

Differentiation of *Macrolophus pygmaeus* (Rambur 1839) and *Macrolophus melanotoma* (Costa 1853) (Heteroptera: Miridae) based on molecular data

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Abstract: Several species of the *Macrolophus* genus are well known predators of small pests such as whiteflies, aphids and mites. *Macrolophus melanotoma* (Costa 1853) (Syn. *Macrolophus caliginosus* Wagner 1951) and *Macrolophus pygmaeus* (Rambur 1839) are the most economically important species. In spite of their economic importance the classification of these two species has not received comprehensive treatment until recently. The lack of discontinuity in the taxonomical characters used to differentiate *M. melanotoma* from *M. pygmaeus* made it difficult to define species boundaries and conspecificity was not excluded. In this work we use molecular information to clarify the identity of *M. melanotoma* and *M. pygmaeus*. The phylogenetic analysis using cytochrome b sequences grouped the specimens studied in two distinct clades, corresponding to insects classified as *M. melanotoma* and *M. pygmaeus* using key morphological characters. Commercial insects, labelled and sold as *M. caliginosus*, were classified as *M. pygmaeus*. Based on molecular phylogenetic and morphological evidences we conclude that *M. melanotoma* and *M. pygmaeus* are two different species. The biology, ecology and role as pest control agents of *Macrolophus melanotoma* and *M. pygmaeus* may have been messed up due to misidentification.

Key words: *Macrolophus* spp., *M. melanotoma*, *M. caliginosus*, *M. pygmaeus*, DNA, phylogeny, cytochrome b, Miridae, Dicyphine, IPM.

Introduction

Several species of the *Macrolophus* genus are well known predators of small pests in vegetable crops. *Macrolophus melanotoma* (Costa 1853) [Syn. *Macrolophus caliginosus* Wagner 1951, (Carapezza, 1995)] and *Macrolophus pygmaeus* (Rambur, 1839) are the most economic important species (Malausa & Trottin-Caudal, 1996; Perdakis & Lykouressis, 2000; Alomar *et al.*, 2003). *Macrolophus caliginosus* is used for pest control in vegetable crops worldwide. In spite of their economic importance, the taxonomical classification of these two species has not received comprehensive treatment until recently (Martinez-Cascales *et al.*, 2006).

Josifov (1992) based the differentiation of *M. melanotoma* and *M. pygmaeus* in the black band-shape macula behind the eye and the colour of the first antennal segment. He reported several major findings, as follows: (1) *M. melanotoma*, band-shape macula behind the eye thin and colour of the first antennal segment clear in the middle part; (2) *M. pygmaeus*, macula as high as half the width of the eye and first antennal segment entirely black. However, several authors pointed out the inconsistency of the characters used to differentiate the two species and conspecificity was not excluded (Kerzhner & Josifov, 1999; Goula *et al.*, 2002).

The paucity of morphologic characters leads to the use of molecular techniques to clarify

the issue. The use of sequence variation in mitochondrial genes has proven most useful in establishing species boundaries (Simon *et al.*, 1994; Sanchez *et al.*, 2006; Martinez-Cascales *et al.*, 2006).

The aim of this work was to investigate whether *M. melanotoma* and *M. pygmaeus* were two different species. We also tested the identity of a *Macrolophus* species labelled and sold as *M. caliginosus*.

Materials and methods

Samples

Macrolophus were collected on *Dittrichia viscosa* and tomato (*Lycopersicon esculentum*) in the Iberian Peninsula and Canary Islands. Only specimens that could undoubtedly be classified, either as *M. melanotoma* or *M. pygmaeus*, according to the key characters listed by Josifov (1992) were used in the phylogenetic analysis: (1) insects with a thin band-shape macula behind the eye and middle part of the first antennal segment clear (*M. melanotoma*); (2) insects with a wide macula and first antennal segment entirely black (*M. pygmaeus*). Two females and two males of each group were used for phylogenetic analyses (Table 1). A female and two males of a commercial *Macrolophus* labelled as *M. caliginosus* were also analysed (Table 1).

Table 1. Sample codes, GenBank accession numbers, localities and host plants, for all taxa used in the phylogenetic analyses. Commercial = commercial *Macrolophus* labelled as "*M. caliginosus*". Localities: Murcia (Southeast Spain); Valverde (Portugal); Tenerife (Canary Islands). Plants: *D. viscosa* = *Dittrichia viscosa*, *L. esculentum* = *Lycopersicon esculentum*.

Species	Sex	Specimen code	GenBank number	Locality	Host plant
<i>M. melanotoma</i>	♀	M017-2	DQ372115	Murcia	<i>D. viscosa</i>
<i>M. melanotoma</i>	♀	M121-5	DQ372117	Tenerife	<i>D. viscosa</i>
<i>M. melanotoma</i>	♂	M037-2	DQ372116	Murcia	<i>D. viscosa</i>
<i>M. melanotoma</i>	♂	M125-2	DQ372117	Tenerife	<i>D. viscosa</i>
<i>M. pygmaeus</i>	♀	M004-3	DQ372110	Murcia	<i>L. esculentum</i>
<i>M. pygmaeus</i>	♀	M015-2	DQ372111	Murcia	<i>L. esculentum</i>
<i>M. pygmaeus</i>	♂	M012-2	DQ372111	Murcia	<i>L. esculentum</i>
<i>M. pygmaeus</i>	♂	M016-2	DQ372113	Murcia	<i>L. esculentum</i>
Commercial	♀	M115-5	DQ372124	-	-
Commercial	♂	M115-3	DQ372124	-	-
Commercial	♂	M115-4	DQ372125	-	-
<i>M. costalis</i>	♀	M240-1	DQ372122	Valverde	<i>Cistus monspeliensis</i>
<i>D. tamaninii</i>	♂	D014-1	DQ232675	Murcia	<i>Epilobium hirsutum</i>
<i>D. cerastii</i>	♂	D003-1	DQ232672	Murcia	<i>Cucurbita maxima</i>
<i>N. tenuis</i>	♂	N006-1	DQ232682	Murcia	<i>L. esculentum</i>
<i>C. geniculata</i>	♂	C001-1	DQ232681	Murcia	<i>Ononis natrix</i>

Macrolophus costalis Fieber was used as an outgroup. This species can be clearly differentiated from other *Macrolophus* species by the black apex of the scutellum. Other dicyphines were also used as outgroups: *Dicyphus cerastii* Wagner, *Dicyphus tamaninii*

Wagner, *Nesidiocoris tenuis* (Reuter), *Cyrtopeltis geniculata* Fieber.

DNA analysis

A fragment of the mitochondrial cytochrome b region (320 bp) was amplified by PCR and sequenced as described by Sanchez *et al.* (2006) and Martinez-Cascales *et al.* (2006). Sampling information and GenBank sequence accession numbers are given in Table 1.

DNA sequences were first explored using MEGA 3.1 (Kumar *et al.*, 2004). All sequences and alignments were checked manually to ensure their correction. Maximum parsimony analysis was carried out using PAUP* 4.Ob10 version (Swofford, 2002). Initial parsimony trees were obtained by branch and bound search. Additionally, Neighbor-Joining method with Kimura 2-parameter substitution model was performed using MEGA 3.1. Nodal support in both analyses was estimated by 2000 bootstrap replicates.

Results and discussion

The insects classified as *M. melanotoma* and *M. pygmaeus* based on the black band-shape macula behind the eye and the colour of the first antennal segment, grouped in two distinct clades in the phylogenetic analyses (Figure 1). *M. pygmaeus* is a sister species of *M. costalis*, and *M. melanotoma* joins the group formed by those two species. Parsimony and Neighbor-Joining analyses produced trees with similar topologies. Nodes at the species level were supported by high bootstrap values. Figure 1 shows the maximum parsimonious tree inferred from cyt b sequence data. The phylogenetic analyses using molecular data confirm the species identity of *M. melanotoma* and *M. pygmaeus*.

The commercial *Macrolophus*, labelled as *M. caliginosus* (Syn. *M. melanotoma*), clustered within the *M. pygmaeus* group (Figure 1); they also shared the wide macula and the entirely black first antennal segment. This result reveals that some of the commercial products are labelled with the wrong species name and we suspect this could be more general for other insects commercialised as *M. caliginosus*. This unfortunate event may have been a great source of mistakes in characterizing the biology, ecology and role as pest control agents of *Macrolophus* species because, many times, insects used in experiments came from natural enemy producers.

The correct classification of the species is one of the key factors for the success of biological pest control programs. Failures in biological control programs due to misidentification of natural enemies are well documented (DeBach 1964). There are several reasons that make the identification of *Macrolophus* species highly important: (1) being zoophytophagous they feed on plants and the degree of phytophagy may greatly vary between species. Goula & Alomar (1994) argued that plant damage information for *M. melanotoma* and *M. pygmaeus* may have been assigned to the wrong species due to misidentification; (2) the characterization of *Macrolophus* species ecology is prior to developing pest management programs based on natural biological control. Many species of *Dicyphus* and *Macrolophus* are restricted to a low number of host plants (Sanchez *et al.*, 2006; Martinez-Cascales *et al.*, 2006). Host plant restrictedness in *Macrolophus* species may be driven by plant preference behaviour, like it has been reported for other dicyphines such as *Dicyphus hesperus* (Sanchez *et al.*, 2004). We predict that differences in host plant preference between *M. melanotoma* and *M. pygmaeus* would limit the movements of each species to the most preferred hosts.

The lack of discontinuous taxonomical characters may sometimes difficult the differentiation between *M. melanotoma* and *M. pygmaeus* using morphological traits (Martinez-Cascales *et al.*, 2006). Molecular techniques can be used to solve the species identity when morphology characters alone were irresolute.

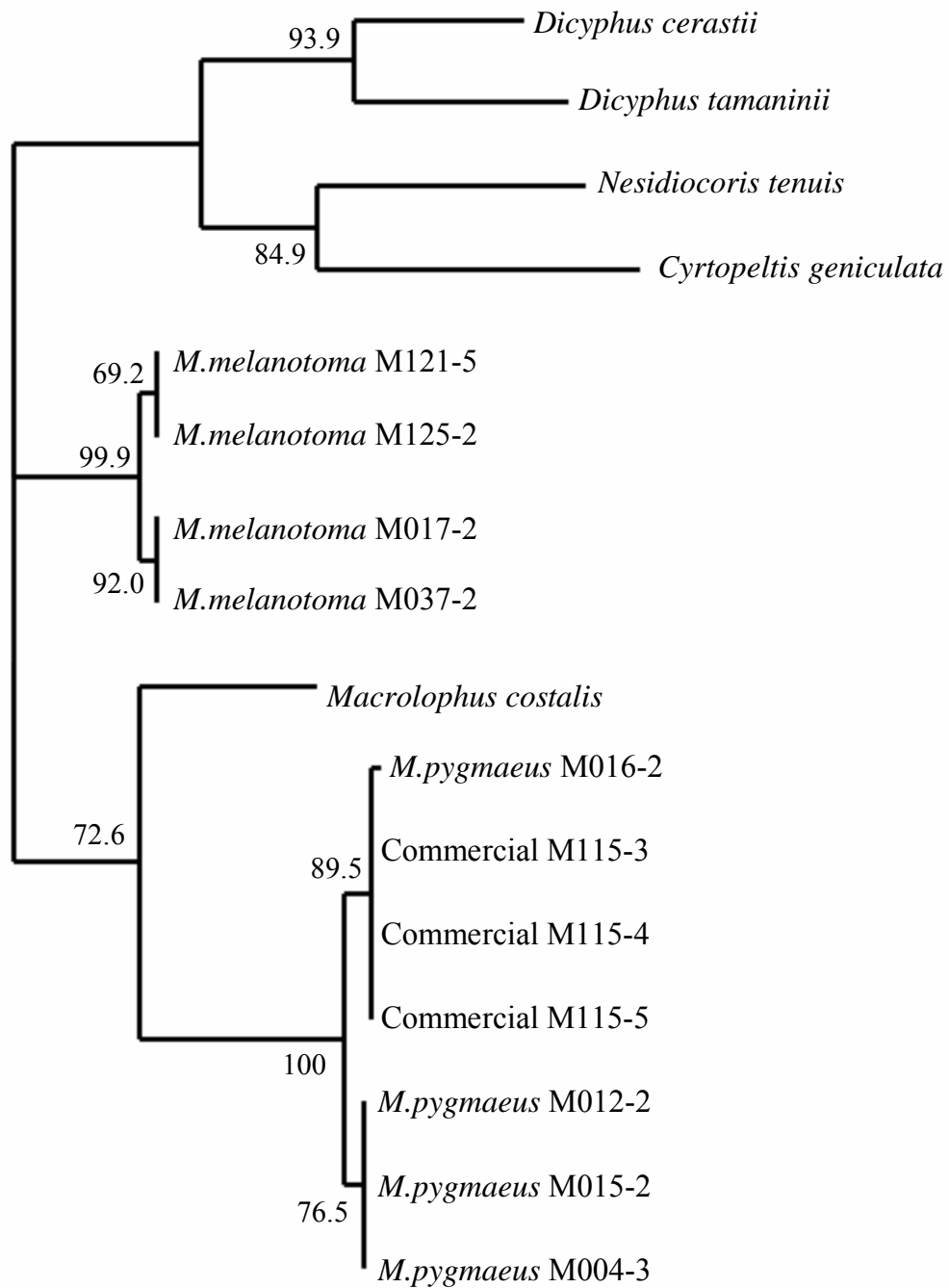


Figure 1. Phylogeny of *Macrolophus* species based on cyt b sequences. Maximum parsimony tree with bootstrap values. Outgroups: *Macrolophus costalis*, *Cyrtopeltis geniculata*, *Nesidiocoris tenuis*, *Dicyphus cerastii* and *Dicyphus tamaninii*.

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