Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence

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Abstract. The oestroid family Tachinidae represents one of the most diverse lineages of insect parasitoids. Despite their broad distribution, diversity and important role as biological control agents, the phylogeny of this family remains poorly known. Here, we review the history of tachinid systematics and present the first quantitative phylogenetic analysis of the family based on morphological data. Cladistic analyses were conducted using 135 morphological characters from 492 species belonging to 180 tachinid genera, including the four currently recognized subfamilies (Dexiinae, Exoristinae, Phasiinae, Tachininae) and all major tribes. We used characters of eggs, first-instar larvae and adults of both sexes. We examined the effects of implied weighting by reanalysing the data with varying concavity factors. Our analysis generally supports the subfamily groupings Dexiinae + Phasiinae and Tachininae + Exoristinae, with only the Exoristinae and the Phasiinae reconstructed as monophyletic assemblages under a wide range of weighting schemes. Under these conditions, the Dexiinae, which were previously considered a well-established monophyletic assemblage, are reconstructed as being paraphyletic with respect to the Phasiinae. The Tachininae are reconstructed as a paraphyletic grade from which the monophyletic Exoristinae arose. The Exoristinae are reconstructed as a monophyletic lineage, but phylogenetic relationships within the subfamily are largely unresolved. We further explored the evolution of oviposition strategy and found that the oviparous groups are nested within ovolarviparous assemblages, suggesting that oviparity may have evolved several times independently from ovolarviparous ancestors. This counterintuitive pattern is a novel hypothesis suggested by the results of this analysis. Finally, two major patterns emerge when considering host associations across our phylogeny under equal weights: (i) although more than 60% of tachinids are parasitoids of Lepidoptera larvae, none of the basal clades is unambiguously associated with Lepidoptera as a primitive condition, suggesting that tachinids were slow to colonize these hosts, but then radiated extensively on them; and (ii) there is general agreement between host use and monophyly of the major lineages.

Introduction

Within the order Diptera, the oestroid family Tachinidae represents the largest lineage of endoparasitoids. Tachinid larvae develop within their hosts (mainly insects, but also chilopods...
According to the recent phylogenetic reconstruction of Wiegmann et al. (2011), cyclorrhaphan Diptera had an early Tertiary explosive radiation. Tachinids were probably radiating rapidly by the mid-Tertiary, becoming widespread and abundant in virtually all terrestrial ecosystems (Wood, 1987b; Tschorsnig & Richter, 1998). There are nearly 8200 named tachinid species (and thousands more undescribed), accounting for about 6.5% of the total diversity of Diptera. Over 90% of tachinid species attack phytophagous insects, and about 60% of these are parasitoids of dipteran Lepidoptera (Stireman et al., 2006; Cerretti, 2010), which comprise the most diverse group of plant-feeding organisms.

Given their broad distribution, great diversity and ecological role as enemies of primarily herbivorous insects, tachinids have attracted considerable attention from basic and applied researchers, as well as taxonomists (Stireman et al., 2006; O’Hara, 2013b). However, the classification and identification of tachinids have posed formidable challenges, and these have hindered the understanding of their biology, ecology and potential usefulness in controlling pests in managed systems. The group is widely regarded as one of the more difficult families of Diptera in terms of identification (Crosskey, 1976), and a universal classification scheme remains elusive despite recent progress.

Here, as part of a larger initiative to understand the phylogeny and evolution of the family Tachinidae on a global scale (Stireman et al., 2013), we present the first quantitative phylogenetic analysis of the family based on morphological data. We briefly review the history of tachinid classification and the hypothesized relationships that key workers have advanced, noting some of the important character states that have been used to define various tachinid clades. We then present an analysis of the phylogenetic relationships of the Tachinidae based on a broad range of taxa (180 genera) and a diverse collection of morphological characters (135). Our aim is to assess the strength of the phylogenetic signal of morphology in defining major tachinid clades and their relationships, despite the great degree of morphological homoplasy in the family. We use our resulting phylogenetic reconstruction to evaluate previous hypothesized relationships and classifications, and to propose novel ones that can be evaluated with subsequent morphological and molecular phylogenetic studies. Finally, we examine the implications of our phylogenetic results for understanding evolutionary patterns of reproductive strategy and host use within the family.

History of the classification of Tachinidae

The purpose of this section is to briefly review the history of tachinid classification and to consider some of the morphological characters that have been used to define fundamental groups in recent classification schemes. For a more comprehensive and detailed account of the history of tachinid classification, see O’Hara (2013b).

Coquillett (1897), in his revision of the Tachinidae of America north of Mexico, recognized the Tachinidae almost as it is known today but without the subfamily Dextrinae. At about the same time, in Europe, Girschner (1893, 1896) was elaborating on a system of chaetotaxy developed by Osten-Sacken (1881, 1884) and used the presence of ‘hypopleural’ (meral) setae to delimit the Tachinidae, a concept for the family equalling that of the present-day superfamily Oestroidea. This classification was largely followed in the influential Katalog der pal¨aarktischen Dipteren (Bezzi & Stein, 1907). Not until the discovery of the value of the ‘metanotum’ (subscutellum; character state 60:1 below) by Malloch (1923) did authors begin to use this character to delimit the Tachinidae along modern lines.

The progressive elements of Girschner (1893, 1896) were further refined by Villeneuve (1924, 1933), whose work was, in turn, further advanced by Mesnil (1939) in his Essai sur les Tachinaires.

Mesnil (1939) recognized six subfamilies of the Tachinidae: Dextrinae, Phasiinae, ‘Larvaevirinae’ (Tachininae), Ameninae (currently in Calliphoridae), ‘Salmaciinae’ and ‘Phorocerinae’ (the last two comprising present-day Exoristinae). He brought together in his Phorocerinae those tachinids possessing a haired prosternum (41:0) (i.e. character 41, state 0 in the present study, see File S2) and a short and fine ‘prealar’ (first postsutural supra-alar) seta (49:1). The Phorocerinae were subdivided into the ‘Phorocerinini’ (Exoristini), Blondelini and ‘Crocutini’ (Siphonini). Each was further characterized in part by: the Phorocerinini by vein M (as ‘4e’) having an angular bend (71:3) and shadow fold (72:1), and subapical scutellar setae divergent (character not coded in analysis below); the Blondelini by vein M having a rounded bend and no shadow fold (71:1, 2; 72:0), and subapical scutellar setae divergent; and Crocutini by vein M having a rounded bend and no shadow fold (71:1, 2; 72:0), and subapical scutellar setae convergent. The current concepts of the Exoristini and Blondelini largely date from Mesnil (1939). The Exoristini are probably monophyletic (Stireman, 2002; Tachi & Shima, 2010), but the Blondelini, according to Wood (1985), are probably not. Wood (1985: 7) noted that the small prealar seta (49:1) ‘is probably a plesiomorphic character’.

Villeneuve’s contemporary in the New World was Townsend, a prolific describer of tachinid genera and species. Townsend’s greatest achievement was his 12-volume Manual of Myiology in which the genera of Tachinidae and allies were diagnosed and assigned to a multitude of tribes and families. This work was also Townsend’s greatest downfall, for unrelated genera were frequently grouped together into tribes. The catalogue of the Tachinidae of America south of the United States by Guimarães (1971) did not significantly
improve the classification of the region’s Tachinidae. The fauna of America north of Mexico has fared better in the successive catalogues of Sabrosky & Arnaud (1965) and O’Hara & Wood (2004), the latter much influenced by the classification of Herting (1984).

Mesnil dominated tachinid taxonomy in Europe for decades after his Essai, particularly through instalments to Die Fliegen der Palaearktischen Region (Mesnil, 1944–1975). During this time, Herting published early works on the female postabdomen of calyptrate flies and the biology of West Palaearctic Tachinidae (Herting, 1957, 1960). Implicit in these works was an attempt to order the Tachinidae phylogenetically. The Salmacinae and Phorocerinae of Mesnil (1939) [also Mesnil (1944–1956, 1956–1965) as Salmacini and Phorocerini] were restructured to form the near-modern Exoristinae with tribes Exoristini, Blondeliini, Acemyini (as ‘Acemyiini’), Siphonini, Ethillini, Winthemiini and Goniini. The Goniini were later split to become the modern Goniini and Eryciini, with the former restricted to species producing microtype eggs (6:1) that are ingested by their hosts (9:1) (Mesnil, 1975a,b; Herting, 1984). Thus constituted, the Goniini are generally considered monophyletic; the Eryciini are regarded as paraphyletic. The Siphonini were later moved to the Tachininae (Herting, 1984).

The modern concept of the Dexiinae, with the main tribes of Dexiini, Voriini and Dufouriini, dates from Herting (1957, 1960). Herting used the as-yet-unpublished discoveries of Verbeke (1962, 1963) to define the subfamily according to unique features of the male terminalia: phallus with a hinged connection between basiphallus and distiphallus (Verbeke’s ‘type II’ connection) (115:1) and pregonite strap-like and connective (‘type C’ of Vebeke’s ‘posterior paramere’) (103:1).

In the first instalment on the ‘Larvaeorini oder Tachinini’ portion of Die Fliegen der Palaearktischen Region, Mesnil (1966) revised the classification of the tachinids he had dealt with in previous instalments and presented the classification he would follow for the Tachinini. The higher classification was summarized in a table (Mesnil, 1966: 882) wherein present-day Tachinidae were treated as a subfamily with six tribes: Voriini, Dexiini and Tachinini (on the left in the table) and Exoristini, Goniini and Phasiini (on the right in the table). Both Mesnil (1966) and Herting (1966) expressed the same views about the two main branches of Tachinidae:

(1) The three groups on the right in Mesnil’s table are oviparous, or originally so, producing planoconvex eggs with a thick curved dorsal surface and a thin and flat underside (state 2:1).

(2) The three groups on the left in Mesnil’s table are ovolarviparous, producing eggs with a thin membrane and without differentiation into an upper and a lower surface (2:0).

Mesnil (1966) recognized the Tachinini as the most diverse group of tachinids and subdivided it into about 30 subtribes. Herting (1984) treated these subtribes as tribes of Tachininae, but reduced them in number and recognized 14 in the Palaearctic Region.

The treatments of Old World non-Palaearctic Tachinidae by Crosskey (1973, 1976, 1980, 1984) and Cantrell & Crosskey (1989) were conservative in their taxonomic approach, treating the Voriini in the Tachininae and the Neaerini and Siphonini in the Exoristinae, recognizing the Dufouriinae as a subfamily, and not dividing the Goniini and Eryciini according to egg type.

The ‘gold standard’ of classifications remains that of Herting (1984), which was followed by Herting & Dely-Draskovits (1993). Although this classification specifically applies to the Palaearctic Region, its hierarchy of subfamilies and tribes provides a good framework for a global classification (Table 1), at least at present (see the Discussion). Only portions of the classification are stable from a phylogenetic perspective, and a greater understanding of tachinid relationships may indicate where changes should be made to better reflect the evolutionary history of the Tachinidae.

Recently, Stireman (2002) and Tachi & Shima (2010) provided the first attempts at reconstructing phylogenetic relationships within the Tachinidae using molecular data. Stireman (2002) used two genes, EF1α and 28S rDNA, while Tachi & Shima (2010) used four (16S, 18S, 28S rDNA and white). These studies focused on the subfamily Exoristinae, generally supporting its monophyly and that of its constituent tribes (with some exceptions). Notably, Stireman (2002) failed to reconstruct a monophyletic Goniini (the microtype egg layers, state 6:1), while Tachi and Shima did find support for this clade. Both studies found a similar progression of clades, with Winthemiini basal, then Exoristini branching off, followed by the Blondeliini and, finally, the Eryciini and Goniini forming a clade. As suggested by this progression, Tachi & Shima (2010) found, using character reconstruction, that ovolarviparous forms (state 7:1) have arisen repeatedly from oviparous ancestors (state 7:0).

Materials and methods

Monophyly of terminal taxa

All terminal taxa included in the analysis are assumed, a priori, to be valid genera following the definitions given in all major monographs (Crosskey, 1984; Tschorsnig, 1985; Wood, 1987b; Tschorsnig & Herting, 1994; Tschorsnig & Richter, 1998; Cerretti, 2010; Cerretti et al., 2012a). We also assumed that each species chosen for character coding is congeneric with the type species of the genus in which it is placed. It is possible that some of these genera are in fact not monophyletic, but assessing this is beyond the scope of this study.

Selection of taxa, coding of character states and terminology

Specimens belonging to 180 tachinid genera (total of 492 species examined) were selected for character state coding
Table 1. Subfamilial and tribal level classification of the Tachinidae, excluding tribes given in Table 2.

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<tr>
<th>Subfamily</th>
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<tr>
<td>Dexiini</td>
<td>Catharosini</td>
<td>Bigonichetini</td>
<td>Myioptini</td>
<td>Anacamptyiini</td>
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<td>Dex.</td>
<td>Cylindromyiini</td>
<td>Brachymerini</td>
<td>Myiophasini</td>
<td>Blondeliini</td>
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<td>Dufourini</td>
<td>Gymnosomatini</td>
<td>Ernestiini</td>
<td>Neanerini</td>
<td>Erycioni</td>
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<td>Epigrimyiini</td>
<td>Hermyni</td>
<td>Germariini</td>
<td>Nemoraeini</td>
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<td>Rutuliini</td>
<td>Leucostomatini</td>
<td>Gariochaeotini</td>
<td>Occisorini</td>
<td>Exoristini</td>
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<td>Sophiini</td>
<td>Parerigonini</td>
<td>Glaurocarini</td>
<td>Ormiini</td>
<td>Gonini</td>
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<td>Teleothyriini</td>
<td>Phasiini</td>
<td>Graphogastriini</td>
<td>Pelatachinini</td>
<td>Thrixioni</td>
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<td>Urmyini</td>
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<td>Icelini</td>
<td>Polideini</td>
<td>Winthemiini</td>
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<td>Voriini</td>
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<td>Macquartiini</td>
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<td>Megaprosopini</td>
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<td>Minthoini</td>
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Table 2. List of exemplar ‘enigmatic’ taxa and their subfamily placement by different authors.

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<td>Imitomyiiini</td>
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<td>P</td>
<td>D</td>
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<tr>
<td>Eutherini</td>
<td>P</td>
<td>P</td>
<td>D</td>
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<tr>
<td>Acemyiini</td>
<td>E</td>
<td>E</td>
<td>T</td>
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<tr>
<td>Masipyini</td>
<td>–</td>
<td>?P</td>
<td>E</td>
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<tr>
<td>Strongygastrini</td>
<td>P</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>Euthelairini</td>
<td>–</td>
<td>E</td>
<td>T</td>
</tr>
<tr>
<td>Palpostomatini</td>
<td>T</td>
<td>T</td>
<td>D</td>
</tr>
<tr>
<td>Litophasia</td>
<td>P</td>
<td>D</td>
<td>–</td>
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Phylogenetic analysis

Cladistic analyses using parsimony were conducted with a data matrix of the states of 135 characters belonging to species of 180 tachinid genera, as well as three calliphorid, one rhiniid and two rhinophorid genera (Files S1–S3). The matrix was produced in mesquite version 2.74 (Maddison & Maddison, 2010) and analysed in tnt version 1.1 (Goloboff et al., 2003). The ‘New Technology Search’ (NTS) options were used because of the large number of taxa. To find the most parsimonious trees, all analyses were run with the following parameters: general RAM of 1000 megabytes and memory set to hold 600 000 trees; multistate characters were treated as unordered and zero-length branches collapsed. The ‘driven search’ was performed by using 14 replications as the starting point for each hit [which is the maximum value as suggested by the program for the most exhaustive search, i.e. when the initial level is set as 100 from ‘set initial level’ dialog box (= maximum value) or 10 from command line (= maximum

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value). Each replicate, initially autoconstrained (previous and Wagner), was conducted with: constraint and random based sectorial searches, no ratchet, tree-drifting (six iterations) and tree-fusing (10 rounds); finding minimum length trees 30 times, not consensusing trees during search, multiplying trees by fusing after hitting best score and saving one tree per replicate. tnt commands from command line were as follows: ‘hold 600000; xmult = level 10 hits 30 rss css fuse 10 drift 6;’.

Analyses were conducted with equal weighting and also with implied weights under the default function k/(es + k), where k = concavity constant and es = extra steps, with k varying continuously between 1 and 10, then for k = 15, 20, 40 (tnt command: hold 600000; Piwe = 1, 2, 3, . . . n; xmult = level 10 hits 30;) in order to examine and compare tree length, total fit and variation in tree topology under various weighting schemes (File S5). Total fit was calculated for most parsimonious trees (MPT) obtained under equal weights for each k-value tested and compared with total fit of MPTs obtained under implied weighting for corresponding k-values (File S5).

Bremer support values were calculated in tnt from 10000 trees up to 10 steps longer than the shortest trees obtained from a ‘traditional search’, using the ‘trees from RAM’ setting. A jackknife resampling was carried out with a traditional search producing 1000 replicates each of 100 random taxa addition subreplicates applying tree bisection-reconnection branch swapping and saving 10 trees per replication. Jackknife removal probability was set at the default value of 0.36, and resampling percentiles were calculated as frequency differences [group present/contradicted (GC)]. Character optimization (using unambiguous changes only) was carried out in winclada version 1.00.08 (Nixon, 1999). Ancestral state parsimony reconstruction of host associations was carried out in Mesquite version 2.74 (Maddison & Maddison, 2010).

Results

Analysis under equal weights

The analysis under equal weights and with all characters unordered produced 87 most-parsimonious trees (tree length = 1109 steps, consistency index for matrix, CI = 0.181, retention index for, RI = 0.731): the strict consensus tree is shown in Fig. 1. We selected one of the three shortest trees with the highest total fit under the whole range of the k-values tested (Fig. 2) (i.e. tree number 7 of the nexus file, File S6) to depict inferred character state changes (File S4), as discussed in the following.

Tachinid monophyly. The Tachinidae (clade A) are reconstructed as a monophyletic assemblage based on eight non-homoplasious apomorphies (1:1, egg dorsal plastron absent; 3:2, egg shell uncoloured; 7:1, ovolarviparous; 10:1, first-instar mandibles reduced; 12:1, first-instar labrum strongly developed; 60:1, subscutellum strongly convex; 133:1, female ovisac very long and generally spiralled when full; 134:1, female ovisac enveloped by a well-developed tracheal network) and two homoplasious apomorphies (80:0; 120:1). Monophyly is well supported by a Bremer support value of 10 and a resampling probability of 95. The calliphorid outgroups formed a paraphyletic grade, consistent with the findings of Rognes (1997), and the two rhinophorids + Rhyncomya (Rhiniidae) were reconstructed as sister group to Tachinidae based on three homoplasious apomorphies (51:1; 52:0; 71:1).

Basal taxa. The Coleoptera-parasitizing clade B (Gnadaochaeta Macquart + Palpostomatini) is reconstructed as sister group of clade C (i.e. all the remaining tachinids). Monophyly of clade C is weakly supported by two homoplasious apomorphies (30:0; 43:2). The macquartine Macprosopsa Brauer & Bergenstamm (clade D) is sister group of clade E ((Ormiini + (Dexiinae + Phasiinae)) + (Tachininae + Exoristinae)) based on three homoplasious apomorphies (57:0; 79:1; 111:2).

Monophyly of clade B is well supported by two homoplasious apomorphies (33:1; 2; 111:1) and one non-homoplasious apomorphy (105:1, medial surface of male pregonite membranous). Taxa comprising this clade have been placed in the Tachininae by several authors and here occupy the most basal position within the Tachinidae.

Clade F (Ormiini + (Dexiinae + Phasiinae)), which is supported by three homoplasious apomorphies (51:0; 65:1; 106:1), is divided into two subclades; clade G (the tribe Ormiini, here represented by Aulacephala Macquart and Therobia Brauer) and clade H (Dexiinae + Phasiinae). Monophyly of clade G is strongly supported by five homoplasious (13:1; 85:0; 88:2; 96:1; 113:1) and three non-homoplasious apomorphies (14:1, ocelli missing or posterior pair strongly reduced; 42:1, female with a very swollen and convex prosternal region; 110:1, basiphallus dorsobasally thickened (Tschorsnig, 1985)).

The Dexiinae. Clade H (Dexiinae + Phasiinae) is supported by two homoplasious (109:1; 114:1) and two non-homoplasious (115:1, dorsal connection between basiphallus and distiphallus membranous; 116:1, basiphallus and distiphallus joined at a right angle) apomorphies. The dorsal membranous connection between basiphallus and distiphallus has long been considered a clear synapomorphy of the subfamily Dexiinae (see historical review). In the present analysis, this character state is interpreted as having undergone a reversal, being secondarily lost in most Phasiinae.

Within clade H, the monophyly of clade I (i.e. the current subfamily Dextinae excluding the Dufouriini) is supported by three homoplasious apomorphies (34:0; 53:0; 95:1). Although additional research is needed to corroborate the phylogenetic position of the genus Phylloomyia Robineau-Desvoidy as sister taxon of clade K (Stomina Robineau-Desvoidy + Dextini), it is interesting to point out that the Dextini (clade L) are nested within the Vorini sensu Herting (1984) and that the genus Stomina takes up a position as sister group of the Dextini based on six homoplasious apomorphies (8:1; 13:1; 30:1; 51:1; 65:0; 85:0). The monophyly of clade L is well supported by four synapomorphic character states: one homoplasious...
(71:3) and three non-homoplasious (97:1, ear-shaped medial side of surstylus; 101:1, boundary between hypandrium and hypandrial apodeme very distinct; 122:1, acrophallus with a granulate or lamellate structure).

Clade M is supported by three homoplasious apomorphies (47:3; 48:0; 79:3,4). The Voria-group (clade N) is well supported by four homoplasious apomorphies (43:4; 45:3; 56:0; 73:0) and one non-homoplasious apomorphy (76:1, crossvein dm-cu exceptionally oblique).

The Phasiinae and Dufourini. Clade O consists of the Phasiinae + Dufourini. The monophyly of clade O is supported by two homoplasious apomorphies (73:3; 90:1).

The Dufourini sensu Herting (1984) (Chetoptilia Rondani, Dufouria Robineau-Desvoidy, Rondania Robineau-Desvoidy, Pandelleia Villeneuve, Microsoma Macquart, Eugymnopeza Townsend, Freræa Robineau-Desvoidy), which are parasitoids of adult beetles, are paraphyletic with respect to the Phasiinae sensu Herting (1984) (clade Q). Clade P
Fig. 2. One of the 87 most parsimonious trees obtained under equal weights with ancestral parsimony reconstruction of host associations mapped in colour. Clades discussed in the text are labelled with letters.

Morphic character states. Rather basal lineages, forms a grade due to lack of any synapomorphic apomorphies (81:1; 119:0; 121:1), the current Rondania, supported by just one homoplasious apomorphy 

Litophasia Girschmer, Imitomyia Townsend and Strongygaster Macquart represent rather basal phasiine lineages. Of these taxa, hosts are unknown for the first two, while the last has a rather complex host spectrum that includes adult Formicidae (S. globula (Meigen); see Herting, 1960), Heteroptera and a plethora of adult Coleoptera (S. triangulifera (Loew); see Reeves & O’Hara, 2004).

Four apomorphic character states support monophyly of clade R (Phasiinae – Litophasia): two homoplasious (16:1; 114:0) and two non-homoplasious (100:1, medial plate of hypandrium elongated; 102:1, pregonite posteriorly connected to hypandrium). Clade S (clade R – Imitomyia) is supported by three homoplasious (103:0; 106:0; 115:0) and two non-homoplasious (91:1, posterior margin of sternite 5 not notched; 93:1, male sternite 6 symmetrical) apomorphies. The monophyly of clade T (i.e. the Phasiinae sensu O’Hara & Wood, 2004), the members of which are all parasitoids of Heteroptera, is rather well supported by five homoplasious apomorphies (7:0; 117:1; 131:1; 133:0; 134:0). Within clade V, the tribe Phasiiine sensu Tschorsnig (1985), here represented by Ope sia Robineau-Desvoidy, Phasia Latreille and Xysta Meigen, is not recovered as monophylectic. The Gymnosomatini (clade W, thus including Trichopoda Berthold), however, are monophyletic and supported by two homoplasious apomorphies (2:1; 96:1) and one non-homoplasious apomorphy (123:1, sperm duct developed into three well-sclerotized ducts).

Monophyly of clade X is supported by two homoplasious apomorphies (65:1; 96:1). Parerigone Brauer and Hermya Robineau-Desvoidy represent basal lineages to clade Y (Cylindromyini + Leucostomatini) whose monophyly is supported by one non-homoplasious apomorphy (125:1, phallos smooth and tubular). The tribe Cylindromyini (clade AA) is supported by two homoplasious (95:1; 131:0) and two non-homoplasious (130:1, female tergite 7 and sternite 7 fused; 132:1, female postgenital plate stylet-like) apomorphies. Monophyly of the Leucostomatini (clade Z) is supported by two homoplasious (16:0; 18:2) and one non-homoplasious (127:1) apomorphies.

The tachinine grade. Clade AB (all remaining Tachinidae, i.e. Tachininae + Exoristinae) is weakly supported by three homoplasious apomorphies (27:1; 35:3; 58:0). Clade AC, supported by one homoplasious apomorphy (73:3), reconstructs the Coleoptera-parasitizing genus Anthomyiops Townsend as sister group of the polyphagous tribe Graphastrini (Graphagaster Rondani, Heraulitia Villeneuve, Phytomyzoptera Rondani). Within clade AD, which is supported by three homoplasious apomorphies (81:1; 119:0; 121:1), the current tribe Minthoini (Rossiomyiops Mesnil, Hyperaea Robineau-Desvoidy, Plesina Meigen, Pseudomintho Brauer & Bergensstamm, Mintho Robineau-Desvoidy), although representing rather basal lineages, forms a grade due to lack of any synapomorphic character states.

Monophyly of the large clade AF is supported by three homoplasious apomorphies (48:0; 81:0; 85:0). This assemblage is divided into two large subclades, AG and AV, representing the remaining Tachininae and entire Exoristinae, respectively.

The tachinine clade AG is supported by three homoplasious apomorphies (32:2; 65:1; 114:1). The Leskiini (Clausiccella Rondani, Leskia Robineau-Desvoidy, Bithia Robineau-Desvoidy, Aphria Robineau-Desvoidy, Demoticus Macquart) are retrieved as a paraphyletic grade of lineages at the base of clade AH. Clade AE, supported by three homoplasious apomorphies (52:1; 78:2; 114:0), is divided into two subclades (AI and AL).

Clade AE, composed of the tribes Brachymerini and Siphonini, is supported by three homoplasious apomorphies (33:2; 85:3; 112:0). Monophyly of the tribes Brachymerini (clade AJ) and Siphonini (clade AK) is supported, respectively, by four homoplasious apomorphies (34:1; 43:5; 44:1; 65:0) and by five homoplasious (21:2; 41:0; 73:3; 74:1; 106:1) and two non-homoplasious (104:1, anterior portion of pregonite membranous; 135:1, female with two spermathecae) apomorphies.

Clade AL represents the Tachinini in the broad sense of Tschorsnig (1985: 70), i.e. an expanded concept with respect to Herting (1984) and most other authors, plus Pelatchina Meade. This clade is supported by five homoplasious apomorphies (95:1; 111:1; 113:1; 117:0; 119:1). The Tachinini s.l. are divided into three main subclades: clades AM and AN supported by two homoplasious apomorphies [(24:4; 66:1,2) and (58:0; 79:2,3), respectively]; and clade AO supported by four homoplasious apomorphies (43:5; 49:0; 65:0; 80:0).

In clade AM, the highly apomorphic and moth-parasitizing genus Sarromyia Pokorny (cf. Cerretti, 2010) is quite surprisingly retrieved as sister to the beetle-parasitizing tribe Megaprosopini (Dexiosoma Rondani, Microphthalma Macquart).

Within clade AN, the genus Loewia Egger clusters with the Polideini sensu O’Hara (2002) (clade AP). The monophyly of clade AP is supported by two homoplasious apomorphies (43:4; 69:2). This clade is, in turn, sister to clade AQ, which is composed of genera currently included in Herting’s (1984) tribes Neaerini (Neaera Robineau-Desvoidy, Neoplectops Malloch), Ernestiini in part (Eloceria Robineau-Desvoidy, Synactia Villeneuve) and Bignonichetini (= Triarthriini) (Triarthria Stephens). The first two of these tribes are reconstructed as polyphyletic.

Clade AO is divided into two subclades: clade AR, supported by three homoplasious apomorphies (39:1; 52:0; 73:0) and clade AS, supported by one homoplasious apomorphies (15:1). In clade AR, Germaria Robineau-Desvoidy and Linnaeuma Robineau-Desvoidy (clade AT) cluster together, forming the sister group of clade AU (Tachinini s.s.). Monophyly of clade AT is supported by just one homoplasious apomorphy (117:1). Clade AU is supported by three homoplasious apomorphies (24:4; 38:1; 44:1) and one non-homoplasious apomorphy (94:1, postabdomen capsule-like). In clade AS, the genera Nemoraea Robineau-Desvoidy (Nemoraeini) and Pelatchina

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(Pelatachinini) are nested within a group of Ernestiini sensu Herting (1984), in part.

A monophyletic Exoristinae. Monophyly of the Exoristinae + Eutherini (clade AV) is supported by four homoplasious apomorphies (2:1; 3:0; 8:0; 45:3). Within this clade the tribe Acemyini (clade AW) is corroborated as monophyletic with strong support of six apomorphic character states: four homoplasious (58:0; 88:1; 103:2; 112:0) and two non-homoplasious (40:1, first flagellomere sharply pointed; 124:1, pronounced shortening of phallus). The Acemyini are sister to clade AX, which represents all the remaining Exoristinae.

Clade AX (Blondellini, Eryciini, Ethillini, Exoristini, Goniini, Winthemini, Eutherini) is supported by two homoplasious apomorphies (52:1; 121:0).

Phylogenetic reconstruction of the lineages of subfamily Exoristinae is ambiguous in our analysis. The Eutherini (clade BA), consisting of two known genera (both included in the present analysis), have been placed either in the Phasiinae or in the Dexiinae, as discussed earlier. Here, the tribe occupies a nested position within the Exoristinae. Monophyly of the Eutherini is strongly supported by nine homoplasious apomorphies (19:0; 51:0; 78:1; 103:1; 106:1; 111:1; 114:1; 117:0; 119:1) and one non-homoplasious apomorphy (5:1, egg with a posterodorsal ‘window’).

Monophyly of each of the tribes Winthemini, Ethillini, Eryciini and Blondellini traditionally ascribed to the Exoristinae is not supported in any of the 87 MPTs, suggesting that these taxonomic groups should be studied in more detail to delineate monophyletic groups and revise their classification accordingly. In the tree chosen for discussion (Fig. 2), the Blondellini, Eryciini and Ethillini are each reconstructed as polyphyletic. There is some support for monophyly of Exoristini (clade BD), Winthemini (+ Phorocerosoma Townsend) (clade BH) and Goniini (clade BJ).

A sister-group relationship is supported between Exoristini (clade BD) and clade BE (Medina Robineau-Desvoidy + (Winthemini + Ethillini (in part))). The tribe Exoristini is supported by three homoplasious apomorphies (71:3; 72:1; 96:1). Clade BF, supported by two homoplasious apomorphies (50:1; 121:1), reconstructs clade BG (Ethilla Robineau-Desvoidy + Prosethilla Herting) as sister group of clade BH (Winthemini and the ethilline Phorocerosoma). The Goniini (clade BJ) are reconstructed as monophyletic with support from three non-homoplasious apomorphies (3:1, egg shell brown or dark brown; 6:1, egg microtype; 9:1, egg hatching stimulated by proteolytic enzymes of host mesenteron) and one homoplasious (8:1) apomorphy.

**Analysis under implied weights**

Using implied weights analyses resulted in different consensus topologies depending on the k-values of the weighting function (Fig. 3). As a consequence, it is not possible to comment in detail upon all the various relationships observed in the resulting trees (especially for distal nodes). Instead, a concise description of the more interesting and significant variations is given in the following (Fig. 3).

$k = 1$ (Fig. 3a). Under these extreme weighting conditions, the genus Litophasia is reconstructed as sister group of all other tachinids due its lack of a well-developed subsutellum. The presence of a strongly convex subsutellum (60:1) represents the only non-ambiguous, non-homoplasious apomorphy present on the consensus cladogram that supports the remaining Tachinidae. In this analysis, Eutherini are reconstructed as a rather basal lineage of the Exoristinae, while the Dexiinae take up a position as sister to clade Phasiinae (sensu Herting, 1984; thus including Imitomyiini and Strongygastrini) + Tachininae (paraphyletic). The Ormiini take up a position of sister group to the Phasiinae.

$2 ≤ k ≤ 5$ (Fig. 3b). Here, the Phasiinae (sensu O’Hara & Wood, 2004, thus excluding Imitomyia and Strongygastrer) take up a sister-group position to the remaining Tachinidae (cf. Richter, 1991). The subfamily Exoristinae is broken up into a paraphyletic grade of lineages from which the monophyletic Acemyini and clad Dexiinae + Tachininae arose. Under these k-values of the weighting function, oviparous groups [i.e. Phasiinae, Winthemini, Ethillini (in part), Exoristini and the blondeline genus Medina] are reconstructed as a basal paraphyletic grade of taxa from which a monophyletic ovolarviparous group arose. More specifically, under these k-values, the laying of fully embryonated eggs (7:1), together with the very long ovisac (133:1) and the presence of a network of tracheae enveloping the ovisac (134:1), seems to have characterized the common ancestor of all the ovolarviparous tachinids, thus rejecting both the reconstruction we obtained under equal weights and previous hypotheses that ovolarvipary evolved several times independently from oviparous ancestors (cf. Stireman et al., 2006; Tachi & Shima, 2010). The membranous, translucent egg shell (3:2) is the only non-ambiguous, non-homoplasious apomorphy supporting clad Dexinae + Tachininae. The Eutherini are reconstructed as nested within a paraphyletic Exoristinae ($3 ≤ k ≤ 5$) or as sister group of clad Tachininae + Dexinae ($k = 2$). The tribe Ormiini and the genus Strongygastrer represent rather basal lineages of clad (Imitomyia + (Litophasia + (Dexinae))), which is in turn supported by two non-ambiguous, non-homoplasious apomorphies (87:4, abdominal tergite 5 more than 1.5 times as long as tergite 4; 115:1, membranous dorsal connection between basiphallus and distiphallus).

$k = 6$ (Fig. 3c). Under these k-values of the weighting function, the oviparous groups noted earlier are still reconstructed as a basal paraphyletic grade of taxa from which ovolarviparous groups arose, with the subfamily Phasiinae representing the most basal tachinid lineage. The most notable difference from the scenario discussed earlier is that the tribe Ormiini takes up the position of sister group of Imitomyia +
Strongygaster and that this clade represents a basal lineage of clade (Eutherini + (Litophasia + (Dexiinae))).

$7 \leq k \leq 8$ (Fig. 3d). The general topology resulting from these analyses is close to that obtained under equal weights, such that the basal tachinid lineage is represented by clade Gnadochaeta + Palpostomatini and the remaining Tachinidae are divided into two large assemblages: Dexiinae + Phasiinae (clade F of Fig. 2) and Tachininae + Exoristinae (clade AB of Fig. 2, but excluding Eutherini). Remarkably, under these conditions, subfamilies Dexiinae (sensu Herting, 1984), Phasiinae (including Imitomyia and Strongygaster) and Exoristinae are reconstructed as monophyletic, while the subfamily Tachininae remains paraphyletic with respect to Exoristinae. The tribes Ormiini and Eutherini are recovered as sister groups of Phasiinae and Dexiinae, respectively.

$k \geq 9$ (Fig. 3e, f). Under these conditions the topology is nearly the same as that obtained under equal weights. Clade Gnadochaeta + Palpostomatini is retrieved as sister group of all the remaining Tachinidae (see earlier for $k = 7$ and 8) and the Dexiinae and Tachininae are both reconstructed...
as paraphyletic with respect to Phasiinae and Exoristinae, respectively. The most notable difference is that the tribe Eutherini does not cluster within the Exoristinae, taking up the sister-group position to clade Dexiinae + Phasiinae (clade H of Fig. 2). For \( k \geq 7 \), which we examined, Ethilla, Prosethilla, Paratryphera Brauer & Bergenstamm and Atylomya Brauer (Ethillini) form a monophyletic group.

**Discussion**

Reconstructing the phylogeny of this large family of parasitoids, a group characterized by extraordinary morphological diversity and impressive radiation in nearly all terrestrial ecosystems, remains a difficult challenge. As mentioned in the introductory sections, the current classification of Tachinidae at the tribal and subfamilial levels is the result of observations on external morphology and male and female terminalia. The phylogenetic value of these traits has never before been analysed using a rigorous cladistic approach on a broad scale at the world level. This analysis, using an extensive morphological data set for a large cohort of taxa, represents the first explicit hypothesis of tachinid phylogenetic relationships. This first analysis has yielded a number of intriguing results (discussed later) and also provides phylogenetic data that can be tested, modified and supplemented in future studies on tachinid evolution.

Despite the widely accepted subdivision of the Tachinidae into four subfamilies (Table 1), clear-cut definitions of these groups remain elusive and specialists are aware that some subfamilies are not monophyletic. As a consequence, certain genera and tribes have been arbitrarily moved from one subfamily to another, based simply upon the opinions of specialists (however informed they may be) (Table 2). For example, in comparing the regional catalogues of Herting (1984) and O’Hara & Wood (2004) – whose authors largely agree on basic ideas about tachinid classification – the following differences are noted (see also historical review and Table 2): the Eutherini and Imitomyini were treated in the Phasiinae by the former and in the Dexiinae by the latter (as proposed by Shima, 1989); the Acemyini were classified in the Exoristinae by the former and moved to the Tachininae by the latter; the Strongygastrini were treated in the Phasiinae by the former and in the Tachininae by the latter.

In addition to inconsistencies in the placement of taxa at the tribal and subfamilial levels among authors, the placement of a key species or small genus into the wrong (though widely accepted) tribe or subfamily is sometimes overlooked. For instance, the genus Litophasia, which is consistently placed in the phasiine tribe Catharosini, is characterized by a very different, dexiine type of male terminalia (cf. Tschorsnig, 1985).

**Equal vs implied weights**

Increasing \( k \)-values of the default weighting function in TNT resulted in a gradual loss of concavity of the function, and hence a reduction in the strength of the weighting function, which at the limit converges on the unweighted linear function. Under implied weighting, a tree that could increase the fit in characters with few homoplasies by reducing the number of steps could also result in an increased number of steps in characters with many homoplasies, i.e. the total fit may be increased at the cost of an increased tree length (Goloboff, 1993; Goloboff et al., 2008). The total fit calculated for MPTs obtained under equal weights for each \( k \)-value tested (TNT commands: piwe = 1, 2, ... 40; fit*) resulted in trees under implied weighting displaying higher fit than the shortest obtained under equal weights (File S5). Also, by increasing values of \( k \), the difference in steps between trees under implied weighting and the shortest under equal weights diminishes, as do the differences in total fit (File S5).

With a \( k \)-value of 1 (Fig. 3a), hence with strongest penalty to homoplasy, the analysis yielded trees 87 steps longer than the shortest trees under equal weights. This extreme degree of concavity gives certain characters excessive influence on the tree, resulting in clades strongly contradicted by numerous characters. For example, with \( k = 1 \) the ‘dexiine-phasine’ genus Litophasia takes up the position of sister group to all the remaining tachinids due its unique lack of a strongly convex subscutellum (60:1). This character state undoubtedly represents a reversal for Litophasia, which shares a large suite of states (i.e. external morphology, male and female terminalia) with the dufourini and the phasiines, thus making the entire reconstruction quite unreliable.

Trees obtained under implied weights with \( k \)-values varying from 2 to 6 (which are still much better in terms of total fit with respect to those obtained under equal weights; see File S5) reconstructed oviparous Exoristinae as a paraphyletic grade of taxa from which ovolarviparous groups such as the Tachininae and Dexiinae arose (Fig. 3b, c). This hypothesis conflicts strongly with previous classification schemes and morphological hypotheses as well as with preliminary molecular data. In fact, the Exoristinae, which are composed of both oviparous and ovolarviparous lineages, have been consistently recovered as monophyletic (see Tschorsnig, 1985; Richter, 1991; Stireman, 2002; Tachi & Shima, 2010). Here, characters associated with egg type and incubation, which characterize large groups (some tribes and subfamilies), are drawing together otherwise unrelated groups and dominating clad structure due to the concave weighting scheme.

For \( k > 6 \), the deeper nodes begin to stabilize around a general pattern (Fig. 3d–f) with the same three basal clades [\( (Gnadochaeta + Palpostomatini), (Dexiinae + Phasiinae) \) and [Tachininae + Exoristinae]] as obtained under equal weights (Figs 1, 2). The notable differences with respect to the analysis under equal weights are that: (i) the tribe Eutherini is recovered as sister group of [Dexiinae + Phasiinae] (for \( k \geq 9 \)) (Fig. 3e, f) or, within this assemblage, as sister to clade [Dexiinae + Litophasia] (for \( k = 7 \) and 8) (Fig. 3d); (ii) for \( k \)-values varying from 7 to 15, the macquartine genus Macroprosopa takes up a position as sister to Anthomyiopsis within the Tachininae grade.
Analyses conducted giving the strongest penalty to homoplasy (i.e. $1 \leq k \leq 6$) appear to be providing excess confidence in very unlikely trees by too severely overweighting characters that change little and underweighting those that are more labile. In the following discussion we focus mainly on the results obtained from the analyses under equal weights and under implied weighting with $k \geq 7$, referring occasionally to certain topologies obtained under implied weighting and $k \leq 6$ that require specific comments.

**Tachinid monophyly and sister group**

The current analysis does not allow us to fully test the monophyly of Tachinidae or determine their sister group, given the inclusion of a relatively small number of outgroup taxa. However, the monophyly of the Tachinidae is here corroborated by ten synapomorphic character states, and supported by a Bremer support value of 10 and a jackknife resampling probability of 95 (Fig. 1; File S4). Surprisingly, some recent phylogenetic reconstructions of calyptrate Diptera, employing mitochondrial and nuclear genes but including only a few tachinid species, have not strongly supported the monophyly of Tachinidae or determine their sister group, under both equal and implied weights ($k \geq 9$) (Fig. 3e, f). Following the traditional definition of the Dexiinae (Tschorsnig, 1985; Wood, 1987b; Shima, 1989; Tschorsnig & Richter, 1998), all members of the subfamily share a strap-like pregonite (103:1) and have the basiphallus and distiphallus joined at a right angle (116:1) through a membranous connection (115:1). In particular, states 115:1 and 116:1, characterizing the dexter-type phallus, are here reconstructed as autapomorphic support for the clade Dexiinae + Phasiinae. These states are interpreted here for the first time as having undergone a reversal, being secondarily lost in the Phasiinae. The strongly autapomorphic phallus of most Phasiinae (Tschorsnig, 1985), often characterized by the lack of a clear border between basiphallus and distiphallus, lends support to this hypothesis. Interestingly, our results support the affinities between Dufourini and Phasiinae identified by Verbeye (1962, 1963) and Tschorsnig (1985), reconstructing the former as a grade from which the latter arose (Figs 1, 2; File S4).

Although previously suggested by Herting (1984), our analysis provides the first rigorous support for the subfamily affiliation of *Litophasia*, *Imitomyia* and *Strongygaster* within the Phasiinae. But, as hypothesized by Tschorsnig (1985), *Litophasia* takes up a position of sister group of the Dexiinae under a wide range of weighting schemes (Fig. 3a–d), in particular, when the Dexiinae are recovered as monophyletic. Interestingly, under both equal weighting and for the lowest $k$-values of the weighting function (Fig. 3a, b), the Eutherini do not cluster with either the Dexiinae or the Phasiinae. The tribe has long been considered a member of the Phasiinae, in part because *Euthera* species (and later confirmed for *Redienbacheria insignis* Egger) are parasitoids of true bugs (Acanthosomatidae, Pentatomidae) (cf. Herting, 1966; Shima, 1999). All Phasiinae, with the exception of *Strongygaster*, are parasitoids of true bugs and all other Tachinidae are parasitoids of other arthropods. But unlike other Phasiinae, *Euthera* Loew and *Redienbacheria* Schiner are ovolarviparous (7:1) (Shima, 1999). Herting (1966) and Mesnil (1966) were both convinced that the egg of *Euthera* is of the planoconvex type (2:1) (File S2, Figures S1, S2) and placement in the Phasiinae

*Phasines from dexterinae?*

Our analysis generally supports the subfamily groupings Dexiinae + Phasiinae and Tachininae + Exoristinae (Figs 1, 2) (as first proposed by Shima, 1989; see earlier), with only the Exoristinae and the Phasiinae reconstructed as monophyletic lineages under a wide range of weighting schemes. This contrasts with the views of Mesnil (1966) and Herting (1966) in which the Tachinidae were grouped into Tachininae + Dexiinae and Exoristinae + Phasiinae based on their oviposition and egg types. Interestingly, the Dexiinae, which were previously considered a well-established monophyletic assemblage, are reconstructed as paraphyletic with respect to the Phasiinae, under both equal and implied weights ($k \geq 9$) (Fig. 3e, f). All members of the subfamily share a strap-like pregonite (103:1) and have the basiphallus and distiphallus joined at a right angle (116:1) through a membranous connection (115:1). In particular, states 115:1 and 116:1, characterizing the dexterine-type phallus, are here reconstructed as autapomorphic support for the clade Dexiinae + Phasiinae. These states are interpreted here for the first time as having undergone a reversal, being secondarily lost in the Phasiinae. The strongly autapomorphic phallus of most Phasiinae (Tschorsnig, 1985), often characterized by the lack of a clear border between basiphallus and distiphallus, lends support to this hypothesis. Interestingly, our results support the affinities between Dufourini and Phasiinae identified by Verbeye (1962, 1963) and Tschorsnig (1985), reconstructing the former as a grade from which the latter arose (Figs 1, 2; File S4).
is warranted despite its ovolarviparous habit. Tschorsnig (1985) was uncertain about the affinities of the Eutherini, recognizing certain similarities with the Dexiinae in the male terminalia, but ultimately retaining the tribe in the Phasiinae. Shima (1989) placed *Euthera* at the base of the Dexiinae, and Shima (1999, 2006), O’Hara & Wood (2004) and Cerretti (2010) continued the placement of the Eutherini in this subfamily. We have observed that the long and platform pregonite in the Eutherini recalls the pregonite of the Dexiinae, as does the position and orientation of the epiphallus in this tribe (File S2, Figure S37). In fact, the only marked difference in the male terminalia of the Eutherini with respect to the Dexiinae is the sclerotized dorsal connection between basiphallus and distiphallus, as noted by O’Hara & Wood (2004). The unusual combination of a planoconvex egg shape (2:1) and possession of a large and tracheated ovisac in which eggs are embryonated (7:1; 133:1; 134:1) appears to play a role in reconstructing the Eutherini at the base of the Exoristinae (Fig. 3a), or nested within the Exoristinae (Figs 1, 2, 3b). However, for k-values ranging from 6 to 40, the Eutherini are retrieved as sister to Dexistinae or sister to Dexiinae + Phasiinae (Fig. 3c–f). The former relationship is only inferred when the Dexiinae are reconstructed as monophyletic (6 ≤ k ≤ 8) (Fig. 3c, d). For higher k-values (Fig. 3e, f), the Dexiinae fragment into a paraphyletic grade from which the Phasiinae arose and the Eutherini become the sister group of Dexiinae + Phasiinae. In all these reconstructions, characters of the male terminalia play a crucial role in defining phylogenetic relationships over those of the egg and the female genitalia, supporting the hypothesis of Shima (1989).

Adults of the small tribe Strongygastrini resemble members of *Phasia* Latreille, and other characters have led most authors to place the genus in the Phasiinae (e.g. Herting, 1960; Verbeke, 1962; Mesnil, 1966; Herting, 1984; Tschorin, 1985), where it is placed in our analysis. However, unlike other phasiines, species of Strongygastrus are not parasitoids of true bugs (see earlier) and are ovolarviparous (7:1). Shima (1989) placed Strongygastrus at the base of the Phasiinae. O’Hara & Wood (2004) and Cerretti (2010) doubted the phasine affinity of Strongygaster and placed the genus in the Tachininae.

**The diverse and complicated Tachininae**

It is generally agreed that the Tachininae are the most weakly supported tachinid subfamily (cf. Tschorin, 1985), being composed of a diversity of morphologically heterogeneous taxa. This is corroborated by our analysis, in which the subfamily is fragmented into a number of para- and polyphyletic lineages. The Coleoptera-parasitizing clade B (Gnadochaeta + Palpostomatina) and the highly derived Ormini (clade G) are widely separated from Tachininae (Figs 2, 3), the subfamily where they are traditionally assigned.

Although not supported by resampling, clade B is recovered as sister to all the remaining tachinids under a wide range of weighting schemes. Interestingly, this hypothesis is similar to that advanced by Mesnil (1966: 881–885). Mesnil (1966) discussed a multitude of morphological and ecological characters of both adults and first instars of all major tachinid tribes and subtribes, and provided a ‘non-rooted’ graphical representation of the relationships among taxa. Mesnil (1966: 881) depicted Myiophasiini and Palpostomatinii in a rather ‘central’, although ambiguous, position from which all other major groups seem to arise. The long and slender distiphallus structure shared by members of clade B recalls that of most daxies; but the shape of the pregonite and postgonite, as well as the shape and position of the epiphallus, recall those of many Tachininae and Exoristinae. Several highly homoplasious character states of the adult chaetotaxy seem to be involved in placing clade B as sister to all remaining tachinids (File S4).

*Macroprosopina* is particularly mobile across phylogenetic analyses, taking up positions at the base of clade C, or, under implied weighting, nested within the Tachininae. It is worth noting that the distiphallal structure in *Macroprosopina* is very similar to that of *Gnadochaeta* and of the Palpostomatina, which is more consistent with the phylogeny obtained under equal weights and for k-values ≥ 20 (Fig. 3f).

The phylogenetic position of the Ormini remains ambiguous regardless of the weighting scheme, taking up positions as the sister to the Phasiinae (Fig. 3a, d), sister to Dexistinae (Fig. 3b, c) or sister to (Phasiinae + Dexistinae) (Figs 1–3e, f). This ambiguity may be due to the highly autapomorphic morphology of this group and we hesitate to make any strong claims as to where this taxon may belong.

All remaining Tachininae are reconstructed as a paraphyletic grade from which the monophyletic Exoristinae arose. Nearly all the currently recognized tachinid tribes are reconstructed as para- or polyphyletic, as already hypothesized by several authors (Tschorin, 1985). Only a few tribes (Graphogastriini, Brachymeriini, Siphonini, Megaprosopini, Tachinini s.s.), nested within this large grade, received support as representing monophyletic groups (File S4). The Siphonini are well supported, confirming conclusions of Andersen (1983) and O’Hara (1989), based largely upon the possession of only two (rather than the usual three) spermathecae in the female reproductive system (135:1). The Polideini (sensu O’Hara, 2002) are not retrieved as monophyletic here due to the (weakly supported) inclusion of *Loewia* in this clade, while the other examined members of the Loewini (sensu O’Hara & Wood, 2004), *Triarthria* and *Eloceria*, are included in a clade with *Neolopectops* and *Neacera* of the Neacerin. However, the two synapomorphies of the male terminalia used to define the recently restructured Polideini (‘sterne 5 V-shaped with micropines on inner margins, and distiphallus with short lateral arms and specialized anterior sclerite’; O’Hara, 2002: 20) were not included in the present analysis. Support for the Tachinini s.s. (*Peleteria* Robineau-Desvoidy, *Tachina* Meigen) is relatively strong, supporting Mesnil (1966) and most subsequent authors (but not Guimarães, 1971) in defining this clade as those Tachininae with the posterior (here posterolateral) margin of the hind coxa setose (83:1). Some other tachinids also have a setose hind coxa, but the position of the setae is not the same throughout the family: in tachinina genera, the setae arise from the posterolateral margin of the hind coxa (83:1), whereas in the
Exoristinae and the genus Sarromyia, they arise from the posterodorsal margin of the hind coxa (82:1) (see File S2). This difference in the position of the setae suggests that the two setal patterns are non-homologous and thus two characters were recognized in our analysis.

**A monophyletic Exoristinae**

Although weakly supported, the clade Exoristinae + Acemyini (AV) is reconstructed as a monophyletic lineage under a wide range of weighting schemes (Figs 1, 3a, d–f). Notably, the Acemyini, which split from Exoristinae under a wide range of weighting schemes (Figs 1, 3a, +), are not recognized in our analysis. A monophyletic Exoristinae are the most diverse subfamily of Tachinidae in terms of number of described species, they are the most morphologically homogeneous subfamily as well. Most of the variation that does exist appears to be highly homoplasious, with similar character states recurring over and over again. The lack of resolution and rampant homoplasy in this subfamily may reflect its relatively recent and explosive evolutionary radiation. Although employing far fewer taxa than here, Tachi & Shima’s (2010) molecular phylogenetic analyses generally supported monophyly of each of the tribes of Exoristinae and indicated sister-group relationships as follows: (((Gonini + Eryciini) + Blondelini) + Exoristini) + Ethillini. Stireman (2002) reached similar, if less clear, conclusions, except he was unable to recover a monophyletic Gonini. Neither the Eryciini nor the Blondelini can be defined on the basis of non-homoplasious apomorphies, and our results suggest that they may not be monophyletic groups. As noted by Wood (1985), the short first poststatural, supra-alar seta (49:1) that is so useful for separating the Blondelini and Exoristini from most other Exoristinae might be the primitive state of the character.

Another issue emerging from this analysis is the probable polyphyly of the Ethillini as currently defined (Cerretti et al., 2012b). Ethillini are traditionally defined as sharing three character states: strongly convex lower calypter, katepimeron setulose, and ovipositor short and unmodified. The first state is shared with several winthemiines, the second is a characteristic feature of the Winthemiini, and the last represents a plesiomorphic condition with respect to the long telescoping ovipositor of winthemiines. These features indicate that the composition of the Ethillini has been weakly founded, and its monophyly was questioned by Tschorsnig (1985). Cerretti et al. (2012b) argued that three monophyletic groups can be identified within Ethillini, and these groups are recognized as monophyletic in the current analysis: a Phorocerosoma group (here represented only by Phorocerosoma); an Ethilla group (clade BG), characterized by the laying of unembryonated eggs (7:0); and a Paratryphera group (clade BI), which lays embryonated eggs (7:1). The laying of unembryonated eggs provides homoplasious autapomorphic support to clade BC, wherein the Ethillini are positioned as sister group of (Medina + (Winthemiini + Phorocerosoma) + Ethilla group). The degree of egg development at oviposition, and hence the reproductive strategy of these flies, plays a key role in the non-monophyly of the Ethillini in our reconstruction, but the resultant pattern of relationships is still not convincing. In fact, the Paratryphera and Ethilla groups share possession of an opercular egg shell (4:1), which is a very rare condition in tachinids (Cerretti et al., 2012b). Under implied weighting this character state is reconstructed as a unique autapomorphy supporting monophyly of the Paratryphera group + Ethilla group, under a wide range of k-values (i.e. k = 1 and k ≥ 7).

**Which came first, the larva or the egg?**

Within the Tachinidae, ovipary (deposition of unembryonated eggs) is generally interpreted as a plesiomorphic condition with respect to ovolarvipary (deposition of embryonated eggs), and it is presumed that the latter evolved several times independently (Wood, 1987b; Tschorsnig & Richter, 1998; Stireman et al., 2006; Tachi & Shima, 2010). In our analysis under equal and implied weights (k ≥ 7), all treated oviparous groups [all the Phasiinae s.s. (clade T) and clade BC, i.e. the exoristine taxa Exoristini, Winthemiini, some Ethillini (clade BG and Phorocerosoma) and the blondelini genus Medina] are nested within ovolarviparous assemblages (Figs 1, 2), suggesting the possible need to re-evaluate the evolutionary history of ovolarvipary vs ovipary. The counterintuitive interpretation that ovolarvipary could have characterized an early tachinid ancestor and that ovipary developed several times independently from an ovolarviparous ancestor is a novel hypothesis generated from this analysis.

The laying of fully embryonated eggs is the most common reproductive strategy in the family and our parsimony reconstruction shows that its occurrence may be more primitive within the Tachinidae than previously thought. Ovolarvipary has several major advantages, the primary being that the egg encloses a fully formed first instar the moment it is laid. Once evolved, ovolarvipary presumably opened new opportunities for the Tachinidae, for instance, the ability to take advantage of an indirect oviposition strategy, which is always associated with ovolarvipary. This means that eggs laid in habitats
frequented by potential hosts hatch almost immediately after deposition and the larvae actively search for a host (e.g. Dexiini) or wait for a host that might eventually pass by (e.g. Tachininae). In Goniini, a special type of ovolarvipary has evolved in which eggs are laid on foliage and must be swallowed by a phytophagous host, subsequently hatching in its midgut through the stimulation of proteolytic enzymes.

The evolution of indirect oviposition undoubtedly allowed Tachinidae to broaden their host spectrum to species that would not be encountered by a searching female, because they are, for example, hidden, buried or nocturnal. In groups characterized by a direct oviposition strategy, the laying of ready-to-hatch eggs is still advantageous because it minimizes the length of time that eggs are exposed and vulnerable during the delicate embryogenesis process.

There are also potential costs associated with ovolarvipary, especially for the adult female parasitoid. Tachininae that lay fully embryonated eggs have a long and distensible ovisac that serves as an incubator and, as a consequence, the time between mating and oviposition is longer than in oviparous females. Indirect oviposition is an inherently risky strategy because the majority of first instars will perish before encountering a host, but inevitably this greater risk of mortality is balanced by higher fecundity (O’Hara, 1985; Mellini, 1991; Stireman et al., 2006). The laying of unembryonated eggs comes at a cost, because such eggs are exposed to an increased mortality risk while the first instar develops, but this risk might be countered by the benefit of the egg being deposited directly onto a potential host. Minimizing the time necessary to produce and lay eggs also minimizes the risk of mortality for females before they can reproduce. Furthermore, these taxa can afford to produce fewer, larger eggs because all that are laid can be certain of reaching the host. We think that various combinations of these factors could have contributed towards making the switch to oviparity sometimes advantageous.

Aside from Goniini, a few other Exoristinae, Dexiini and most Tachininae, which lay eggs ready to hatch in habitats frequented by hosts, all other ovolarviparous tachinids lay eggs directly on or inject eggs directly into the body of the host, as in all oviparous tachinids. Given our results, it is conceivable that reversals to oviparity occurred in ancestors characterized by a direct oviposition strategy.

Interestingly, with \( k \)-values varying from 2 to 6, analyses under implied weights reconstruct ovipary as a plesiomorphic state with respect to ovolarviparity (Fig. 3b, c), more in line with previous hypotheses (see earlier), but with some important differences. Under these implied weights, ovolarviparous groups are retrieved as a monophyletic lineage, rejecting the hypothesis that ovolarvipary may have evolved several times independently from oviparous ancestors. As discussed earlier, this result has been achieved at the cost of reconstructing Exoristinae as a paraphyletic grade. Morphological and molecular evidence (Tschorsnig, 1985; Stireman, 2002; Tachi & Shima, 2010) converges on a monophyletic Exoristinae, making this scenario quite unlikely.

**Host associations**

Two major patterns emerge when considering host associations across our phylogenetic reconstruction: (i) there is general agreement between host use and monophyly of major lineages (see the Results section); and (ii) the host preferences of more basal groups tend to be either hemimetabolous orders or Coleoptera (Fig. 2). More than 60% of tachinids are parasitoids of lepidopteran larvae (cf. Cerretti, 2010: 43), but none of the four basal clades (Fig. 2; B, D, F, AB) or the sister groups of all major, more distal, assemblages, are unambiguously associated with Lepidoptera as the primitive condition. Gnadochaeta and the Palpostomatini (clade B) are both Coleoptera parasitoids. Gnadochaeta species attack concealed endophytic weevil larvae by means of actively seeking first-instar planidia, as do the Dexiini. Palpostomatines are nocturnal, or semi-nocturnal, parasitoids of adult scarabaeoids. Clade F (Ormiini + (Dexiinae + Phasiinae)) is ambiguous, being composed of three assemblages (clades G, I and O) with diverse host spectra. Clade G (Ormiini) are nocturnal parasitoids of Orthoptera; female ormiines are able to locate singing male hosts by means of an unusual tympanic organ located between the fore coxae (42:1). Within clade I (Dexiinae – Dufouriini), the Vorini i.s. (clade M) are composed of Lepidoptera parasitoids, without exception, while clade J has the sawfly parasitoid Phyllomyia as sister group of clade K (Stomina + Dexiini). The hosts of Stomina are unknown, while the Dexiini are, with a few exceptions, parasitoids of soil or wood-dwelling beetle larvae. One of the exceptions is the genus Trixa Meigen, which is unusual among Dexiini in parasitizing caterpillars (Belshaw, 1993; Tschorsnig & Herting, 1994). The nested position of Trixa in clade L suggests a relatively recent shift to larval Lepidoptera in this taxon. However, ecologically, Trixa is similar to other dexionines in that its Hepialidae hosts (Belshaw, 1993; Tschorsnig & Herting, 1994) are soil dwellers. Within clade O, the Dufouriini form a paraphyletic grade of adult Coleoptera parasitoids from which the Phasiinae arose (clade Q). The only dufouriines that do not directly attack adult beetles are those belonging to the genus Dufouria, which lay eggs on larvae but emerge from the adults (Kaufmann, 1933; Mellini, 1964). Unfortunately, there are no host records for two key basal groups, Litophasia and Imitomyia, but the position of Strongygaster, as sister to the Phasiinae s.s. (clade T), is particularly interesting. Strongygaster has a diverse host range that encompasses adult beetles as well as adult ants (Tschorsnig & Herting, 1994; Reeves & O’Hara, 2004; Shima, 2006), whereas the Phasiinae s.s. are exclusively parasitoids of Heteroptera. A shift from adult Coleoptera to Heteroptera may have characterized the developmental strategy of the phasine ancestor. The only other tachinids known as Heteroptera parasitoids are the Eutherini. Under equal weights, the Eutherini are nested within the Exoristinae, thus suggesting that parasitism of Heteroptera evolved independently in the Eutherini and Phasiinae. As discussed earlier, analyses under implied weighting and \( k \geq 6 \) yield an entirely different and probably more likely phylogenetic scenario, reconstructing the Eutherini as more...
closely related to Dexiinae and Phasiinae (Fig. 3c–f), although never as sister to the Phasiinae. Interestingly, however, all ancestral state reconstructions of host associations across phylogenetic analyses for k ≥ 6 agree in interpreting parasitism of Heteroptera as independently evolved in these two tachinid lineages.

Non-lepidopteran hosts also characterize basal lineages of clade AB (Tachininae + Exoristinae). Species in the genus Anthomyiopsis parasitize chrysomelid beetle larvae (cf. Herting, 1960). Similar to Dafuria, Anthomyiopsis species can complete development in the adult host, although in this genus it seems to be facultative, being triggered by specific environmental conditions (Mellini, 1957, 1964). It is worth noting that, among parasitoids of Holometabola, larval-adult parasitoids have been recorded almost exclusively in coleopteran hosts (Mellini, 1964), suggesting that these groups may have retained some degree of presumably ancestral plasticity.

Within clade AB (Tachininae + Exoristinae), parasitoids of Lepidoptera larvae become dominant at clade α (which is also the most species-rich clade worldwide) and this preference may have characterized the ancestor of this clade. As a consequence, associations with sawflies (clade Pseudopachystylum Mik, Hyalurgus Brauer & Bergenstamm, Staurochaeta Brauer & Bergenstamm, Blondelia Robineau-Desvoidy (in part), Catagonia Brauer & Bergenstamm, Phebellia Robineau-Desvoidy (in part), Myxesoristops Townsend], crickets and grasshoppers (clade AZ, Leiophora Robineau-Desvoidy, Phorocerosoma), beetles [Microphthalma, Cleonice Robineau-Desvoidy, Chetogena Rondani (in part), Medina, Istochema Rondani, Picconia Robineau-Desvoidy, Zaira Robineau-Desvoidy, Erythocera Robineau-Desvoidy], earwigs (Triarthria, Ocytata Gistel) and chilopods (Eloceria, Loewia) (Fig. 2) probably represent secondary shifts from primitively Lepidoptera-parasitizing ancestors.

Interestingly, our cladograms agree in reconstructing clade α as arising from a grade of taxa for which hosts are mostly unknown (several Graphogaster, Heraliduia, Hyperaea, Plesina) or from taxa characterized by associations with saprophagous micro-moths (most Phytomyzya, a few Graphogaster and some mimothines) or with Embioptera (Rossimyops, one Phytomyzya, some undescribed Graphogaster) (Cerretti et al., 2009, and unpublished data from the E.S. Ross collection at California Academy of Sciences, San Francisco, CA, USA). Except for Phytomyzya, most of these taxa are very rarely collected and it is possible that they may parasitize rarely reared groups of arthropods.

The possibility that the major groups of ditrysian parasitoids (i.e. clades M and α) may have arisen independently from ancestors that parasitized Heterometabola or Coleoptera leads to the hypothesis that tachinids could have had an early Tertiary origin and that their massive radiation took place later in the Tertiary and Quaternary, after that of angiosperms (which date back to the Jurassic–Cretaceous boundary: Sun et al., 2002) and parallel to that of major ditrysian Lepidoptera lineages (Grimaldi & Engel, 2005). However, Tachinidae are exceptionally rare in the fossil record and there are no unquestionable fossils from the Eocene or older (O’Hara, 2013a).

A recent molecular phylogenetic analysis of the Diptera suggests that the Tachinidae may have arisen as recently as 30 million years ago (Wiegmann et al., 2011). Under this scenario, the radiation of tachinids would have experienced a time lag with radiations that took place in ditrysian host groups. Such non-parallel clade diversification, where parasitoids radiated into an existing and independently diversified resource, has recently been documented for other host/parasite associations (Gómez-Zurita et al., 2007) and would be necessary to account for host-use patterns in other tachinid lineages, most notably for heterometabolous insect parasitoids (e.g. Ormiini, Phasiinae, Acemyini).

Caterpillars would seem to be excellent hosts for parasitoids in general. They are often abundant, exposed (exophytic) and unable to flee from attack. Given these traits, it is expected that they would be colonized by parasitoids at the first opportunity. But, if there was a lag in their colonization by tachinids, as is suggested by the current phylogenetic reconstruction and their estimated age, what was the reason? Also, it is interesting to note that many other families of Diptera have adopted the parasitoid lifestyle (e.g. acrocerids, nemestrinids, pipunculids, conopids, rhinophorids, many phorids and bombyliids, some muscids, sarcophagids and calliphorids), but none have colonized lepidopteran hosts as extensively as have tachinids. There are several factors that probably played a role in the radiation of Tachinidae on lepidopteran hosts. The groundplan of the Tachinidae was likely characterized by the formation of a respiratory funnel within the host (Clausen, 1940), which is the method by which most tachinids avoid encapsulation during their larval endoparasitic stage. Even with this preadaptation, the parasitization of larval Lepidoptera must have posed significant problems. Due to their long evolutionary history with hymenopteran endoparasitoids, caterpillars may have evolved particularly effective defenses (e.g. encapsulation responses; Strand, 2008; Smilanich et al., 2009), which make it difficult for non-coevolved parasitoid taxa to colonize them. These defenses may have been partly overcome by further specialized refinements to respiratory funnel formation and/or other means to circumvent the hosts’ immune response (e.g. Ichiki & Shima, 2003). Additionally, many caterpillars are known to use secondary chemicals from their host plants for their own defense (Bowers, 1993; Dyer, 1997) and it is possible that tachinids have had to develop special physiological adaptations to avoid or tolerate these chemical defenses before they could effectively colonize and radiate upon plant-feeding lepidopteran larvae. Similar and independently evolved adaptations may have allowed other tachinid lineages to radiate onto other groups of plant-feeding and chemically protected insects such as Heteroptera and certain Coleoptera (e.g. chrysomelids).

In addition to surviving the immune response and chemical defenses of lepidopterans, other adaptations have been necessary for tachinids to take full advantage of this diverse lineage of potential hosts. These include the complex synchronization of development between parasitoid and host, which as a consequence is one of the determinants of host range among Tachinidae. Often, hormonal changes within the host that trigger developmental changes are also used as cues by tachinid
larvae to alter their behaviour, location, feeding and growth (Mellini, 1975, 1991; Baronio & Sehnal, 1985).

Innovations and adaptations in the adult stage have probably also contributed to the successful radiation of Tachinidae on Lepidoptera and insects in general. In particular, the evolution of specialized mechanisms of host location (e.g. use of specific plant volatiles by Exoristinae and host pheromones by Phasiinae; Roland et al., 1995; Aldrich & Zhang, 2002; Ichiki et al., 2012) and the diversification of oviposition strategies (O’Hara, 1985; Stireman et al., 2006) may have contributed to the rapid, host-related diversification of tachinids.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article under the DOI reference:
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File S1. Exemplar taxa.
File S2. Character list.
File S3. Data matrix.
File S4. Tree with character states traced.
File S5. Equal vs implied weighting.
File S6. Data matrix in nexus format.

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