

INSECT PEST DIAGNOSTICS & SPECIES DISCOVERY UNDER iBOL: THE CASE OF *OROSIUS* LEAFHOPPERS

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Introduction

Orosius Distant (Hemiptera: Cicadellidae) is a leafhopper genus of economic importance in Africa, the Middle East, Asia, Australia and the Pacific. At least half of the eight described species have been reported as vectors of plant pathogens, including phytoplasmas and viruses. Despite their economic importance, the identities and distributions of *Orosius* species are unclear and the genus has a checkered taxonomic history. In addition, like many leafhoppers, only adult males of *Orosius* are identifiable to species. The resulting uncertainty over the identity of vector species has hampered research into phytoplasma transmission and management.

We performed an integrative taxonomic study of the genus in order to provide tools for elucidation of the vector-capability of *Orosius* species. DNA barcodes available for seven of the eight recognised species and several putative novel species were used to group ♂ & ♀ specimens into distinct genetic species clusters using a general mixed Yule-coalescent (GMYC) modeling procedure ⁽³⁾. These clusters were compared with morphospecies clusters identified through male genitalic characters.

Results & Discussion

Multiple threshold GMYC modeling applied to 97 unique *Orosius* haplotypes identified 18 genetic clusters (Fig.1). These clusters resolved as seven previously described morphospecies, three novel morphospecies and five novel species represented by females only (Fig 2). Average sequence difference within morphospecies was 14 times less than that observed between morphospecies (0.78% vs 11.4%). Monophyly at each morphospecies was well supported by bootstrap analysis. There was strong congruence between species clusters defined by genetic and morphological methods; DNA barcoding was very useful for delineating morphologically cryptic species where male genitalic character variations were subtle (eg., *O. argentatus* vs. *O. orientalis*). Use of ♀ samples in genetic analyses allowed detection of additional putative novel species (Fig. 2).

Conclusions

Our study doubles the known species diversity of the genus and clarifies the identity and distributions of the species. The implications of newly discovered cryptic species for studies of plant disease transmission are significant. Our new diagnostic tools can be used as a standard for future investigations of leafhopper-phytoplasma species associations.

References

⁽³⁾ Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595-609.

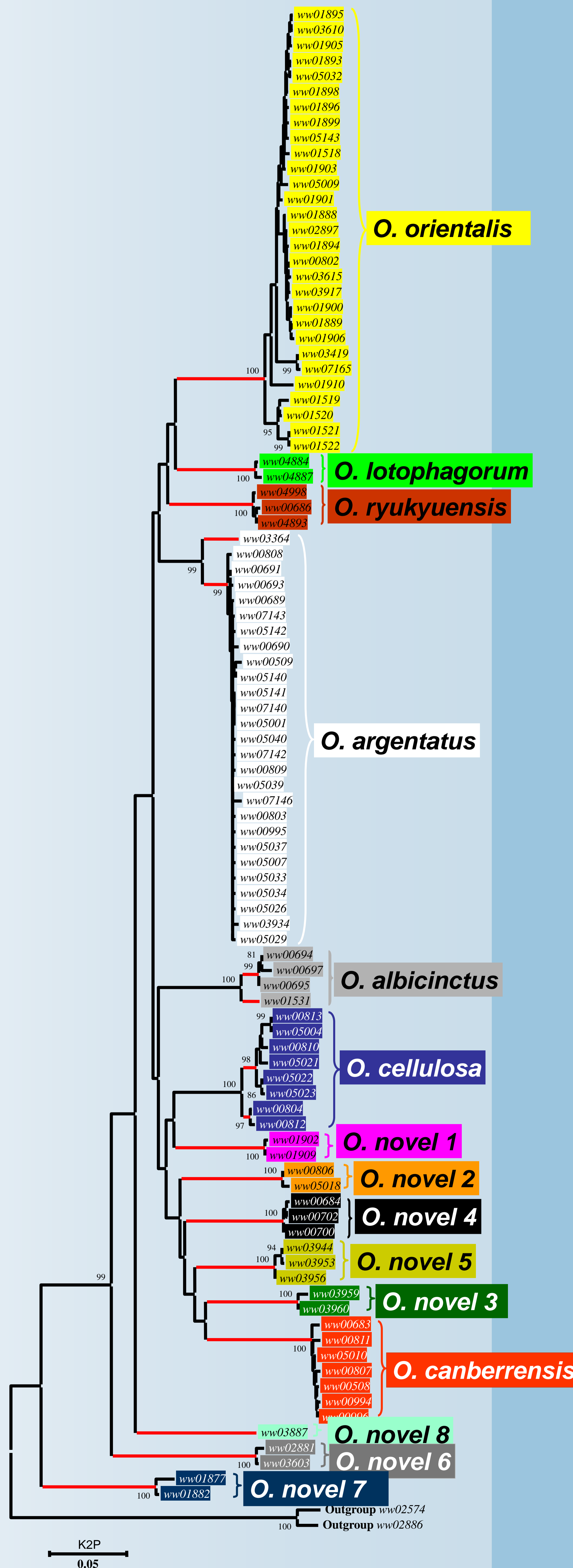


Figure 1. NJ tree of 97 unique *Orosius* haplotypes derived from 179 specimens. Red branches indicate genetic species identified by multiple threshold GMYC model.

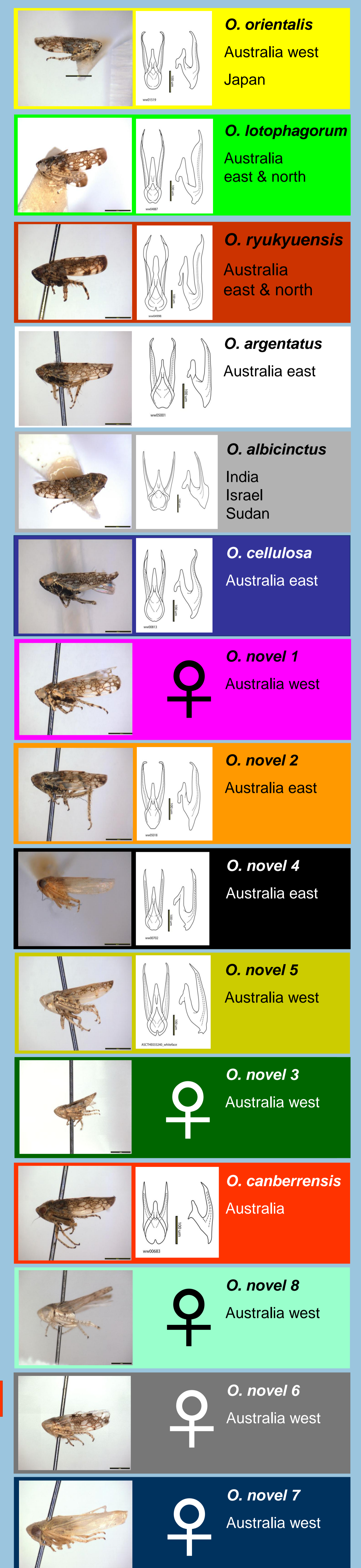


Figure 2. *Orosius* morphospecies, male genitalic characters and sample distributions. ♀ = female specimens only, putative novel species delineated exclusively by genetics.