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The dead can talk: Museum specimens show the origins of a cryptic species used in biological control

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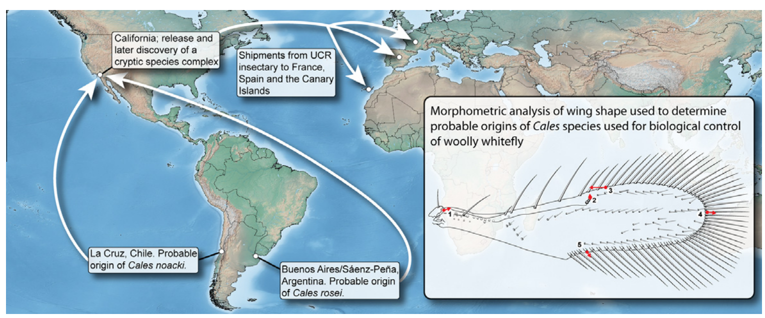
HIGHLIGHTS

- Biological control populations of *Cales noacki* comprise a cryptic species complex.
- We analyze wing shape to determine the South American origin of each species.
- Shape analysis is useful for morphological character discovery.
- Specimen vouchering should be a critical component of biological control programs.

GRAPHICAL ABSTRACT



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ABSTRACT

The parasitoid wasp species *Cales noacki* Howard (Aphelinidae) is an important biological control agent against woolly whitefly, *Aleurothrixus floccosus* (Aleyrodidae), in citrus-growing regions worldwide. We recently discovered two cryptic species of *Cales* on citrus in California: *C. noacki* and *Cales rosei* Mottern. Examination of historical biological control records is combined with a geometric morphometric analysis of fore wing shape to reconstruct aspects of the biological control history of *Cales*. Our analyses indicate that *C. rosei* is most likely descended from populations introduced from Argentina in the mid 1970s, with newly collected specimens from California clustering with Argentinian slide-mounted specimens from the original importation. Our analyses support a Chilean origin of *C. noacki*. Morphometrics confirms the earlier synonymy of *Diaspidophilus pallidus* Brèthes with *C. noacki*. A potential third species was imported from Brazil and Tucumán, Argentina, although it does not appear to have established in the field. The implications of these results for future studies on the bionomics of *Cales* and also the utility of geometric morphometric analyses for species identification and description are discussed.

1. Introduction

Correct species identification of both pests and their potential natural enemies is a critical early step in biological control programs (DeBach, 1974; Rosen, 1986). Successful biological control is not only dependent on clear delineation of species based on reproductive isolation (Mayr, 1942), but also the suite of behavioral and physiological traits that may vary both among and within species (Rosen, 1986). This effort can be severely complicated by the presence of cryptic species in a biological control system. Cryptic species can be defined as two or more populations of organisms that are morphologically indistinguishable (or nearly so), but sufficiently reproductively isolated to merit classification as separate species (Bickford et al., 2007). A growing body of literature suggests that cryptic species complexes may be common among insect parasitoids, and may differ substantially in host preferences and other bionomic traits (Campbell et al., 1993; Kazmer et al., 1996; Heimpel et al., 1997; Fernando and Walter, 1997; Stouthamer et al., 1999, 2000; Kankare et al., 2005a,b; Smith et al., 2006, 2007, 2008; Heraty et al., 2007; Bernardo et al., 2008; Kath-

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irithamby, 2009; Desneux et al., 2009; Heraty, 2009). Consequently, the presence of cryptic species may have important consequences for the efficacy and safety of biological control programs (DeBach, 1960, 1969; Rosen, 1986; Clarke and Walter, 1995; Stouthamer et al., 2000; Pinto et al., 2003; Heraty, 2009). However, the taxonomic challenges posed by small, soft bodied and morphologically uniform parasitoid wasps will necessitate studies combining molecular data with detailed quantitative analyses of morphological data (e.g. Polaszek et al., 2004).

The woolly whitefly, *Aleurothrixus floccosus* Maskell, is a significant pest in citrus-growing regions throughout the world, including California (DeBach and Rose, 1976), the Mediterranean region (European and Mediterranean Plant Protection Organization, 2002), and parts of tropical Africa (Legg et al., 2003). Following the discovery of the woolly whitefly in a residential area of San Diego in 1966 (Robinson, 1969), Paul DeBach and colleagues conducted surveys in Central and South America to find and import natural enemies (DeBach and Rose, 1976). Their initial efforts resulted in the introduction and release of four parasitoid wasp species into California and Baja California: *Amitus spiniferus* (Bréthes) (Hymenoptera: Platygastridae) from Mexico, an unidentified *Encarsia* species (Hymenoptera: Aphelinidae) from El Salvador, *Eretmocerus paulistus* Hempel (Hymenoptera: Aphelinidae) from Mexico and *Cales noacki* Howard (Hymenoptera: Aphelinidae) from Chile. The *Cales, Amitus* and *Eretmocerus* species were successfully established, with *Amitus* and *Cales* becoming the dominant natural enemies of woolly whitefly in California citrus (DeBach and Rose, 1976; Rose and Woolley, 1984; Miklasiewicz and Walker, 1990). Shipments of California *Cales* colonies were subsequently sent to France, mainland Spain and the Canary Islands between 1970 and 1972 (DeBach and Rosen, 1976). Contemporaneously with the work of DeBach and Rose, biological control workers from France were conducting their own foreign explorations for parasitoids of woolly whitefly, successfully importing and rearing *Cales* from Chile (Onillon and Onillon, 1972; Onillon 1974).

Cales has historically been a taxonomically challenging genus, with its placement within Aphelinidae only recently affirmed by Heraty et al. (2013). Recent molecular and morphological studies have indicated that *Cales* is far more diverse than previously thought (Mottern and Heraty, 2014). Their revision increased the number of described Neotropical species from one (*C. noacki*) to 10, and focused on species that have the *C. noacki* phenotype (c.f. Mottern et al., 2011). These species are exclusively Neotropical in distribution, with the exception of introduced populations in citrus-growing regions of the world. Molecular analyses indicated that at least two species of *Cales* were present on Citrus in its biological control range: *C. noacki* and *Cales rosei* Mottern. While almost indistinguishable morphologically, the two species have a distinctly different molecular signature, differing by 2.2–2.3% in 1109 bp of 28S D2–5 rDNA and 9.2–9.7% in 390 bp of cytochrome oxidase subunit I (COI) mtDNA (Mottern and Heraty, 2014). By contrast, there is very little within-species divergence (0.0–0.09% for 28S D2–5 and 0.0–3.3% for COI). This divergence in 28S between the two species is comparable to that expected between distantly related species (Campbell et al., 1993; Babcock et al., 2001), and exceeds the genetic divergence associated with reproductive incompatibility found in cryptic species complexes of chalcidoid parasitoids (Triapitsyn et al., 2006; Heraty et al., 2007).

The distribution of *C. rosei* was somewhat perplexing as its unique haplotype was only recovered from specimens collected in an unsprayed "biological control" citrus orchard at the University of California, Riverside (UCR-bc), where it exists in both temporal and spatial sympatry with *C. noacki* and attacks the same host. Though almost certainly introduced in the 1970s along with the more common *C. noacki* (a genetic match to more recently -collected specimens from Chile and established populations in Italy), the native range of *C. rosei* remained unclear.

Here, we use two sources of data to determine the probable origin of *C. rosei*: (1) importation and release records from the Insectary and Quarantine facility at the University of California, Riverside (UCR-I&Q), and (2) geometric morphometric analysis of fore wing shape in both slide-mounted specimens from the original importations and recent collections of *Cales* specimens that have been determined based on molecular analysis. We discuss the relevance of these results for future studies on the ecology and systematics of *Cales* in both its native and biological control ranges, and also discuss the utility of geometric morphometric analysis for species description and identification.

2. Materials and methods

2.1. Biological control records

2.1.1. Importations

In order to determine the timing and geographic origins of all *Cales* populations imported into the UCR-I&Q, we examined the UCR Sender's and Receiver's Reports from explorations for entomophagous insects. This process was facilitated by an electronic database maintained by the UCR Entomology Research Museum that could be queried for the taxon of interest. For records prior to 1971, which have not been entered in the database, we examined hard-copy files maintained at the UCR-I&Q. These records are somewhat variable in the data they contain, but generally include shipping and receiving dates, collector, country of origin, host identifications, natural enemy identifications, approximate number of natural enemy individuals, and a ship-receive (SR) number that remained associated with laboratory colonies derived from the original shipments. The map showing source populations and transcontinental movements of *Cales* was generated using SimpleMappr (Shorthouse, 2010).

2.1.2. Releases

Currently, there is no electronic database of biological control releases originating from the UCR-I&Q. Therefore, we examined hard copies of the reports of natural enemy releases that were prepared two times per year and submitted to the California State Department of Food and Agriculture, County Agricultural Commissioners, and other parties who may be interested in biological control programs in California. These reports include the dates of release, species of natural enemies, number of natural enemies released, country of origin, the release locality, and usually the SR number for the original source population. These records exist in two forms, the *Colonization of Beneficial Organisms* report that is submitted to state agencies, and the *Parasite/Predator Release Report* that contains greater detail regarding release dates, release localities, and the source populations.

2.2. Geometric morphometrics

We used geometric morphometric analyses of fore wing shape to examine *Cales* populations used for biological control of woolly whitefly. The use of fore wings is a natural choice for such analyses because they are relatively easy to slide-mount within a single plane of focus, minimizing error due to tilting within the mounting medium. Also, many specimens that were destructively sampled during DNA extraction could be included in the analysis because their wings were retained and mounted.

The analysis included a total of 158 specimens collected during the 1970's as part of a biological control program against the woolly whitefly in citrus (DeBach and Rose, 1976). Collection local-