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## ARE COCHINEAL INSECTS ERIOCOCCIDS?

### ABSTRACT

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Scale insects of the genus *Dactylopius* Costa, which all feed on cacti, are commonly called cochineal insects. Currently there are nine described species placed in their own family, the Dactylopiidae, based on a few unique morphological features. Here we review available biological, morphological and karyotype information on *Dactylopius* and report on cladistic analyses of morphological data (from first-instar nymphs plus adult females, and adult males separately) and molecular data (from the nuclear gene 18S rDNA and the mitochondrial gene cytochrome oxidase II) from *Dactylopius* and its potential relatives. We suggest that *Dactylopius* belongs with the eriococcids and we discuss the nomenclatural implications of this placement.

Key words: dye, Eriococcidae, *Apiomorpha*, *Stictococcus*, phylogeny, Acleridae, Asterolecaniidae, Coccidae, Diaspididae, Eriococcidae, Kermesidae, Kerriidae, Lecanodiaspididae, Phenacoleachiidae, Pseudococcidae, Putoidae, Ortheziidae, *Eriococcus*, karyology, chromosomes, life history, pigment chemistry.

### INTRODUCTION

Members of the genus *Dactylopius* Costa have attracted human attention for centuries because of the beautiful carmine dye, called cochineal, which can be extracted from their bodies and used to colour textiles and some foods (Moran, 1981; Guerra & Kosztarab, 1992). There are only nine recognised species of *Dactylopius* (De Lotto, 1974; Guerra & Kosztarab, 1992) and all are native to the New World, mostly in the deserts of south-western United States, Mexico and South America (Miller, 1991), where they feed on the stems and/or cladodes of Cactaceae.

Currently *Dactylopius* is placed in its own family, the Dactylopiidae (De Lotto, 1974; Guerra & Kosztarab, 1992), although Ferris (1955) used this family name to include all genera currently placed in the Eriococcidae, before later recognising the Eriococcidae as a distinct family that included *Dactylopius* (Ferris, 1957). Hoy (1963) later placed *Dactylopius* in the family Eriococcidae because of the presence in *Dactylopius* of 'typical eriococcid tubular ducts'. The action of Ferris and Hoy in giving priority to the name Eriococcidae is nomenclaturally invalid because, as pointed out by Williams (1969), the name Dactylopiidae is much older than the name Eriococcidae. Subsequent authors have not followed Ferris' and Hoy's recommendation: the

Dactylopiidae is recognised universally by coccidologists as a distinct, monotypic family. However, prior to 1983 (ICZN, 1983), the use of Dactylopiidae as a family-group name was unstable due to uncertainty concerning the type species of *Dactylopius* (Miller, 1974). Following the ruling by the International Commission on Zoological Nomenclature that *Dactylopius coccus* Costa be designated as the type species of *Dactylopius* (ICZN, 1983), the name Dactylopiidae was placed on the Official List of Family-Group Names in Zoology.

The main morphological features that distinguish *Dactylopius* from related genera are: (i) the presence of clusters of quinquelocular pores surrounded by a sclerotised rim (so-called “wide-rimmed pores”) in the crawlers and in the second-instar and adult females and, in later instars, usually associated with one or more tubular ducts - (clusters of similar pores, but not on sclerotised plates, also occur in the eriococcid genus *Phacelococcus* Miller); (ii) the presence of truncate dorsal setae in the crawlers and in the second-instar and adult females - (truncate dorsal setae also occur in some *Eriococcus* spp.); (iii) the absence in all instars of a cellular anal ring bearing setae; and (iv) the absence of microducts in all instars (De Lotto, 1974; Hodgson, 1997). These important diagnostic features of *Dactylopius* are autapomorphies for the genus and thus provide no information of use in assessing the relationships of *Dactylopius* to other scale insect genera. Only shared derived features (synapomorphies) furnish phylogenetically informative data.

Miller (1991) stated that the relationships of cochineal insects were in need of study because, although they appear to be related to the Eriococcidae and the Kermesidae (based on female characters), some coccidologists (e.g., Boratynski & Davies, 1971) believed that their affinities lay with the Pseudococcidae. Borchsenius (1958) grouped *Dactylopius* closest to *Stictococcus* Cockerell and *Apiomorpha* Rübsaamen, whereas Koteja (1974) reported that the mouthparts of the Dactylopiidae were acanthococcid (= eriococcid) in appearance. Williams (1969) believed that a thorough study of the adult males of *Dactylopius* held the key to understanding whether the genus is distinct from the Eriococcidae. However, although morphological data on the adult males of a few *Dactylopius* species are available (Loubser, 1966), there are no explicit phylogenetic analyses based on many adult male characters from a range of coccoid families.

A recent cladistic analysis (Foldi, 1997) of combined first-instar, adult female and adult male data placed the Dactylopiidae as a more derived group than the Pseudococcidae, Eriococcidae or Kermesidae and sister to a clade containing the other lecanoid families *sensu* Boratynski & Davies (1971) and also most of the diaspidoids, but the relationships among these families were very poorly supported (no non-homoplasious apomorphies at any of

the crucial nodes). The only recent taxonomic study of *Dactylopius* (Guerra & Kosztarab, 1992) included an intuitive phylogram for the genus based on selected morphological features of adult females, but did not assess the relationships of *Dactylopius* to other genera and families. Here we review all available biological, morphological and genetic data on *Dactylopius* and provide the first comprehensive phylogenetic study of the genus and its putative relatives. We show that the relationships of *Dactylopius* clearly are with the eriococcids and we consider the implications of this postulated relationship for family-level nomenclature.

## MATERIALS AND METHODS

We performed a cladistic analysis on 84 morphological characters (49 binary and 35 multistate) of the first-instar nymphs and adult females (L.G.Cook, unpublished data) for several species of Coccidae and Pseudococcidae, one species of Kermesidae (*Allokermes kingi* (Cockerell)), one species of Kerriidae (*Austrotachardia angulata* (Froggatt)) and 28 species of Eriococcidae, selected to represent a range of potential relatives of *Dactylopius*.

We also carried out a cladistic analysis of 43 morphological characters of the macropterous adult males of *Dactylopius* and the families Aclerididae, Asterolecaniidae, Coccidae, Diaspididae, Eriococcidae (represented by *Eriococcus sensu lato*), Kermesidae, Kerriidae, Lecanodiaspididae, Phenacoleachiidae, Pseudococcidae and Putoidae, using the Ortheziidae as an outgroup. We selected many of our characters and obtained much of our data from Afifi (1968, table A, p. 52), Koteja & Zak-Ogaza (1972, table 1) and Hodgson (1997, table 1.1.3.4.3). We used slide-mounted specimens of the adult males of *D. opuntiae* (Cockerell) and information from Loubser (1966) to score *Dactylopius*, slide-mounted specimens of an Australian *Austrotachardia* sp. to score the Kerriidae, and data from Ghauri (1962), Koteja (1986), Beardsley (1962), Theron (1962) and R.C. Henderson and T.K. Qin (pers. comm.) to score the Diaspididae, Ortheziidae, *Puto* Signoret and *Phenacoleachia* Cockerell. Phylogenetically uninformative characters were deleted from the matrix prior to analysis, leaving the final matrix with 33 binary characters and 10 multistate characters.

We obtained sequence data for a nuclear ribosomal RNA gene, 18S rDNA, for two species of *Dactylopius* (*D. austrinus* De Lotto and *D. confusus* (Cockerell)), a range of potential relatives from the Coccidae, Eriococcidae and Pseudococcidae, and *Newsteadia* sp. (Ortheziidae) as an outgroup. Nucleotide sequences for *D. confusus* and *Dymasicoccus neobrevipes* (Pseudococcidae) were obtained from Genbank (accession numbers U20402 and U20429) but all other sequences were obtained by L.G. Cook. DNA was

extracted from fresh, dried or ethanol preserved specimens using the salt extraction method of Sunnucks & Hales (1996). Sequencing templates for the 5' region of 18S rDNA were generated by PCR under standard conditions using the primers 2880 and B- (von Dohlen & Moran, 1995). Templates were sequenced in both directions and analysed on an automated DNA sequencer (Applied Biosystems, Inc.). Sequences were initially aligned using Clustal W v1.6 (Thompson *et al.*, 1994) and then adjusted by eye. Regions of alignment ambiguity in the 18S rDNA data set were omitted from the phylogenetic analyses.

Taxon sampling for each of the three data sets was very different and thus all data sets were analysed separately. We used the computer program PAUP\* test version 4.0d64 (Swofford, 1996) for all phylogenetic analyses. The adult male and the 18S rDNA data sets were small enough to allow use of the branch-and-bound algorithm to ensure that optimal trees were found, but the large size of the adult female + first-instar data set necessitated the use of heuristic searches using random addition sequences to overcome the possibility of converging on a single island in the data. Bootstrap support for each clade was estimated based on 500-1000 bootstrap replications. Bremer support (the Decay Index) was calculated and T-PTP tests of monophyly were carried out for important nodes in the adult-male and 18S rDNA trees.

## RESULTS

*Morphological data.* The full cladistic analysis of the morphological data set from the adult females plus first-instar nymphs is not presented here but the main findings relevant to *Dactylopius* are as follows. *Dactylopius* fell within the large eriococcid clade and, although its position was not stable (sensitive to taxon deletion), it always fell within, rather than basal to, the other eriococcids.

Cladistic analysis of the adult male data matrix produced four minimum-length trees (Fig. 1), in which *Puto* was basal and the Pseudococcidae was sister to the rest of the taxa. *Dactylopius* formed a clade with *Eriococcus s.l.* and this clade formed a trichotomy with *Phenacoleachia* and a clade containing the remainder of the taxa. The *Dactylopius-Eriococcus* clade had high bootstrap and significant T-PTP support.

*Molecular data.* Analysis of the 18S rDNA data under different assumptions produced slightly different trees but in all trees the two *Dactylopius* species were always embedded within the larger eriococcid clade (Fig. 2). The coccids formed a polytomy with multiple eriococcids whereas the pseudococcids were more basal, although the latter node had no support. The results of a phylogenetic analysis of nucleotide sequence data from a mitochondrial protein-coding gene, cytochrome oxidase II (COII) (L.G. Cook,

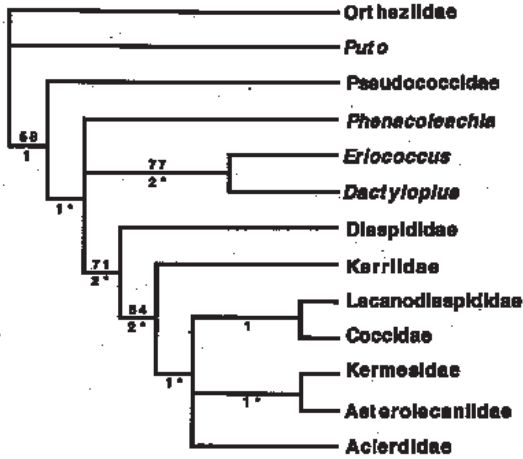


Fig. 1 - A strict consensus of the four minimum-length cladograms from morphological data of the adult males (tree length = 109; CI = 0.495; RI = 0.560). Numbers above the nodes are bootstraps (only values of 50 and above shown), below the nodes are Bremer values and the asterisks indicate nodes for which, by T-PTP tests, a prior hypothesis of monophyly is corroborated.

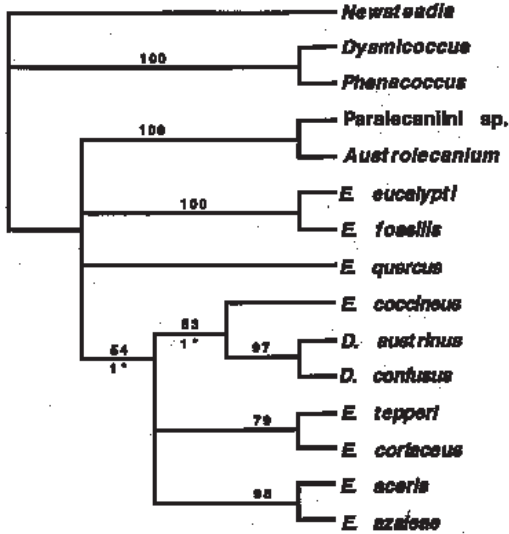


Fig. 2 - A strict consensus of the six minimum-length cladograms from nucleotide sequence data from the 18S rDNA gene (tree length = 129; CI = 0.720; RI = 0.783). Numbers above the nodes are bootstraps; Bremer support (below the node) and T-PTP significance (asterisks) were calculated only for the eriococcid (including *Dactylopius* clade) and the *Dactylopius* + *E. coccineus* clade.

unpublished data) did not contradict the above findings from the 18S rDNA analysis but were inconclusive, partly because the COII data were uninformative at the required level and also because the taxon set was smaller and did not include coccids.

## DISCUSSION

Our morphological data from first-instar nymphs plus adult females and from adult males and our molecular data from the 18S rDNA gene all show that *Dactylopius* belongs with or, at least, is more closely related to the Eriococcidae than to either the Coccidae or Pseudococcidae, as suggested by Koteja (1974) based on mouthpart morphology. Furthermore, the Pseudococcidae may be basal to both the eriococcid (including *Dactylopius*) clade and the coccid (or even the remainder of the lecanoid) clade. This is contra to the schemes suggested by Boratynski & Davies (1971), based on adult males, and by Foldi (1997), based on all life stages. The mitochondrial gene data do not contradict the relationship of *Dactylopius* suggested by the nuclear gene and the morphological data.

*Karyology and chromosome systems.* Both the Comstockiella and the lecanoid chromosome systems have been reported for *Dactylopius* (Nur, 1980, 1982). Such information is of little phylogenetic value because both of these chromosome systems and intermediates are widespread in the various lecanoid families, including the Eriococcidae (Brown, 1967; Nur, 1980). *Dactylopius* has a diploid number of  $2n = 10$  (Nur, 1982; Moharana, 1990), which is similar to some pseudococcids and at least one coccid species (Nur *et al.*, 1987; Moharana, 1990) and different from that of typical eriococcids (Brown, 1967). Few species of the other lecanoid families have been examined chromosomally but none have a karyotype like that of *Dactylopius*.

*Life history.* Cochineal insects have three female instars and five male instars, which is the same life-history pattern as found in eriococcids, aclerdids, asterolecaniids, kermesids and lecanodiaspidids (Miller, 1991). In contrast, the ortheziids, pseudococcids and most, but not all, coccids have four female instars (Miller, 1991), which is apparently the ancestral instar number for female neococcoids. Thus, the life-history pattern of dactylopiids is derived compared to mealybugs, but is uninformative concerning relationships with the other lecanoid families.

*Pigment chemistry.* Banks (1977) classified coccoid pigments into two chemical groups (the alpha-methyl and beta-methyl systems) and reviewed the taxonomic distribution of these pigments among coccoid families. *Dactylopius* possesses the alpha-methyl system, which is also found in representative taxa of the Coccidae, Kermesidae, Kerriidae and Margarodidae, whereas *Eriococcus* (represented by *E. confusus* Maskell and *E. coriaceus* Maskell) have the beta-methyl system typical of the Asterolecaniidae

(represented only by *Callococcus acaciae* (Maskell), which L.G. Cook's unpublished molecular data suggest is an eriococcid) and the Pseudococcidae. Thus, if *Dactylopius* belongs with the eriococcids, then eriococcid taxa are heterogeneous with respect to pigment chemistry, as suggested by Banks (1977), and thus we must accept that similarities in pigments are due to convergent evolution.

In summary, neither chromosomal nor life-history data assist with the phylogenetic placement of *Dactylopius*, whereas the pigment chemistry of *Dactylopius* is more similar to that of coccids than of eriococcids. However, from analysis of available morphological and molecular data, we conclude that *Dactylopius* falls within a clade containing representatives of several undisputed eriococcid genera. In the recent past, *Dactylopius* has been set apart from other members of this clade due to its autapomorphic morphology. In addition, the cactus host association of *Dactylopius* species is unusual for eriococcids (although not for pseudococcids). Among the eriococcids, perhaps only four species, including *Eriococcus coccineus* Cockerell, feed exclusively on Cactaceae (Hoy, 1963; Miller & Miller, 1992). Interestingly, *E. coccineus* is the putative sister of *Dactylopius* in some cladograms derived from the 18S rDNA data.

If the status of Dactylopiidae as a monotypic family is to be maintained, then this would necessitate the elevation to family level of a number of other groups currently placed firmly within the Eriococcidae. Some reclassification may be warranted given that Cox & Williams (1988) argue that the Eriococcidae, as presently interpreted, cannot be regarded as a monophyletic group because it has no defining autapomorphies. Furthermore, our suggestion that *Dactylopius* belongs with the eriococcids has other serious nomenclatural implications because Dactylopiidae is an older name than Eriococcidae (Williams, 1969). However we do not support either of the potential solutions to this dilemma, i.e. either break the eriococcids into a number of smaller families (in order to maintain the Dactylopiidae as a separate taxon) or transfer all eriococcids to the Dactylopiidae. In the interests of nomenclatural stability, we instead argue that formal recognition of the valid family-group name(s) for these genera should await the completion of comprehensive phylogenetic studies. In the interim, we propose that the current usage of the names Dactylopiidae and Eriococcidae be maintained.

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