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A simple molecular approach for identifying a rare *Acronychia* (Rutaceae) provides new insights on its multiple hybrid origins

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Abstract

Hybridization plays an important role in the evolution of plant diversity. Nonetheless, it is often difficult to clearly ascertain hybrid origin by assessing morphological characters alone. *Acronychia littoralis* (Rutaceae) is a recently described rare tree from littoral rainforests in eastern Australia. Two forms have been tentatively distinguished on the basis of different leaf morphology however, because of the variability of the discriminatory characters across populations (and in some cases across single individuals), compiling data on the exact distribution of each form has been a difficult process. To further complicate matters, some of these distinguishing morphological characters are intermediate between those of closely related, sympatric species. As a result, simple molecular techniques were developed to assist the development of adequate conservation and management strategies for *A. littoralis* and to better understand its evolutionary history. Sequence data from the ITS2 region were obtained and haplotype specific primers that distinguished the two forms and the related taxa were developed. Sequence data suggest that the two forms of *A. littoralis* are each of hybrid origin but both involve different parental taxa. The findings provide a perspective on the evolutionary dynamics of the local rainforest flora and highlight the need for phylogenetic evaluation of rare taxa where there is taxonomic ambiguity.

Keywords: Hybrid; ITS; Rainforest; Rutaceae; Rare flora

1. Introduction

Only recently described, *Acronychia littoralis* T. Hartley and J. Williams (Rutaceae) is listed as a rare species restricted to a number of littoral rainforest remnants along the northern New South Wales (NSW) and southern Queensland coasts of Australia (Fig. 1). This species is difficult to differentiate from closely related taxa based on simple morphological characters alone. Drupe morphology is a good discriminatory character (Table 1) but its usefulness is limited to the breeding season which can be erratic. The type and frequency of oil dots in the leaves is another potentially useful field character, but within-individual variability is such that identification often remains tentative. To further complicate matters, morphologically distinct populations were more recently discovered at the southern end of the species' distribution range (Horton, 1997).

Recent surveys have subsequently identified two potentially distinct *A. littoralis* forms apparently differing slightly in leaf morphology: form 1 (AL1) found mostly across the northern distribution range of the species and bearing morphologic similarities to *A. imperforata* and *A. wilcoxiana*, and form 2 (AL2) more southern in distribution and bearing similarities to *A. oblongifolia*. Despite these cross-species similarities the suggestion of possible hybrid origin was originally rejected on the basis of high pollen viability (Hartley and Williams, 1983). On the whole, it is extremely difficult to accurately identify *A. littoralis* and differentiate between the two forms in the field. Consequently, conservation efforts are hampered by the uncertainty surrounding the characters that should be used to distinguish between these two forms and by their ambiguous taxonomic

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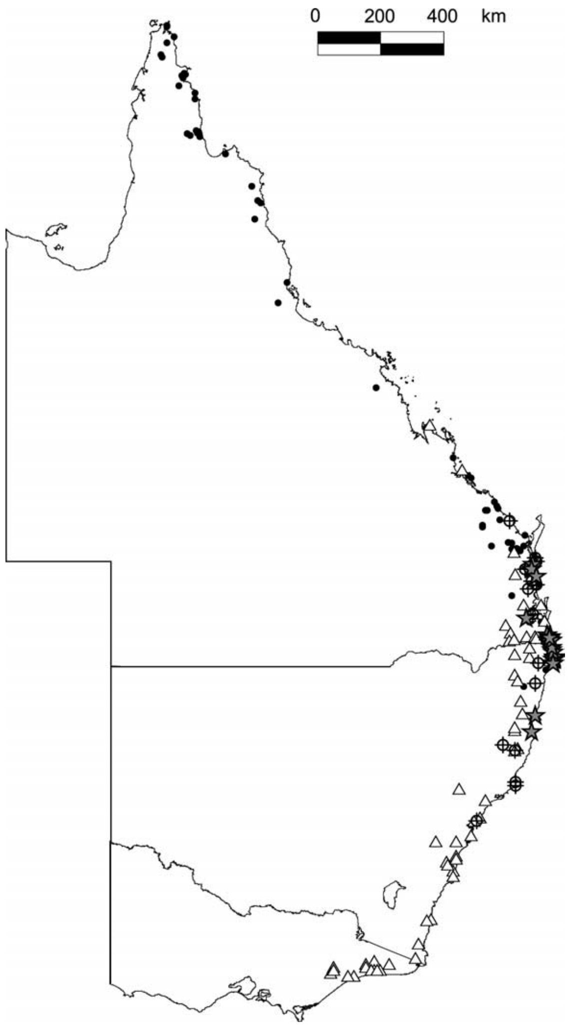

Fig. 1. Distribution details for *A. littoralis* (star symbol), *A. imperforata* (black circle), *A. oblongifolia* (white triangle) and *A. wilcoxiana* (crossed circle) in Eastern Australia.

status. This is particularly relevant as *A. littoralis* occurs in areas where the pressure for coastal development is intense and the presence of rare species is considered as an important factor supporting the preservation of valuable remnant habitat.

Hybrids can be defined as organisms formed by the cross-fertilization between individuals originating from populations distinguishable on the basis of one or more heritable characters (Rieseberg, 1997). They are often found along hybrid zones where two distinct evolutionary units meet, and their successful establishment as a hybrid species relies on the evolution of reproductive barriers (that prevent backcrossing to the parental species) and/or the existence of fitness advantages (Rieseberg, 1997). It is now recognized that hybridization makes a considerable contribution to the evolution of flora diversity with Ellstrand et al. (1996) estimating that around 11% of plant species are of hybrid origin. In fact, it is likely that the evolutionary influence of hybridization has been underestimated because of difficulties in ascertaining hybrid origin based on traditional approaches alone.

The description of hybrid speciation should preferably rely on gene phylogenies as well as other lines of evidence (Rieseberg, 1998). Molecular techniques provide a powerful means of identifying hybrid genotypes and investigating historical as well as current levels of gene flow (Rieseberg, 1998). DNA markers can provide additivity of genetic characters that correspond to the character intermediacy of morphological investigations (Rossetto et al., 1997) and comparative analysis of homologous DNA sequences can be used to identify interbreeding among related taxa (Sang et al., 1995; Franzke and Mummenhoff, 1999; McKinnon et al., 2001).

The paucity of variable sites in plastid DNA (cpDNA) usually limits its differentiating potential at lower taxonomic levels, although a number of non-coding regions have been successfully used to measure between-genus introgression (Bleeker and Hurka, 2001; McKinnon et al., 2001). Hybridization events can generally be inferred more readily from nuclear sequences, as these tend to accumulate nucleotide substitutions

Table 1
Some distinguishing characters of the *Acronychia* species from Northern NSW

Species	Preferred habitat	Flower colour	Flower time	Drupe colour	Fruit time	GenBank ITS
A. baeuerlenii T. Hartley	ST	Cream	October–Feburary	Green	March–May	AY588596
A. imperforata F. Muell	L	Cream	Feburary–April	Yellow	August–December	AY588597
A. littoralis T. Hartley and J. Williams	L	Yellow	January–Feburary	Cream	May–August	–
A. oblongifolia (Cunn. Ex Hook.) Endl. Ex Heynh	RF	Cream	January–March	White	May–November	AY588598
A. pauciflora C. White	D	Green	Feburary–May	White	September–November	AY588593
A. pubescens (Bailey) C. White	WT	Green	Mar–June	Yellow	January–March	AY588594
A. suberosa C. White	WT	White	January–April	Cream	March–June	AY588595
A. wilcoxiana (F. Muell) T. Hartley)	L/W	White	January–May	White	March–May	AY588592

Rainforest types: D (dry rainforest), L (littoral rainforest), RF (most rainforest types), ST (subtropical rainforest), W (warm temperate rainforest), WT (warm tropical rainforest).

GenBank accession numbers for the nuclear sequences are included.