Parallel host range expansion in two unrelated cossid moths infesting *Eucalyptus nitens* on two continents

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Abstract. 1. Two cossid moths, *Coryphodema tristis* and *Chilecomadia valdiviana*, have recently become pests on *Eucalyptus nitens* in South Africa and Chile, respectively. Both *C. tristis* and *C. valdiviana* have large host ranges and high levels of similarity in their host distributions. Their infestations of *E. nitens* are the first records of these moths on Myrtaceae.

2. The contemporaneous adoption of *E. nitens* as a novel host, despite widespread availability of native and introduced Myrtaceae, suggests a non-random pattern of invasion. Phylogenetic relatedness among the two species linked to cryptic invasion of one or both moths at some time in the recent past provides a possible explanation for this pattern.

3. To test this hypothesis, variation in mtDNA sequences for the COI gene of *C. tristis* and *C. valdiviana* were analyzed. The COI mtDNA sequence data showed that *C. tristis* and *C. valdiviana* are highly divergent genetically, indicating that both are native on their respective continents with independent evolutionary trajectories.

4. The parallel host range expansions to *E. nitens* on different continents appear to be unrelated events, likely driven by characteristics of the biology and / or ecology of the host.

Key words: *Coryphodema tristis*, *Chilecomadia valdiviana*, COI gene, host association

Introduction

Wood boring moths in the family Cossidae are widespread, diverse, and are commonly cryptic and understudied. The family comprises of at least 110 genera and approximately 700 species (Davis *et al*., 2008). Members of the Cossidae are often of serious concern to forestry and horticulture due to their aggressive, often gregarious wood-boring behaviour, and association with a wide range of trees and shrubs (Nair, 2001; FAO, 2009). Approximately 20% of the species are known to be polyphagous, with host ranges spanning up to seventeen families for a single species (Powell, 1980). There is widespread speculation that even those species recorded from only one or a few hosts are also likely to be generalists (Powell, 1980; Powell *et al*., 1999).

During the course of the past 20 years, two cossid moths, *Coryphodema tristis* and *Chilecomadia valdiviana*, have become an important pest on *Eucalyptus nitens* in South Africa and Chile, where they were recorded in 2004 and 1992 respectively (Cerda, 1995; Gebeyehu *et*

al., 2005). Both insects are of growing importance to plantation forestry in these two countries because there are few commercial alternatives to *E. nitens* that can be planted in areas prone to low temperatures, including frost and snow.

The roughly contemporaneous colonization of *E. nitens* by *C. tristis* and *C. valdiviana* is intriguing as neither insect had previously been recorded on a myrtaceous host. This is despite the fact that native and introduced members of this family, including *Eucalyptus*, have long been present within their ranges. Recorded hosts include *Acacia*, cherry, apple, quince, loquat, avocado, willow and elm for *C. valdiviana* (see Cerda, 1996; Gonzalez, 1989; Angulo & Olivares, 1991), and pear, apple, quince, loquats, olives, vines, avocado, bush willow, oak, elm and hawthorn for *C. tristis* (Petty, 1917; Hoppner, 1994). The apparent specificity on *E. nitens* within the Myrtaceae, together with the broad overlap in host range of these two geographically distant species, has led to speculation that the recent new host association represents a non-random pattern that could help reveal elements of the biology and/or ecology of these species (Janz *et al*., 2001; Weingartner *et al*., 2006).

Several plausible and non-exclusive hypotheses exist that might help to explain the two independent shifts of related cossid moths to *E. nitens* in the Southern Hemisphere. First, overlapping abiotic requirements in the two cossid species could result in preferential exposure to *E. nitens*, which is planted under roughly similar ecological conditions on the two continents. Further, long term exposure to a similar suite of cultivated hosts (eg. apple, quince, loquats, avocado, bush willow, and elm (Pettey, 1917; Gonzalez, 1989; Angulo & Olivares, 1991; Hoppner, 1994)) could lead to convergent evolution and non-random overlap in the preference for or suitability of novel hosts (Mardulyn *et al*., 1997). Finally, phylogenetic relatedness among

the two species linked to cryptic invasion of one or both moths at some time in the recent past could drive overlap in the responses of herbivorous insects to host plant cues and/or defenses (Janz & Nylin, 1998; Heidel-Fischer *et al*., 2009).

In this study we report on relatedness between *C. tristis* and *C. valdiviana* using sequence similarity in the cytochrome oxidase subunit I mitochondrial DNA (COI mtDNA), placing them in a phylogenetic context within the Cossidae using available data from NCBI. Membership in the same or closely related clade could in part explain the curious similarity in host use and shed light on the hypothesis of a more recent common origin. This study also reports the first molecular identification of *Coryphodema tristis* and *Chilecomadia valdiviana*.

Materials and Methods

Sample collections, DNA Extraction, Polymerase Chain Reaction and Sequencing

One hundred and thirty two specimens of *Coryphodema tristis* and *Chilecomadia valdiviana* were collected from South Africa and Chile (Table 1). The total genomic DNA was extracted from the thorax tissues of the larvae using the protocol described by Goodwin *et al*. (1992). PCR amplification was optimized using 1µl of total genomic DNA template (50-100 ng/µl) for all samples. PCRs were run in 25 µl reactions including 10x PCR buffer, 3 mM $MgCl_2$, 1 mM dNTP's, 1.0 U of Taq polymerase, 16.75 µl of Sabax water and 0.4 µM of each primer set of LCO1490 and HCO2198 (Folmer *et al*., 1994), and C1-J-2183 (Jerry) and TL2-N-3014 (Pat) (Simon *et al*., 1994). Sequencing was performed bidirectionally using the ABI Prism^{TM} 3100Genetic Analyzer (Applied Biosystems) to produce an overlap of 657 and 743 bp of the COI gene region for all samples.

Table 1. Samples of *Coryphodema tristis* collected from *E. nitens* and *V. vinifera* in South Africa and *Chilecomadia valdiviana* from *E. nitens* in Chile.

No.	Sample site	Latitude	Longitude	Elevation (m)	Sample size	Host plant
1	Rooihoogte1	S26.05655	E30.32772	1645	10	*E. nitens*
2	Rooihoogte2	S26.06038	E30.32549	1691	10	*E. nitens*
3	Bonnie Braes	S26.16803	E30.74489	1701	10	*E. nitens*
4	Isabelladale	S26.21773	E30.63133	1718	10	*E. nitens*
5	Lothair	S26.31445	E30.62239	1666	10	*E. nitens*
6	Meadowland	S26.28522	E30.69000	1552	10	*E. nitens*
7	Elandsport1	S26.11923	E30.74505	1552	10	*E. nitens*
8	Elandsport2	S26.11616	E30.74162	1529	10	*E. nitens*
9	Ndubazi	S25.55425	E30.29552	1592	10	*E. nitens*
10	Helvetia	S25.56159	E30.29077	1622	10	*E. nitens*
11	Bambi Hotel	S25.51008	E30.37118	1677	10	*E. nitens*
12	Vredandal	S31.66435	E18.50594	-	12	*V. vinifera*
13	Chile	-	-	-	10	*E. nitens*

Molecular Data Analyses

Sequence data for the forward and reverse strands were edited manually using CLC bio workbench v. 6 (www.clcbio.com) and aligned with MAFFT v. 6 (Katoh *et al*., 2002). Pairwise nucleotide diversity was calculated using combined COI sequence data in MEGA 5.0 (Tamura *et al*., 2011). For phylogenetic analysis, sequence data generated from primer set of LCO1490 and HCO2198 were combined with 11 sequences for Cossidae obtained from GenBank. Phylogenetic relationships were estimated based on 1000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on best trees only (Swofford, 2002). Bootstrap analyses were based on 1000 replications.

Results and Discussion

Sequence divergence between *C. tristis* and *C. valdiviana* was high, ranging from 16.3 – 17.7% using combined COI data (Table 2). Such levels of molecular dissimilarity strongly suggest that the two cossid species have been separated evolutionarily for considerable time (Sobti *et al*., 2007). We therefore reject the hypothesis of parallel cryptic invasion, or that host use overlap arises from close phylogenetic relatedness. A total of five COI haplotypes present in ten individuals of *C. valdiviana* collected from a single *E. nitens* site showed sequence divergence ranging between 0.1 – 3.6% (Table 2). This diversity was partitioned into two well-supported clades (Fig 1). Sequence divergence within *C. tristis* on *E. nitens* was considerably lower (0.0 – 0.1%). Combined divergence values for *C. tristis* was marginally higher (0 – 1.9%) when including the samples from grapevine (*Vitis vinifera*; Fig 1, Table 2), despite geographic separation of ~1400 km between populations.

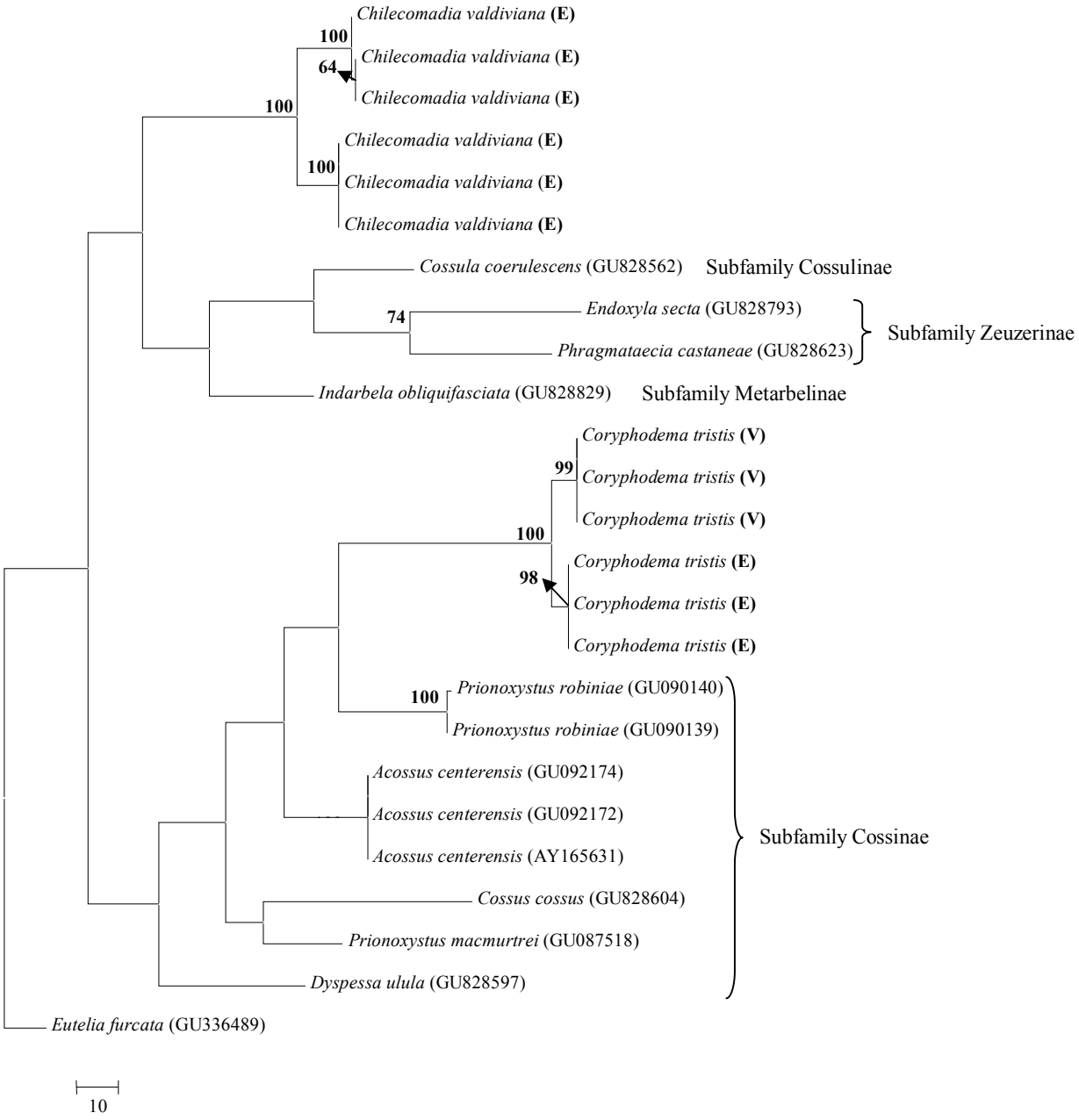

Figure 1. Maximum parsimony tree of *Coryphodema tristis* and *Chilecomadia valdiviana* from this study, and other Cossidae represented in GenBank, produced from nucleotide sequences from 657bp mtDNA COI sequence data produced with the primer set of LCO1490 and HCO2198. The percentage of replications supporting each branch is shown. *Eutelia furcata* was used as an outgroup. Family designations are from Edwards *et al*. (1999) and some internet electronic sources. For *Chilecomadia valdiviana* and *Coryphodema tristis*, the letter "E" and "V" represents their host plant *E. nitens* and *V. vinifera*.

Table. 2. Nucleotide divergence in pairwise comparisons based on combined COI mtDNA sequence data of *Coryphodema tristis* and *Chilecomadia valdiviana*.

Haplotype	*Coryphodema tristis*				*Chilecomadia valdiviana*				
Name	*Eucalyptus*		Grapevine		*Eucalyptus*				
	E1	E2	G1	G2	V1	V2	V3	V4	V5
E1	0.000								
E2	0.001	0.000							
G1	0.019	0.019	0.000						
G2	0.018	0.017	0.001	0.000					
V1	0.165	0.164	0.167	0.166	0.000				
V2	0.164	0.163	0.166	0.165	0.001	0.000			
V3	0.165	0.164	0.167	0.166	0.001	0.001	0.000		
V4	0.177	0.176	0.175	0.174	0.034	0.035	0.036	0.000	
V5	0.176	0.176	0.174	0.174	0.033	0.034	0.035	0.001	0.000

Placing *C. tristis* and *C. valdiviana* within a broader systematic context proved difficult based on mtDNA sequence data. Very little mtDNA sequence data are available on GenBank for the Cossidae – only 30 sequences from nine species were present on GenBank as of April 2012 (Mutanen *et al*., 2010). Based on the COI data, *C. tristis* and *C. valdiviana* formed distinct clades, with the latter falling outside the Cossinae (Figure 1), which contradicts the species' current designation (see Edwards *et al.* 1999). In contrast, the clade including *C. tristis*, which has no subfamily designation, included only members of the Cossinae (Figure 1). Thus, either the subfamily is polyphyletic or *C. valdiviana* is not a true member of this group. While our

sampling across the Cossidae was minimal and limits inference regarding evolutionary relationships, the family is clearly in need of taxonomic revision.

Genetic diversity for the *C. valdiviana* samples was greater than expected. Sequence divergence rates ~3.6% were particularly surprising given the limited spatiotemporal scope of our sampling, and the fact that the insect has become established on a novel, introduced host on which it has likely only been present for a little over 20 years. This diversity was partitioned into two well-supported clades, which suggests that colonization of *E. nitens* by *C. valdiviana* occurred at least twice, and may in fact occur as regular spillover from various source populations. Low sequence divergence within *C. tristis* on *E. nitens* is more congruous with one or a few recent colonization events.

As the areas of commercial cultivation of *Eucalyptus* and other plantation tree species expand, these non-native trees are exposed to a broader range of native insect and pathogen species with the potential to colonize them (Paine *et al*., 2011). Thus, understanding patterns and mechanisms driving host range expansion of native insects is of critical concern. Insect pests and pathogens typically interact intimately with the environment and their hosts. In the case of *C. tristis* and *C. valdiviana* on *Eucalyptus*, we hypothesized that parallel host use was driven by specific traits of the insects in question. Specifically, we tested the idea that a high degree of relatedness might be driving similarities in host use, namely the recent shift to *E. nitens*. We reject this hypothesis based on conclusive evidence that the two insect species share only a distant evolutionary relationship. Several alternative explanations remain. Host use overlap may simply stem from shared ecological requirements between *C. tristis* and *C. valdiviana*, may reflect convergent suite of traits in artificially selected, cultivated potential hosts, or may stem

from the increasing homogenization of potential food sources linked to changing land use patterns. While more work will need to be done to further elucidate patterns of host use in cossids, the current case represents an interesting example where molecular tools aid in the understanding of evolutionary relationships with a bearing on crops of economic importance.

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References

Angulo, A.Y.T. & Olivares, T. (1991) *Chilecomadia valdiviana* (Philippi) (Lepidoptera: Cossidae) associated with a pendulous *Ulmus glabra* Hudson (Laud.) Rehder (" Olmo pendulous ") in the VIII Region, Forest, **12**, 67 – 68.

Cerda, L. (1995) Estudio y seguimiento del taladrador de la madera *Chilecomadia valdiviana* (Lepidoptera: Cossidae) en plantaciones de *Eucalyptus nitens* en el patrimonio de FORMIN S. A. Informe de avance de temporada 1994-1995. pp 15.

Davis, S., Gentili-Poole, P. & Mitter, C. (2008) A revision of the Cossulinae of Costa Rica and cladistic analysis of the world species (Lepidoptera: Cossidae). *Zoological Journal of the Linnean Society*, **154**, 222 – 277.

Edwards, E.D., Gentili, P., Horak, M., Kristensen, N.P. & Nielsen, E.S. (1999) The

Cossoid/Sesioid Assemblage. Pages 181-197 in: Lepidoptera: Moths and Butterflies. 1. Evolution, Systematics, and Biogeography. Handbook of Zoology Vol. IV, Part 35. N. P. Kristensen, ed. De Gruyter, Berlin and New York.

FAO (2009) Global review of forest pests and diseases. A thematic study prepared in the framework of the Global Forest Resources Assessment 2005. FAO Forestry Paper 156, Viale delle Terme di Caracalla, 00153 Rome, Italy.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial Cytochrome C Oxidase Subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294 – 297.

Gebeyehu, S., Hurley, B.P. & Wingfield, M.J. (2005) A new lepidopteran insect pest discovered on commercially grown *Eucalyptus nitens* in South Africa. *South African Journal of Science*, **101**, 26 – 28.

Gonzalez, R. (1989) Insects and mites and quarantine of agricultural importance in Chile. University of Chile - BASF. Santiago, Chile, 310 p.

Goodwin, S.B., Drenth, A. & Fry, W.E. (1992) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics*, **22**, 107 – 115.

Heidel-Fischer, H.M., Freitak, D., Janz, N., Soderlind, L., Vogel, H. & Nylin, S. (2009) Phylogenetic relatedness and host plant growth form influence gene expression of the polyphagous comma butterfly (*Polygonia c-album*). *BMC Genetics*, **10**, 506 – 514.

Hoppner, G.F.J. (1994) The trunk borer of grapevines. p14.

Janz, N., Nyblom, K. & Nylin, S. (2001) Evolutionary dynamics of host-plant specialization: a case study of the tribe Nymphalini. *Evolution*, **55,** 783–796.

Janz, N. & Nylin, S. (1998) Butterflies and plants: a phylogenetic study. *Evolution*, **52**, 486 – 502.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059 – 3066.

Mardulyn, P., Milinkovitch, M.C. & Pasteels, J.M. (1997) Phylogenetic analyses of DNA and allozyme data suggest that Gonioctena leaf beetles (Coleoptera; Chrysomelidae) experienced convergent evolution in their history of host-plant family shifts. *Systematic Biology*, **46,** 722 – 747.

Mutanen, M., Wahlberg, N., & Kaila, L. (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 2839-2848.

Nair, S.S.K. (2001) Pest outbreaks in tropical forest plantations: Is there a greater risk for exotic tree species. Center for international forestry research, Indonesia. Website-http://www. cifor.ogiar.org.

Paine, T.D., Steinbauer, M.J. & Lawson, S.A. (2011) Native and exotic pests of *Eucalyptus*: A worldwide perspective. *Annual Review of Entomology*, **56**, 181 – 201.

Petty, F.W. (1917). The Quince Borer and its Control. Department of Agriculture, Bulletin no. 2. Pretoria.

Powell, J.A. (1980) Evolution of larval food preferences in microlepidoptera. *Annual Review of Entomology*, **25**, 133 – 159.

Powell, J.A., Mitter, C. & Farrell, B.D. (1999) Evolution of larval feeding habits in

Lepidoptera. Pages 403-422. **In**: Lepidoptera: Moths and Butterflies. 1. Evolution, Systematics, and Biogeography. Handbook of Zoology Vol. IV, Part 35. N.P. Kristensen, ed. De Gruyter, Berlin and New York.

Simon, C., Frati F., Beckenbach, A., Crespi, B, Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annual Entomological Society of America*, **87**, 51 – 701.

Sobti, R.C., Sharma, V.L., Kumari, M., Gill, T.K., Singh, J., Sodhi, M., Mukesh, M., Bansal, S., Arya, S. & Bisnoi, S. (2007) Genetic relatedness of six North-Indian butterfly species (Lepidoptera: Pieridae) based on 16S rRNA sequence analysis. *Molecular and Cellular Biochemistry***, 295,** 145 – 151.

Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **10**, 2731 – 2739.

Weingartner, E., Wahlberg, N. & Nylin, S. (2006) Dynamics of host plant use and species diversity in Polygonia butterflies (Nymphalidae). *Journal of evolutionary biology***, 19,** 483 – 491.