

Phylogenetics informs host range testing for *Acacia auriculiformis* in Florida

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Abstract: **Background:** *Acacia auriculiformis* (earleaf acacia), an Australian perennial tree, is an increasingly problematic invasive species in Florida. **Objective:** Create a host range molecular phylogeny by generating test plant patristic distances to earleaf acacia (using *rbcl* sequences). **Methods:** DNA was extracted and sequenced from 98 Fabaceae species and an additional 28 sequences were downloaded from NCBI. **Results:** Molecular phylogenetics and patristic distance inform which plant species should be tested starting with closely related

(usually native) plants, especially those threatened or endangered, then extending to less related plants of commercial value. **Conclusions:** We show the Mimosoideae clade, while monophyletic, appears within the Caesalpinioideae clade. We also suggest 33 mostly threatened or endangered species for the initial test list from the Mimosoideae and Caesalpinioideae, including nine Papilionoideae commercial species. Finally, as a secondary test list, if additional testing is required, we suggest 30 additional Papilionoideae species be added.

Keywords: Fabaceae; Invasive Species; Host Range; Molecular Phylogeny; Patristic Distance

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1. Introduction

Acacia auriculiformis A.Cunn. ex Benth (earleaf acacia; Fabaceae), a tree native to Papua New Guinea, northern Australia, Philippines, and Indonesia, is an established invasive species in peninsular Florida. This species thrives in areas previously disturbed by invasive plants [e.g., *Melaleuca quinquenervia* (Cav.) S.T. Blake] and at the urban wildland interface. High reproductive and germination rates coupled with rapid growth and nitrogen fixation allow *A. auriculiformis* to outcompete native vegetation (Minter et al., 2020), especially in critical habitats such as upland hardwood hammocks and pinelands in the Florida Everglades, a United Nations Educational, Scientific and Cultural Organization (Unesco) World Heritage Site.

The environmental and economic damage caused by earleaf acacia in Florida is reviewed in Minter et al. (2020), who provide a case for the use of biological control (henceforth biocontrol) in the management of this species. Minter et al. (2020) also provide an extensive list of potential biological control agents collected from this tree over several years of field surveys in Australia (76 Lepidoptera, four Coleoptera, two Hemiptera, two Hymenoptera, three Diptera, one Acari, and one fungal pathogen). Recently, McCulloch et al. (2021) reported, while using Genotyping by Sequencing, that Florida populations of *A. auriculiformis* have their probable origin in Northern Territory, Australia. Additionally, Nawaz et al. (2021) reports on the continued search in the native range for useful biocontrol agents.

Critical to the successful use of biocontrol is predicting the fundamental and ecological host range (i.e., host specificity) of potential agents. Open field-testing, to gauge the realized (ecological) host range when all ecological cues are provided, is often performed first in the native range, narrowing the potential agents, followed by quarantine testing at a site in the adventive range where native plants are available (Briese, 1999; Sutton et al., 2021). Test plants are usually chosen following the centrifugal-phylogenetic method (CPM) (Wapshere, 1974) where testing begins with the native plants taxonomically most related to the target weed and moves outward to more distant species. Primary concerns for host-range testing are for threatened or endangered native species, followed by other natives and plants of economic importance.

The CPM assumes host shifts are most likely to occur onto plants of similar taxa (Madeira et al., 2008; Pearse, Hipp, 2009; Paynter et al., 2018). Because the secondary chemistry of plants is critical to the evolution of insect plant relationships, plants

hosting taxa related to the proposed agent and invasive plant are more likely to share similar suites of secondary plant compounds (Simmons, Blossey, 2023; US Department of Agriculture, 2024). However, there are cases where similarities in plant chemistry have evolved independently in disparate plant families, which may provide an avenue for acceptance of a more distantly related species.

The CPM, with minor modifications, has successfully predicted the host range of biological control agents since its inception (Suckling, Sforza, 2014; Hinz et al. 2019). At its core the CPM relies on phylogenetic conservatism, acknowledging that the majority of specialist herbivores retain their associations with host plants for millions of years (Futuyma, Agrawal, 2009; Simmons, Blossey, 2023). As a result, phylogenetically related plants typically share more conserved traits than more distantly related plants, and since herbivores typically use those traits to select their host plants, the result is if biocontrol agents feed on alternative plants they are generally closely related.

Briese (2003) notes that the CPM usage of taxonomy may not accurately reflect a true evolutionary history due to polyphyletic or paraphyletic features. In comparison, molecular phylogenetics are more likely to be useful since they more accurately represent true evolutionary relationships. Supporting this, the Technical Advisory Group for Biological Control Agents of Weeds Manual (US Department of Agriculture, 2024) recognizes molecular taxonomy as a “more advanced approach that uses plant molecular systematics in test plant species choice” and states these “alternative host plant selection strategies may also be used” in creation of test plant lists. Further, Chen et al. (2024) point out that using the patristic distance from the host plant as a guide for testing makes the best use of molecular phylogenetics and recommend it for prioritizing species for host specificity testing.

Plant biochemistry, morphology, and physiology are all traits that impact insect host-acceptance decisions (Futuyma, Agrawal, 2009; Simmons, Blossey, 2023). For example, plant-insect relationships are influenced by the secondary chemicals produced by the plants, and these are more likely to be similar where plants share an evolutionary history (Futuyma, Agrawal, 2009). Therefore, for plants chosen using either the CPM or molecular phylogenetics, the more closely related the plants are to the targeted weed, the more likely they will be acceptable to the biocontrol agent. However, this does not always hold true, especially where there is the independent development of similar plant chemistry in a disparate taxon (Rapo et al., 2019). Shared host acceptance behaviors may also reflect host plant choices of congeners of the potential biocontrol agents. Madeira et al. (2008) notes that, where a relationship exists between a related species to the biocontrol insect and a related alternate host plant, the biocontrol insect may also develop on the alternate host. Additionally, ancestral host usage by an insect's progenitors may provide an avenue for acceptance of an ancient but somewhat less related species.

The USDA-ARS Invasive Plant Research Laboratory and the University of Florida Institute of Food and Agricultural Sciences have already begun host range testing on two biocontrol agents, for *A. auriculiformis* *Calomela intemerata* Lea (Coleoptera: Chrysomelidae) and a *Trichilogaster* sp. (Hymenoptera: Melanosomellidae). The genus *Calomela* is native to Australia and primarily feeds on the genus *Acacia* (Selman, 1979). The other six genera which *Calomela* species feed on are all endemic to Australia. *Trichilogaster* comes from a small genus of chalcid wasps which, with one exception, are Australian species of gall-formers on Australian *Acacias*. This genus can, for all practical purposes, be regarded as being host-specific on species of *Acacia* (Prinsloo, Neser, 2007). As for any evidence that secondary chemicals might influence host choice, Hattas et al. (2010) looked at phylogenetically related *Acacia* species, along with species with similar life history, morphological, and functional traits, and found that they had different foliar low molecular weight phenolic profiles. It appears unlikely, therefore, that alternate hosts, past evolutionary history, or chemical profiling would change the basic makeup of the test list.

Minter et al. (2020) presented a prioritized list of plants for earleaf acacia host-range testing, but it did not include a molecular phylogeny. They suggested this phylogeny would follow separately. Based on the analyses by Minter et al. (2020), a candidate test plant list designed using the CPM, was submitted to the Technical Advisory Group for the Biological Control of Weeds (TAG) for review and approval in 2019. The TAG generally was in agreement with the taxa included on the list and offered only a few suggested additions from the U.S. Fish and Wildlife Service to include endangered taxa native to the U.S./Mexico border. The objective herein is to present that aforementioned molecular phylogeny and to provide the data to support the full test list. This phylogeny places emphasis first on relationships to the Mimosoideae, of which *Acacia* is a member, but also to the Florida species of the Caesalpinioideae and Papilionoideae, sister clades to the Mimosoideae.

Earleaf acacia nests within *Acacia* *sensu stricto*, a genus restricted to Australasia. No native *Acacia* species exist in the Americas as systematists have retained the genus name for Australian species while giving new generic names to American species (e.g., *Acaciella* and *Vachellia*) (Karlin, Karlin, 2018). The Atlas of Florida Plants (<https://florida.plantatlas.usf.edu/>), Missouri Botanical Garden database (<https://www.tropicos.org/>), and the PLANTS Database (<https://plants.usda.gov/>) were used to identify native Florida Fabaceae taxa for the molecular phylogeny.

2. Materials and Methods

DNA was extracted from 98 members of the Mimosoideae, Caesalpinioideae, and Papilionoideae subfamilies. Plant material was obtained from Fairchild Tropical Gardens (Coral Gables, Florida, USA), seed companies, native plant

nurseries and private collections. A graduated sampling strategy was used, starting with extensively sampling the taxa most likely closely related to *A. auriculiformis* (Minteer et al., 2020). Taxa of the sub-family Mimosoideae, either from our samples or sequences downloaded from National Center for Biotechnology Information (NCBI), contained 28 representatives, of which 10 are Florida native species, five are North American, six are Caribbean/Central American, and seven are native to tropical America (see Figure 1A). We were unable to obtain sequences for two Florida Mimosoideae natives, *Pithecellobium bahamense* Northr. and *Vachellia macrantha* (Humb. & Bonpl. ex Willd.) Seigler & Ebinger, however other members of these genera were sequenced. Phylogenetically more distant from *A. auriculiformis*, eleven Caesalpinioideae species native to Florida were included (also Figure 1A), with only *Caesalpinia pauciflora* (Griseb.) C. Wright ex Sauvalle and *Chamaecrista lineata* (Sw.) Greene var. *keyensis* (Pennell) H.S. Irwin & Barneby missing among Florida natives. Four taxa of North American and/or Caribbean/ Central American origins were included. For the large sub-family Papilionoideae (see Figure 1B), at least one species was selected from 21 of the 35 genera containing a Florida native. Additionally, nine taxa of economic importance were included.

The ribulose 1,5 biphosphate carboxylase/oxygenase (*rbcl*) gene was chosen to construct the molecular phylogeny. The *rbcl* gene has been widely sequenced and provides a large reference database of Fabaceae species within NCBI. Even among chloroplast coding genes, *rbcl* evolves slowly, making it useful for assessing phylogenetic relationships at the interfamilial level (Soltis et al., 1990). Since the primary goal here was to establish the genetic distances from host range candidate species to *A. auriculiformis*, the slowly evolving *rbcl* sequences likely present more consistent evolutionary rates than would be found in faster evolving genes. One downside, *rbcl* is less able to distinguish between closely related species than faster evolving sequences, which may also result in lower bootstraps (posterior probabilities) for clades. However, as elucidating the genetic distances to *A. auriculiformis* was our primary goal, these limitations were accepted.

DNA preservation, DNA extraction, PCR cleanup, sequencing and chromatogram processing follow Madeira et al. (2016). The primers *rbcl*-F [ATGTCACCACAAACAGAGACTAAAGC] and *rbcl*-724R [TCGCATGTACCTGCAGTAGC] were used. Cycling parameters included initial denaturation (98 °C, 2 min.); 35 cycles of denaturation (98 °C, 10 sec.), annealing (65 °C, 30 sec.), extension (68 °C, 1 min.); ending with final extension (68 °C, 1 min.). Eurofins MWG Operon (Louisville, KY) performed cycle sequencing using BigDye terminator technology (Life Technologies, Carlsbad, CA.). Sequences aligned unambiguously using MAFFT v7.017 (<https://mafft.cbrc.jp/>). Model testing was carried out in Topali V2 (<http://www.topali.org/>) with the 'best' PHyML model (BIC criteria) being K81uf+G+I. This tree was bootstrapped 1000 times. Bayesian analysis was conducted using

MrBayes 3.2.7 (<http://nbisweden.github.io/MrBayes/>) with a 'best' BIC model of HKY+G+I. This analysis used 5,000,000 generations with a burn-in of 10%. Posterior probability numbers are for 1,000 iterations. Numbers in the nodes reflect bootstrap values greater than 50% or posterior probabilities greater than 0.50. The PHyML tree and the calculated patristic distances (distance across tree branches) are presented in Figure 1. The MrBayes tree is omitted for brevity, however the posterior probabilities are displayed in parentheses in the PHyML phylogeny to allow for comparison. For further details see the Figure 1 legend.

3. Results and Discussion

The taxa's native status (F- Florida; A- North America; C- Caribbean / Central America; a- tropical America) is presented along with environmental qualifiers [E- endemic; T- threatened Florida; N- endangered Florida; D- endangered US; \$- commercial value] and the maximum likelihood patristic distance from the taxa to *A. auriculiformis* (Figure 1). Table 1 adds to this information with primary and secondary test lists containing patristic distances ordered by increasing distance from *A. auriculiformis*. Square brackets [] represent actual distances. Braces {} represent distance to a species in the same genera.

Starting in the Mimosoideae, of which *A. auriculiformis* is a member, three of the closest related species are the state-endangered *Acaciella angustissima* (Mill.) Britton & Rose [0.0142], the state-threatened *Pithecellobium keyense* Britton ex Britton & Rose [0.0141], the native *Pithecellobium unguis-cati* (L.) Benth. [0.0242], and the state-endangered *Pithecellobium bahamense* Northrop. Also appearing in close patristic distance are *Pseudosamanea cubana* (Britton & P.Wilson) Barneby & J.W.Grimes [0.0161], a Cuban endemic, and *Lysiloma sabicu* Benth. [0.0202] from the Bahamas, Cuba and Hispaniola. Other close relatives include *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald [0.0328] and *Neptunia pubescens* Benth. [0.0328], both native. Additional Mimosoideae species of conservation concern, comprising five native endangered species, are listed in Table 1, bringing the suggested total of Mimosoideae species for testing to fifteen.

Mimosoideae, while monophyletic, appeared within the Caesalpinioideae clade. The Legume Phylogeny Working Group (LPWG) also observed that the traditional subfamily Mimosoideae is a distinct clade nested within a re-circumscribed Caesalpinioideae (Azani et al., 2017). The LPWG currently refers to this clade as the "mimosoid clade" pending a future classification. In terms of patristic distance, some Caesalpinioideae taxa are closer to *A. auriculiformis* than some in the Mimosoideae and are threatened or endangered (TE) native Florida species. The test list (Table 1) includes eight members of the Caesalpinioideae.

The subfamily Papilionoideae is large, with many Florida natives. The closest species appearing in the phylogeny is *Poitea florida* (Vahl) Lavin [0.0562]. However, as it is native to the Virgin Islands and Puerto Rico and it need not be tested

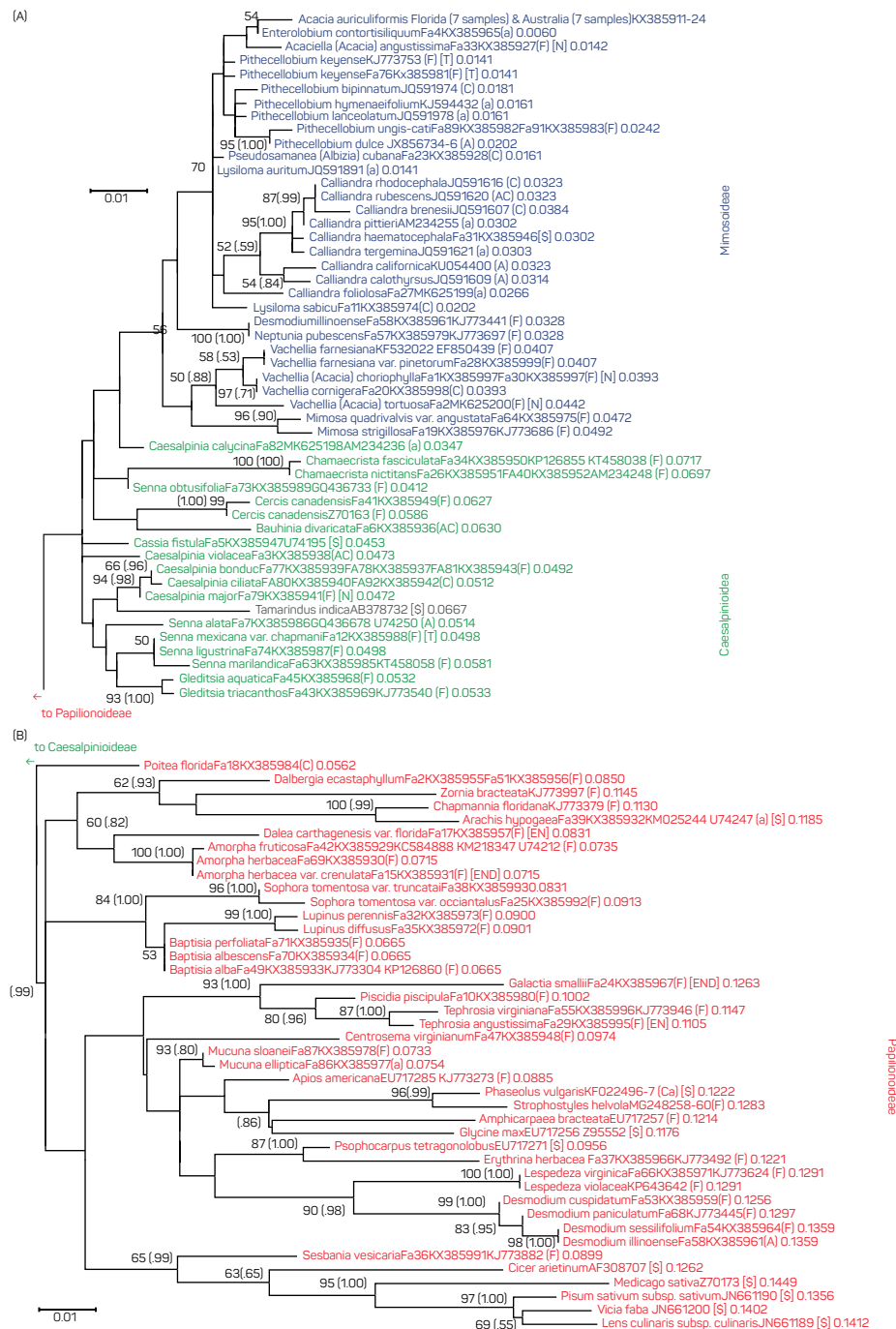


Figure 1 - PHyML phylogenetic tree informing host range testing for *Acacia auriculiformis*. Model testing and PHyML phylogeny was carried out in Topali V2 with the 'best' model (BIC criteria) being K81uf+G+I. This tree was bootstrapped 1000 times. Bayesian analysis was also conducted using MrBayes 3.2.7 with a 'best' BIC model of HKY+G+I. Numbers in the nodes reflect PHyML bootstrap values greater than 50% or MrBayes posterior probabilities greater than 0.50 (only the posterior probabilities are displayed for MrBayes, in parentheses). The Mimosaideae species are presented in blue; the Caesalpinioideae in green, and the Papilionoideae in red. Following the taxa name, sequences generated by this lab start with an 'FA' sample number, followed by the NCBI accession number both italicized & bold (i.e. KX385927), while unaccented accession numbers (i.e., KJ773753) indicate that NCBI was the source. Some NCBI sequences did not cover the full alignment (632 base pairs) and this is indicated following the NCBI accession number by a 'P' for 'partial sequence'. The taxa's native status (F- Florida; A- North America; C- Caribbean / Central America; a- tropical America) follows, to better quantitate the possibility of their exposure to an introduced agent. Next, qualifiers [E- endemic; T- threatened Florida; N- endangered Florida; D- endangered US; S- commercial value] help assess the biological danger. Last, the maximum likelihood patristic distance from the taxa to *A. auriculiformis* is shown to quantitate the degree of relationship.

Primary test list					Secondary test list				
			# Florida	on			# Florida	on	
	patristic	status	natives	CPM		patristic	status	natives	CPM
	distance	in Florida	in genera	list?		distance	in Florida	in genera	list?
Mimosoideae (15)					Papilionoideae				
<i>Enterolobium contortisiliquum</i>	[0.0060]	horticultural	0	N	Florida threatened/ endangered & natives	30			
<i>Acaciella angustissima</i>	[0.0142]	endangered	1	Y	<i>Amorpha herbacea</i> var. <i>crenulata</i>	[0.0715]	endangered	3	Y
<i>Pithecellobium keyense</i>	[0.0142]	threatened	3	Y	<i>Dalea carthagenensis</i> var. <i>floridana</i>	[0.0831]	endangered	8	Y
<i>Pseudosamanea cubana</i>	[0.0161]	Cuban endemic	0	Y	<i>Tephrosia angustissima</i> var. <i>angustissima</i>	[0.1105]	endangered	8	Y
<i>Lysiloma sabicu</i>	[0.0202]	Cuba, Bahamas	0	Y	<i>Tephrosia angustissima</i> var. <i>corallicola</i>	[0.1105]	endangered	8	Y
<i>Pithecellobium unguis-cati</i>	[0.0242]	FL native	3	N	<i>Galactia smallii</i>	[0.1263]	endangered	14	Y
<i>Pithecellobium bahamense</i>	{0.0242}	endangered	3	Y	<i>Dalbergia brownei</i>	{0.085}	endangered	2	Y
<i>Calliandra haematocephala</i>	[0.0302]	horticultural	0	Y	<i>Clitoria fragrans</i>	M	endangered	2	Y
<i>Desmanthus illinoensis</i>	[0.0328]	FL native	1	N	<i>Lupinus aridorum</i>	{0.091}	endangered	5	Y
<i>Neptunia pubescens</i>	[0.0328]	FL native	1	N	<i>Lupinus westianus</i> var. <i>westianus</i>	{0.091}	threatened	5	Y
<i>Vachellia choriophylla</i>	[0.0393]	endangered	4	Y	<i>Aeschynomene pratensis</i>	M	endangered	2	Y
<i>Vachellia farnesiana</i>	[0.0407]	native	4	Y	<i>Crotalaria avonensis</i>	M	endangered	4	Y
<i>Vachellia tortuosa</i>	[0.0442]	endangered	4	Y	<i>Stylosanthes calcicola</i>	M	endangered	3	Y
<i>Mimosa quadrivalvis</i> var. <i>angustata</i>	[0.0472]	native	2 (3*)	N	<i>Desmodium ochroleucum</i>	{0.126}	endangered	20	Y
<i>Mimosa strigillosa</i>	[0.0492]	native	2	N	<i>Indigofera trita</i> ssp. <i>scabra</i>	M	endangered	3	Y
					<i>Centrosema arenicola</i>	{0.097}	endangered	2	Y
					<i>Rhynchosia parvifolia</i>	M	threatened	9	Y
Caesalpinioideae (9)									
<i>Caesalpinia (Guilandina) major</i>	[0.0472]	endangered	2	Y	<i>Rhynchosia swartzii</i>	M	endangered	9	Y
<i>Caesalpinia (Denisophytum) pauciflora</i>	{0.0472}	endangered	1	Y	<i>Lespedeza violacea</i>	[0.1291]	native	9	Y
<i>Caesalpinia (Guilandina) bonduc</i>	[0.0492]	native	2	N	<i>Zornia bracteata</i>	[0.1145]	native	1	Y
<i>Senna mexicana</i> var. <i>chapmanii</i>	[0.0498]	threatened	4	Y	<i>Chapmannia floridana</i>	[0.1130]	native	1	Y
<i>Chamaecrista lineata</i> var. <i>keyensis</i>	{0.07}	endangered	5 (6*)	Y	<i>Mucuna sloanei</i>	[0.0733]	native	1	Y
<i>Senna obtusifolia</i>	[0.0412]	native	4	Y	<i>Apios americana</i>	[0.0885]	native	1	Y
<i>Cassia fistula</i>	[0.0453]	horticultural	1	N	<i>Amphicarpaea bracteata</i>	[0.1214]	native	1	Y
<i>Cassia grandis</i>	{0.0453}	horticultural	0	Y	<i>Orbexilum virgatum</i>	M	endangered	3	Y
<i>Delonix regia</i>	M	horticultural	0	Y	<i>Baptisia calycosa</i>	{0.0665}	endangered	9	Y
					<i>Baptisia megacarpa</i>	{0.0665}	endangered	9	Y
Papilionoideae					<i>Baptisia hirsuta</i>	{0.0665}	threatened	9	Y
Commercial (9)	9				<i>Baptisia simplicifolia</i>	{0.0665}	threatened	9	Y
<i>Psophocarpus tetragonolobus</i>	[0.0956]	commercial	0	Y	<i>Vicia ocalensis</i>	{0.1482}	endangered	6	Y
<i>Glycine max</i>	[0.1176]	commercial	0	Y	<i>Sophora tomentosa</i>	[0.0831]	native	1	Y
<i>Arachis hypogaea</i>	[0.1185]	commercial	0	Y	<i>Piscidia piscipula</i>	[0.1002]	native	1	Y
<i>Phaseolus vulgaris</i>	[0.1222]	commercial	0	Y					
<i>Cicer arietinum</i>	[0.1262]	commercial	0	Y	key:				
<i>Pisum sativum</i>	[0.1356]	commercial	0	Y	patristic distance: [actual distance to <i>A. auriculiformis</i>];				
<i>Vicia faba</i>	[0.1402]	commercial	0	Y	{distance to <i>A. auriculiformis</i> of taxa in same genera}; M, no distance available				
<i>Lens culinaris</i>	[0.1412]	commercial	0	Y	# Florida native species in genera				
<i>Medicago sativa</i>	[0.1449]	commercial	0	Y	(*) includes varieties or subspecies				
					on CPM list? - whether appears on test list in Minter et al. (2020)				
					created using guidelines for the centrifugal-phylogenetic method [CPM]				
					and as submitted to TAG				

(agents are unlikely to naturally migrate to either place and they do not currently host any *A. auriculiformis* on those islands). All other members of the Papilionoideae are at a patristic distance of 0.066 or greater, making them unlikely hosts. Nevertheless, they are represented in the primary test list (Table 1) by nine commercial Papilionoideae.

Strophostyles helvola (L.) Elliott. [0.0392] appears in the Caesalpinoideae clade; however, this species belongs to the Papilionoideae which suggests a new sample should be collected and re-sequenced.

Generally, screening tests, usually no-choice (where insects are confined to one potential host plant species at a time), are applied to the broadest range of test plants, followed by multiple choice tests focusing on those plants where the screening has showed any positive responses, as well as those of greatest concern. We suggest that the 15 plants identified in the Mimosoideae, the nine members of the Caesalpinoideae, plus the nine commercial species might constitute an initial screening list. We also identified 30 species (21 TE), members of the Papilionoideae clade, as secondary candidates for testing (if regulatory agencies request them).

Lonsdale et al. (2001) presented a case for risk analysis in weed biological control, stressing the importance of analyzing both direct and indirect effects of introducing an agent. They discuss predictive tools such as climate-matching, agent selection, phylogeny and host-screening procedures. It should be noted that while much of the justification for centrifugal testing lies in the reasoning that closely related species are more likely to support similar taxa of herbivores, the link between a plants' biochemistry and its phylogeny is not necessarily that straightforward. Wheeler et al. (2021) showed that similar volatile signatures, not relatedness, was a better predictor of oviposition in an approved agent. However, Wheeler et al. (2017) found that phylogeny was a good predictor of host range in *Pseudophyllothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae), a biological control agent for *Schinus terebinthifolia* Raddi (Anacardiaceae). However, a systematic examination of all possible plant volatiles produced from relatives is not available for these Fabaceae. As discussed earlier, the likelihood of non-target use on host plants shared by closely related insect taxa appears unlikely due to their different foliar low molecular weight phenolic profiles (Hattas et al., 2010).

Finally, it is of interest to compare the CPM test list with the molecular phylogenetic list presented here. Nine of the 15 Mimosoideae species listed in the molecular phylogeny appear in the CPM test list. Five Florida natives appear in the molecular phylogeny that do not appear in the CPM list, probably because they are not TE. However, as they are close in patristic distance from *A. auriculiformis* they were included on the molecular test list. Similarly, seven of the phylogenetic test plant selections in the Caesalpinoideae also appeared in the CPM test list, while two (one native, one horticultural) did not. There was complete agreement on the nine commercial species in the Papilionoideae to be tested. On the secondary test list 23 of the 30 listed

on the phylogenetic test list also appear on the CPM list. For the comparison in reverse the molecular test list does not include Category 2 (species in the same genus as *Acacia auriculiformis*, native range), Category 5 (species in other families within the Florida Fabales), or Category 6 (species in other orders important in the targeted area). Within the other categories there was substantial overlap. There were seven species on the CPM list that were not native and nine that were native but not TE which did not appear on the molecular test list. The advantage of the molecular list appears to be the inclusion of Florida natives that were phylogenetically close to *A. auriculiformis* and the prioritizing of the other test samples by patristic distance. Lesieur et al. (2020) have demonstrated cost and effort savings result from testing a few prioritized phylogenetically related species from the native range first, especially as non-specific agents could be discarded earlier.

4. Conclusions

Classical biological control of invasive plants has been broadly successful in introducing host specific agents, supporting the validity and effectiveness of the centrifugal phylogenetic method (Hinz et al., 2019). Increased use of molecular phylogenetics should only increase the methodological efficacy. Heimpel and Cock (2018) note that a recent focus on risks has been important to the maturation and safety of biological control, however this has also decreased the pace of introductions. They encourage a new paradigm where the potential benefits and risks of biological control introductions are carefully balanced, especially by effective dialogue among regulators, scientists, and other stakeholders.

Authors' contributions

PTM, CRM, FAD, GSW, and MCS: conceptualization of the manuscript and development of the methodology. PTM, CRM, and FAD: execution of the experiments. PTM and FAD: data collection and analysis. PTM, CRM, FAD, GSW, and MCS: data interpretation. FAD, GSW, and MCS: funding acquisition and resources. FAD, GSW, and MCS: project administration. FAD, GSW, and MCS: supervision. PTM and FAD: writing the original draft of the manuscript. PTM, CRM, FAD, GSW, and MCS: writing, review, and editing.

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