*X. spinosum* and *X. ambrosioides*), we report in total four *Xanthium* species present in Australia, each of which can be genetically identified by the match of their DNA barcodes to those reported by Tomasello (2018).

Interestingly, DNA barcodes of the Mediterranean-based species *X. strumarium* (sensu stricto) obtained from Northern hemisphere vouchers (Tomasello 2018) were entirely absent among our samples from Australia. Therefore, we argue against future taxonomic reference to a presence of this species in Australia, despite its pervasive listing in all earlier reports of *Xanthium* in the country. The prior reporting of *X. strumarium* (in either the general or strict sense) in Australia is attributed to the widely accepted provisional classification by Löve and Dansereau (1959) which conservatively placed all proposed *X.* sect. *Xanthium* species under a polymorphic *X. strumarium* complex. Also, we argue against further reference to the earlier species identities reported for the four burr complexes by McMillan (1975) and or by Hocking and Liddle (1986). Those species identities differentially reported by McMillan (1975) and Hocking and Liddle (1986) are genetically reduced under two of the *X.* section *Xanthium* species recognised by Tomasello (2018), as we discussed above and presented in Table 1.

Our study here does not investigate the reliability of descriptive morphological features reported by Hocking and Liddle (1986) for diagnostic identification of the four burr complexes in Australia. Nor have we determined their potential utility to differentiate between the two *Xanthium* Sect. *Xanthium* species that we have genetically recognised in Australia. These informal descriptions, and the *Xanthium* species key reported by Tomasello (2018), are subject to analyses we have in progress to provide a formal dichotomous key for identification of the *Xanthium* taxa in the country. As a caveat, we note that the defining morphological features of these complexes reported by McMillan (1975) and/or by Hocking and Liddle (1986) are subtle, partly overlapping and potentially phenotypically plastic under various environmental conditions and plant growth stages (Martin 1981; Moran et al. 1981). This feature plasticity may have partly confounded earlier taxonomic identification and distribution records of the complexes in Australia. Promisingly, we note from the literature that measurement of palynotaxonomic features assisted efforts to identify *Xanthium* taxa in the Iberian Peninsula (Coutinho et al. 2021). Analyses of pollen features and/or other potentially species informative micro-phenotypic traits such as trichomes (Eyvazadeh Khosroshahi and Salmaki 2019) may provide robust approaches to morphologically distinguish the *Xanthium* species and/or the complexes in Australia,

Our work here has relevance for future diagnostic genetic identifications of the *X.* sect. *Xanthium* species in Australia. In preparation for our broader all-specimen examination, we conducted a pilot genetic analysis of burr exemplars to evaluate the effectiveness of nine loci targeted for the DNA barcode identification of *Xanthium* species. This included four loci reported by Tomasello (2018) for species delimitation of vouchered *Xanthium*, and five other loci used for reported DNA barcoding of other plant species (Table 2). The later five loci were excluded as uninformative or unreliable for use with *Xanthium*, and we similarly excluded the two nuclear loci (ITS and D35) reported by Tomasello (2018) because they lacked informative nucleotide variation for genetic separation of the Australian specimens of *X.* sect *Xanthium* (at the ITS locus) and/or they exhibited low PCR recovery from specimens (at the D35 locus). These two nuclear loci were useful for delimiting infrageneric sections in *Xanthium* but lacked power to individually delimit each of the five species recognised by Tomasello (2018). Subsequently we

chose two maternally inherited chloroplast loci (*rps*16-*trn*Q and *psb*A-*trn*H) reported by Tomasello (2018) for our DNA barcode analysis of Australian specimens. Both loci can be used as "stand-alone" target loci for congruently delimiting the four burr complexes to two genetic clades. However, we observed higher levels of nucleotide variation and PCR amplification reliability at the *rps*16-*trn*Q locus. Furthermore, the phylogenetic relationship between *X. chinense* and *X. orientale* references was unresolved at the *psb*A-*trn*H locus, with some evidence of incomplete sorting of haplotypes among northern hemisphere references of the two species. Therefore, we recommend use of the *rps*16-*trn*Q locus as a stand-alone DNA barcode locus for reliable and informative delimitation of *Xanthium* species from single complex populations in Australia. The current absence of any biparentally inherited nuclear loci reliably informative as DNA barcodes for delimitation of all the *X.* sect *Xanthium* species poses a challenge for diagnostic surveys that encompass hybrid plants (next section). We recommend further screening of other potentially informative nuclear loci to fill this purpose, and/or the use of contemporary genomic sequencing approaches (our work in progress).

Hybrid *Xanthium* populations in Australia

Xanthium hybrids have been documented in natural populations and also in controlled breeding experiments (Millspaugh and Sherff 1919; Widder 1923; Löve and Dansereau 1959; McMillan 1973a, 1974, 1975). They are apparent within each of the two *Xanthium* sections, but never between the sections (Widder 1923; Löve 1975; McMillan 1975). McMillan (1975) documented the existence of natural hybrids among sympatric *X.* section *Xanthium* complexes in NSW Australia. The hybrids were detected as morphological intergrades between complexes, and were observed in sympatric populations of Noogoora burr (*X. chinense*) and Hunter burr (cited as *X. italicum*, now *X. orientale*) (McMillan 1975), but not in sympatric populations of Noogoora burr (*X. chinense*) and South American burr (cited as *X. cavanilessii*, now *X. orientale*). Intergrades of the latter two complexes were evident in a historical plant collection from the Nepean River area west of Sydney (McMillan 1975). McMillan (1975) noted that populations of Californian burr (cited as *X. californicum*, now *X. chinense*) were geographically isolated from the other complexes in Australia and lacked any observed natural hybrids.

Experimental crosses between various *X.* sect. *Xanthium* taxa from the Americas and the Australian complexes in most instances produced intermediate forms with high pollen viability at F1 and F2 progenies (McMillan 1974, 1975). In contrast, F1 crosses between Australian complexes identified here to *X. chinense* and *X. orientale* were chlorotic and stunted but were improved when their soils where enriched. McMillan (McMillan 1974, 1975) proposed that natural populations of interfertile hybrids in Australia are more likely to be sustained at high-nutrient habitats favourable for their development. McMillan (1975) noted, for example, that intergrades between Noogoora burr and Hunter burr are common in floodplain populations but are rare or absent at roadside populations of parental plants. Our sampling and genetic analyses of intergrade burr populations and/or co-existing burr complexes (Table 3) generally agree with McMillan's reporting, although some differences are also apparent.

For example (Table 3), we sampled plants from Baan Baa which exhibited as morphological intergrades between Californian burr (*X. chinense*) with either South American burr or Hunter burr (both *X. orientale*). All intergrade specimens had DNA barcodes of *X. chinense* typically observed in single complex populations of Californian burr or Noogoora burr. The complete absence among the intergrade specimens of any *X. orientale* DNA barcodes (typically observed in single complex populations of South American burr and Hunter burr) indicates the maternally inherited cpDNA contained in the ovules of Californian burr was possibly transmitted to and fixed among hybrid intergrade plants via a process of chloroplast capture (Rieseberg 1991). Also, this may be evident at Gunnedah consisting entirely of intergrades between Hunter burr and other complexes, which at that site were all fixed for *X. orientale* barcodes typically observed at single complex populations of Hunter burr. In contrast to the above, intergrades of Californian and South American burrs at Ashley exhibited either *X. orientale* or *X. chinense* DNA barcodes. This would be expected under a scenario whereby bidirectional nuclear exchange between sympatric plants (with or without subsequent backcrosses) produces plants with admixed combinations of chloroplast and nuclear genomes. Our genetic analysis does not include biparentally inherited informative genes for direct comparisons to the maternally inherited cpDNA barcodes that we assayed; subsequently, we cannot explicitly comment on the degree and direction of nuclear exchange among the putative hybrid complexes reported here. We have work in progress involving representative genomic screening of the plants to report this aspect of our *Xanthium* sample.

We sampled three sites that were each sympatric for Hunter burrs and Noogoora burrs but lacking any obvious morphological intergrades between the two complexes (Table 3). At Bullawa Creek and Griffith, the two complexes were found to be genetically segregated as *X. orientale* and *X. chinense*, in accordance with their species identities observed at single complex populations of each burr. Despite their sympatric proximity, there was no evidence of hybridisation between the complexes at these sites. In contrast at Wee Waa, co-occurring and morphologically distinct Hunter burr and Noogoora burr plants shared *X. orientale*

and *X. chinense* DNA barcodes, evidencing some level of interspecies hybridisation and/or backcrossing between the complexes in the absence of any discernible morphological intergrades.

Based on the variable genetic profiles evidenced among the sites containing intergrades and/or sympatric *Xanthium* complexes (Table 3), we propose that hybridisation among the complexes in Australia is subject to the genetic backgrounds of each complex and their responses to environmental factors where they are present. Hybrid emergence at sites of sympatry may only be promoted by habitat and environmental conditions that favour genetic exchange and subsequent growth of interfertile crossed plants. Reported *Xanthium* complex distributions in Australia suggest single and/or sympatric complex populations are more prevalent than hybrid populations. This may not be surprising as the complexes in Australia are mainly self-pollinated (autogamy), and as they are derived from different geographic sources, they exhibit little or no overlap in their reproductive timing and photoperiodic requirements for flowering (McMillan 1974, 1975; Moran et al. 1981). Genetically separate *X.* sect. *Xanthium* species in Italy are similarly autogamous in their mating systems, and possibly, this is an adaptation in the genus to ensure continuity of successful progenitor genotypes in diverse habitats (Dinelli et al. 2003).

To a lesser extent, *Xanthium* are wind-pollinated, and in some circumstances, this may favour hybrid emergences at sites where environmental conditions allow for overlap of the reproductive strategies between co-occurring complexes. Edaphic factors may also limit hybrid emergence and/or persistence at sites. The controlled breeding experiments by McMillan (1974) clearly indicated hybrids of the two most widespread complexes (Noogoora and Hunter burrs) were stunted and chlorotic in depleted soils otherwise suitable for growth of the parental complexes. The hybrids successfully developed to burr production after soils were improved with nutrients and chelating agents. Subsequently McMillan (1975) proposed the *Xanthium* hybrids in Australia are more likely to be sustained at floodplain sites containing high-nutrient soils as opposed to sites containing depleted soils.

Our genetic analysis of hybrid and sympatric complexes obtained from NSW floodplains containing agricultural quality soils (Ashley, Baan Baa, Gunnedah, and Wee Waa), and from sandy creek bank sites (Bullawa and Griffith) containing organically depleted soils (Table 3) generally is consistent with McMillian's proposal. At the latter two depleted soil sites, there was no evidence of genetic exchange between sympatric populations of Noogoora burr (*X. chinense*) and Hunter's burr (*X. orientale*). In contrast, DNA barcode sharing between the two complexes was evident at a floodplain site in Wee Waa, in the absence of any apparent morphological intergrades. Three sites containing morphological intergrades between otherwise genetically distinct complexes were all sampled from floodplain sites, and each showed some form of genetic exchange.

Implications for biocontrol

Management of invasive and naturalised weeds is underpinned by a robust understanding of their species taxonomy. Such information is critical for accurate reporting of weed biology, ecology, and distribution. Further, effective use of biological control agents (BCAs) for the management of a specific host weed is reliant on accuracy of taxonomic assignments at the species and or sub-species level, of both the host and its controlling agent. BCAs that evolved with and detrimentally impact a specific host plant lineage may have little or no effect when exposed to other related hosts (Morin 2009). The genetic evidence here of two genetically distinct but outcrossing populations of *X.* sect. *Xanthium* in Australia needs to be considered relative to the ecological and physiological aspects of each, and this may have direct relevance regarding the efficiency of proposed BCAs for affecting the genetic "species" and hybrids present in Australia.

Biological control programmes in Australia targeting Noogoora burr date back to 1929, and since then, a variety of BCAs have been trialled and/or released in the country with outcomes ranging from failure to partial success (see review (Van Klinken and Julien 2003)). The most successful of these is the exotic rust fungus, *Puccinia xanthii* Schw., which was accidentally or illegally introduced during 1975 and subsequently impacted on the "Noogoora burr complex" (inclusive of the four complexes) in eastern Australia but had limited effect on populations in the far north of the country (Morin et al. 1994). Failure of the introduced rust strain to impact on the northern *Xanthium* populations was explained as tropical conditions and environmental constraints affecting the rust itself (Van Klinken and Julien 2003). Novel tropical strains of *P. xanthii* impacting on other *Xanthium* taxa in the Dominican Republic and Mexico were later trialled under quarantine but failed to infect any Australian Noogoora burr (Morin 2009). Failure of this latter rust strain to affect Noogoora burr was evidenced as variation in resistance among genetically unrelated *Xanthium* taxa in Australia and the American central tropics. Our sample of 24 Noogoora burr specimens from Northern Australia all had DNA barcodes which conformed with the identification of Noogoora burr in eastern Australia to *X. chinense*. Our genetic results offer some support to the proposal that environmental constraints on the BCA rather than host genetics have limited the effectiveness of the rust as a control of the

weed in Northern Australia (Van Klinken and Julien 2003). *Verticillium dahliae* Kleb. is another fungal patho-agent which shows promise for the control of some portion of the *Xanthium* in Australia. One particular strain of the pathogen present in Australia (Kirkby et al. 2022) was characterised by its necrotic impact on Noogoora burr (genetically identified as *X. chinense*) during controlled pathogenicity testing experiments. The efficacy of this pathogen to control *X. chinense* more broadly in Northern Australia and the other burrs genetically placed to *X. orientale* is yet to be determined.

Conclusions

Our study provides a foundational reference for the future taxonomic reporting of introduced *Xanthium* section *Xanthium* species in Australia, with the intention of being used by both federal and state research institutions, as well as their related reporting and regulatory agencies dealing with these weeds. A crucial aspect of this implementation will involve the retrospective amendment of online taxonomic records concerning the presence and distribution of *Xanthium* species within the country. For instance, we assert that it is no longer justifiable to claim the presence of *X. strumarium* (sensu stricto) in Australia, as the reference DNA barcode sequences from northern hemisphere specimens of this species do not match any sequences from the *Xanthium* samples collected in Australia. Given the increasing rate of international biological introductions associated with global trade, it is plausible that exotic *X. strumarium* will eventually crossover international borders into Australia. In such scenarios, it is imperative that diagnostic genetic identification of this potential new weed is recognised, and that biosecurity agencies are promptly alerted and act in response to its invasive presence, rather than being hindered by ongoing taxonomic ambiguities regarding its previously and erroneously reported presence in the country.

Information on Electronic Supplementary Material

Online Resource 1. Specimen sample summary.
Online Resource 2. PCR primers used in pilot tests of nine loci.
Online Resource 3. Neighbour–joining tree of (*rps*16-*trn*Q) pairwise genetic distances.
Online Resource 4. Neighbour–joining tree of (*psb*A-*trn*H) pairwise genetic distances.
Online Resource 5. All specimen FASTA aligned sequence data for the *rps*16-*trn*Q locus.
Online Resource 6. All specimen FASTA aligned sequence data for the *psb*A-*trn*H locus.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00606-025-01959-w.

Acknowledgements We thank the following for their sampling assistance: Sally Dickinson, Greg Kauter, Bert Lukitsch, Peter Michael, Janelle Montgomery, Cathy Semmler, Amanda Thomas, Annabel Twine, Stacey Vogel, Jo Williams. We gratefully acknowledge comments on the manuscript by Dr. Karen Bell, Dr. Bernie Dominiak, and anonymous reviewers.

Author contributions Conceptualization, GC, KK, SJ, DG; Data curation, DG, BA, SJ; Formal analysis, DG; Funding acquisition, GC, SJ; Investigation, XZ, RR, BA, HS; Methodology, DG; Project administration, DG, HW, SJ, GC; Resources, BA, HS, SJ; Supervision, DG, HW, SJ, GC; Validation, JH, KK; Writing—original draft, DG; Writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This research project was funded through the Australian Government Department of Agriculture and Water Resources, "Control Tools and Technologies for Established Pest Animals and Weeds Programme", grant number CT10. The project received additional financial support from the Cotton Research and Development Corporation, NSW Dept Primary Industries and the University of Queensland.

Data availability statements Haplotype sequences reported here are deposited at NCBI GenBank (accessions numbers PQ669692—PQ669705) and listed to total project specimen records reported in Supplementary materials: Online Resource 1. FASTA format aligned sequence data of all specimens are available at Online Resource 5 for the *rps*16-*trn*Q locus, and at Online Resource 6 for the *psb*A-*trn*H locus.

Declarations

Conflict of interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Calviño CI, Downie SR (2007) Circumscription and phylogeny of Apiaceae subfamily Saniculoideae based on chloroplast DNA sequences. Molec Phylogen Evol 44:175–191. https://doi.org/10.1016/j.ympev.2007.01.002

Cole RJ, Cutler HG, Stuart BP (1989) Carboxyatractyloside. In: Cheeke PR (ed) Toxicants of plant origin: glycosides. 1st edn. CRC Press, Boca Raton, pp 253–263. https://doi.org/10.1201/9781003418276-9

Coutinho AP, Silveira P, Pita C, Santos MJ, Saraiva C, Perpétuo NC (2021) Pollen morphology of *Xanthium* L. (s.l.) (Asteraceae, Asteroideae, Heliantheae, Ambrosiinae) in the Iberian Peninsula—a palynotaxonomic approach to a poisonous, allergenic and invasive genus. Grana 60:35–56. https://doi.org/10.1080/00173134.2020.1737729

Dinelli G, Bonetti A, Viggiani P (2003) Genetic structure and mating system of Italian *Xanthium strumarium* complex. Weed Sci 51:69–77. https://doi.org/10.1614/0043-1745(2003)051[0069:GSAMSO]2.0.CO;2

Eyvazadeh Khosroshahi E, Salmaki Y (2019) Evolution of trichome types and its systematic significance in the genus *Phlomoides* (Lamioideae–Lamiaceae). Nordic J Bot. https://doi.org/10.1111/njb.02132

Frohne D, Pfänder HJ (2005) Poisonous plants: a handbook for doctors, pharmacists, toxicologists, biologists and veterinarians. Timber Press, London

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Molec Ecol 8:521–523. https://doi.org/10.1046/j.1365-294X.1999.00510.x

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proc Roy Soc B Biol Sci 270:313–321. https://doi.org/10.1098/rspb.2002.2218

Hicks AJ (1971) Systematic Studies of *Xanthium* (Compositae: Ambrosieae). The Cockleburs of Tazewell County, Illinois. PhD Thesis, University of Illinois at Urbana-Champaign, Urbana-Champaign

Hocking P, Liddle M (1986) The biology of Australian weeds: 15. *Xanthium occidentale* Bertol. complex and *Xanthium spinosum* L. J Austral Inst Agric Sci 52:191–221

Khan Y, Shah S, Ullah S (2020) Ethnomedicinal, pharmacological and phytochemical evaluation of *Xanthium strumarium* L. Int J Biol Sci 11:587

Kirkby K, Webster J, Landa BB, Olivares C, Roser S, Falconer L, Gopurenko D, Chapman TA (2022) First report of the presence of *Verticillium dahliae* VCG6 in Australia. Austral Pl Dis Notes 17:9. https://doi.org/10.1007/s13314-022-00455-1

Van Klinken RD, Julien MH (2003) Learning from past attempts: does classical biological control of Noogoora burr (Asteraceae: *Xanthium occidentale*) have a promising future? Biocontrol Sci Technol 13:139–153. https://doi.org/10.1080/0958315021000073420

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molec Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lechowicz MJ (1984) The effects of individual variation in physiological and morphological traits on the reproductive capacity of the common cocklebur, *Xanthium strumarium* L. Evolution 38:833–844. https://doi.org/10.2307/2408394

Linnaeus Cv (1753) Species Plantarum. Impensis Laurentii Salvii, Holmiae

Löve D (1975) The genus *Acanthoxanthium* (DC.) Fourr. revived. Lagascalia 5:55–72

Löve D, Dansereau P (1959) Biosystematic studies on *Xanthium*: taxonomic appraisal and ecological traits. Canad J Bot 37:173–208. https://doi.org/10.1139/b59-016

Martin RJ (1981) Distribution, ecology and control of *Xanthium* species. Australian National University, Canberra

McMillan C (1973a) Partial fertility of artificial hybrids between Asiatic and American cockleburs (*Xanthium strumarium* L.). Nat New Biol 246:151–153. https://doi.org/10.1038/newbio246151b0

McMillan C (1973b) Photoperiodic adaptation in *Xanthium strumarium* populations introduced to Australia. Canad J Bot 51:221–229. https://doi.org/10.1139/b73-030

McMillan C (1974) Experimental hybridization in *Xanthium strumarium* of American complexes with diverse photoperiodic adaptation. Canad J Bot 52:849–859. https://doi.org/10.1139/b74-107

McMillan C (1975) The *Xanthium strumarium* complexes in Australia. Austral J Bot 23:173–192. https://doi.org/10.1071/BT9750173

Millspaugh CF, Sherff EE (1919) Revision of the North American species of *Xanthium*. Field Museum of Natural History, Chicago

Mitich LW (1987) Cockleburs. Weed Technol 1:359–360. https://doi.org/10.1017/S0890037X00029894

Moran GF, Marshall DR (1978) Allozyme uniformity within and variation between races of the colonizing species *Xanthium strumarium* L. (noogoora burr). Austral J Biol Sci 31:283–292

Moran GF, Marshall DR, Müller WJ (1981) Phenotypic variation and plasticity in the colonizing species *Xanthium strumarium* L. (Noogoora burr). Austral J Biol Sci 34:639–648

Morin L, Auld BA, Brown JF, Cholil MA (1994) Pathogenic fungi occurring on the Noogoora burr complex in Australia. Proc Linn Soc NSW 114:133–148

Morin L (2009) Enhancing Noogoora burr biocontrol in Northern Australia. Land and Water Australia, Canberra

Noedoost F, Vaezi J (2021) Taxonomic investigation of the *Xanthium strumarium* L. complex (Asteraceae) distributed in Iran inferred from morphological, palynological and molecular data. Biodiversitas J Biol Diversity 22:1961–1974. https://doi.org/10.13057/biodiv/d220444

Oveisi M, Rahimian Mashhadi H, Yousefi AR, Alizade H, Baghestani MA, Gonzalez-Andujar JL (2013) Predicting maize yield in a multiple species competition with *Xanthium strumarium* and *Amaranthus retroflexus*: comparing of approaches to modeling herbicide performance. Crop Prot 45:15–21. https://doi.org/10.1016/j.cropro.2012.11.008

Qureshi H, Anwar T, Arshad M, Osunkoya OO, Adkins SW (2019) Impacts of *Xanthium strumarium* L. Invasion on vascular plant diversity in Pothwar region (Pakistan). Ann Bot (Rome) 9:73–82

Rieseberg L (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. Evol Trends Pl 5:65–84

Shaik RS, Lepschi BJ, Gopurenko D, Urwin NAR, Burrows GE, Weston LA (2016) An integrative morphological and molecular approach to identification of three Australian cucurbitaceous invasive weeds: *Citrullus colocynthis*, *C. lanatus* and *Cucumis myriocarpus*. Austral Syst Bot 29:247–264. https://doi.org/10.1071/SB16027

Tomasello S (2018) How many names for a beloved genus? – Coalescent-based species delimitation in *Xanthium* L. (Ambrosiinae, Asteraceae). Molec Phylogen Evol 127:135–145. https://doi.org/10.1016/j.ympev.2018.05.024

Tomasello S, Heubl G (2017) Phylogenetic Analysis and Molecular Characterization of *Xanthium sibiricum* Using DNA Barcoding, PCR-RFLP, and Specific Primers. Pl Med (Stuttgart) 83:946–953

Tomasello S, Manzo E (2023) Phylogenomics and the biogeography of the Old-World *Xanthium strumarium* (Asteraceae). XVII Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area (OPTIMA) Meeting, Erice

Villesen P (2007) FaBox: an online toolbox for fasta sequences. Molec Ecol Notes 7:965–968. https://doi.org/10.1111/j.1471-8286.2007.01821.x

Wang AS, Gopurenko D, Wu HW, Lepschi B (2017) Evaluation of six candidate DNA barcode loci for identification of five important invasive grasses in eastern Australia. PLoS ONE 12:14. https://doi.org/10.1371/journal.pone.0175338

Widder FJ (1923) Die Arten der Gattung *Xanthium*: Beiträge zu einer monographie. Verlag des Repertoriums, Akademieverlag, Berlin

Zhu X, Gopurenko D, Haegi LAR, Wu H (2018) Molecular identification of *Solanum elaeagnifolium* in Australia using DNA barcoding, a solution for better management. 21st Australasian Weeds Conference. Weed Society of New South Wales, Sydney, New South Wales, pp 307–311

Keil DJ, Baldwin BG (2023) *Xanthium orientale*. In: Jepson eFlora. Available at: https://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=5618. Accessed 7 Nov 2024

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.