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Molecular phylogeny of telenomine egg parasitoids (Hymenoptera: Platygastridae s.l.: Telenominae): evolution of host shifts and implications for classification

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Abstract. Parasitoid wasps of the subfamily Telenominae (Hymenoptera: Platygastroidea, Platygastridae) develop as immatures within the eggs of other insects (Lepidoptera, Hemiptera, Diptera and Neuroptera). Rearing records indicate that individual species are restricted to attack hosts within only one of these four main groups. We conducted a phylogenetic analysis of the group using sequence data from multiple genes (*18S*, *28S*, *COI*, *EF-1α*) to assess the pattern of shifts among host groups and to test the monophyly of and relationships among genera and species-groups. Telenominae *sensu* Masner – that is, including only the nominate tribe Telenomini – is not monophyletic. Representatives of the *Psix* group of genera (*Psix* Kozlov & Lê and *Paratelenomus* Dodd) form a monophyletic group that is sister to *Gryon* Haliday (Scelioninae: Gryonini) and are excluded from the subfamily. The remaining telenomines are monophyletic. The genus *Phanuromyia* Dodd and the *crassiclava* group of *Telenomus* Haliday, both recorded as parasitoids of planthopper eggs (Hemiptera: Auchenorrhyncha, Fulgoroidea), form a monophyletic group that is sister to all other telenomines exclusive of the *Psix* group. Twenty-nine species of the *crassiclava* and *aradi* groups of *Telenomus* are transferred to *Phanuromyia* as new combinations. Basal elements of the remaining species are all in groups reared from the eggs of true bugs (Heteroptera), primarily the stink bugs (Pentatomoidea) and seed bugs (Lygaeoidea). A shift to parasitism of lepidopteran eggs evolved within a single clade, occurring either one or two times. From this clade a small group of species, the *Telenomus tabanivorus* group, subsequently shifted to parasitism of egg masses of true flies (Tabanidae and Stratiomyiidae). *Aholcus* Kieffer and *Platytelenomus* Dodd both belong to the clade of lepidopteran parasitoids and are considered as junior synonyms of *Telenomus* (new synonymy for *Aholcus*). The monophyletic status of the two core genera, *Telenomus* and *Trissolcus* could not be resolved using these data. The phylogenetic pattern of host shifts suggests comparisons among taxa that may be fruitful in elucidating mechanisms by which parasitoids locate their hosts, the proximate factors that determine the host range, and the changes in these factors that influence host changes.

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Introduction

The mechanisms and evolution of host finding among parasitoid insects is an area of great significance given the important role such species play in regulating the populations of their hosts, both naturally and in biological control programmes. Wasps of the superfamily Platygastroidea (Hymenoptera; Fig. 1) are idiobiont endoparasitoids of the eggs of insects and spiders (Scelioninae, Teleasinae and Telenominae) or koinobiont parasitoids of the immature stages of Auchenorrhyncha (Hemiptera), Sternorrhyncha (Hemiptera) or Cecidomyiidae (Diptera) (most Platygastrinae and Sceliotrachelinae). The major groups within the superfamily show a clear pattern of host group specificity (Austin *et al.*, 2005). However, because of the current paucity of well-supported hypotheses of phylogenetic relationship, the sequence of shifts from one host taxon to another is uncertain. The many species of the subfamily Telenominae are particularly diverse in their host relationships, having been recorded from Lepidoptera, Diptera, Neuroptera and the suborders Heteroptera and Auchenorrhyncha within the Hemiptera (summarized in Bin & Johnson, 1982; Kozlov & Kononova, 1983; Johnson, 1984a). Several species of telenomines have been shown to find their egg hosts by cuing in to semiochemicals produced by the adult host, in some cases synergized by chemicals released by the plant upon which the host feeds or oviposits (for example, Colazza *et al.*, 2009; Moraes *et al.*, 2009; Conti *et al.*, 2010; Arakaki *et al.*, 2011; Peñaflor *et al.*, 2011). No telenomine species, to our knowledge, has been reared from hosts from more than one order. Therefore, the change from, for example, Heteroptera to Lepidoptera (or vice versa), seems to be an evolutionarily 'difficult' and significant step. Insights into how it takes place may emerge from comparisons of the chemosensory capabilities of closely related species of wasps that attack the two different groups of hosts. The goal of this work is to contribute to a better understanding of relationships among telenomines to facilitate such work and, in the process, test the monophyly of its genera, species groups and the subfamily itself.

The scope of the subfamily Telenominae is the subject of some disagreement. Kozlov (1970) and Kozlov & Kononova (1983) divided it into three tribes: Telenomini, Tiphodytini (with one genus, *Tiphodytes* Bradley) and Aradophagini (also monobasic, *Aradophagus* Ashmead). Masner (1972, 1976) and Masner & Huggert (1979) argued that the latter two tribes were misplaced because they possess metasomal laterosternites (absent in Telenomini). They treated Aradophagini and Tiphodytini as members of the subfamily Scelioninae and restricted Telenominae to the nominate tribe. The concept used here follows Masner's (1976) restricted version. Within the subfamily 51 genus-group and 1057 species-group taxa have been described, of which 20 genera and 904 species are considered to be valid (Johnson, 2013). The bulk of species are placed in one of two genera, either *Trissolcus* Ashmead (179 valid species) or *Telenomus* Haliday (652 valid species), both of which are cosmopolitan. Of the remaining genera, 14 have only 1–2 described species. Modestly sized genera remaining are *Eumicrosoma* Gahan (13 spp.), *Paratelenomus* Dodd (13 spp.), *Phanuromyia* Dodd (9 spp.) and *Psix* Kozlov & Lê (22 spp.). Species of *Trissolcus* parasitize the eggs of stink bugs and their allies (Kozlov & Kononova, 1983; Johnson 1984a,b, 1987, 1991); *Telenomus* shares these hosts, but also attacks a wider range of Heteroptera, as well as Auchenorrhyncha, Lepidoptera, Diptera and Neuroptera (Bin & Johnson, 1982; Johnson & Bin, 1982; Kozlov & Kononova, 1983; Johnson, 1984a). The host records for *Eumicrosoma* are limited to seed bugs (Lygaeoidea: Blissidae) (Gahan, 1913; Ryu & Hirashima, 1989); *Paratelenomus* to turtle bugs (Pentatomoidea: Plataspidae) (Johnson, 1996); *Phanuromyia* to auchenorrhynchous Hemiptera (Issidae, Fulgoridae) (Johnson & Musetti, 2003); and *Psix* to Coreoidea and Pentatomoidea (Johnson & Masner, 1985).

Fig. 1. Female *Trissolcus euschisti* (Ashmead) on egg mass of a pentatomid, probably a species of *Podisus*. Photo © Charles Eiseman (www.charleyeiseman.com)

Murphy *et al.* (2007) included seven representative species of the Telenominae in their molecular phylogeny of the Platygastroidea: one *Phanuromyia*, two *Trissolcus*, and four *Telenomus*. The subfamily emerged as a monophyletic group in their analyses, sister to the genus *Gryon* Haliday (Scelioninae: Gryonini, three species represented). *Phanuromyia* grouped together with *Telenomus crassiclava* at the base of the telenomine clade, and the two *Trissolcus* species grouped together at the apex. The remaining *Telenomus* species emerged as a paraphyletic basal grade in the subfamily between these two. Johnson (1985c, 1988a) grouped together the genera *Psix, Paratelenomus*, *Nirupama* Nixon and *Mudigere* Johnson as the *Psix* group of genera. The relationship of this group to the bulk of Telenominae, however, was unclear. Johnson & Musetti (2003) recognized *Phanuromyia* as a distinct and valid genus, a conclusion subsequently rejected by Mineo (2006). Some progress in understanding relationships at a finer level has been made in the attempts to circumscribe natural groups within *Telenomus* (Kozlov & Kononova, 1983; Johnson, 1984a) and *Trissolcus* (Kozlov & Lê, 1976; Kozlov & Kononova, 1983; Johnson, 1984b, 1985a,b).

We report here the results of phylogenetic analyses of an extensive species sampling of Telenominae based on sequence data from four molecular markers: *18S* and *28S* rDNA,

the mitochondrial protein-coding gene *COI*, and the nuclear protein-coding gene *EF-1α* F2 copy. Also included in the analyses were a wider range of species of *Gryon*, designed to test the putative sister-group relationship or, if possible, to test whether Telenominae are more closely related to a subset of *Gryon* species. The results serve as a framework for interpreting the evolution of host relationships within the subfamily and, in addition, have repercussions for the classification of Telenominae.

Materials and methods

Taxonomic sampling and specimen vouchering

A total of 62 species were sequenced: 51 species for the ingroup Telenominae, and 11 species in the outgroups (File S1). The ingroup comprises representatives of six genera: *Telenomus* (eight species groups), *Trissolcus* (three species groups), *Psix* (three species), *Paratelenomus* (one species), *Phanuromyia* (two species) and *Eumicrosoma* (one species). The generic names *Platytelenomus* Dodd and *Aholcus* Kieffer are still recognized as valid by some authors (e.g. Kononova, 2008; Mineo *et al.*, 2011). Representatives of each were also included: *Platytelenomus* sp1 and *Telenomus dalmanni* (Ratzeburg), respectively. Other telenomine genera were not sampled because they are fossil taxa (e.g. *Sinoprotelenomus* Zhang) or are rare, and fresh material was unavailable. Within *Trissolcus* all three recognized major groups (Johnson, 1984b, 1985a,b) are represented by multiple species: *thyantae* group by *Tr. thyanate* Ashmead, *Tr*. THY1 and *Tr*. THY2; the *flavipes* group by *Tr. euschisti* (Ashmead), *Tr. strabus* (Johnson) and *Tr. urichi* Crawford; and the *basalis* group by *Tr. basalis* (Wollaston), *Tr. hullensis* (Harrington), *Tr. latisulcus* (Crawford), *Tr*. BAS1, *Tr*. BAS2 and *Tr*. BAS3. The Australian species *Tr. ogyges* (Dodd) is an unusual species that cannot be placed in one of these species groups. For *Telenomus*, representatives of eight species groups are included: the *floridanus* group (parasitoids of Lygaeoidea) by *Te. consimilis* Ashmead, *Te. nysivorus* Huggert, *Te*. FLO1, *Te*. FLO2 and *Te*. FLO3; the *crassiclava* group (Auchenorrhyncha) by *Te*. CRA1, *Te*. CRA2, *Te*. CRA3, *Te*. CRA4 and *Te*. CRA5; the *podisi* group (Pentatomoidea) by *Te. podisi* Ashmead, *Te. grenadensis* Ashmead and *Te. sechellensis* Dodd; the *laricis* group (Miridae) by *Te*. LAR1, *Te*. LAR2 and *Te*. LAR3; the *phymatae* group (Reduviidae) by *Te. dolichocerus* (Ashmead); the *californicus* complex (Lepidoptera) by *Te. dalmanni, Te*. CAC1, *Te*. CAC2, *Te*. CAC3, *Te*. CAC4 and *Te* CAC5; the *tabanivorus* group (Diptera) by *Te. goniopis* Crawford and *Te*. TAB1; and the *longicornis* group (with unknown aquatic hosts) by *Te*. LON1, *Te*. LON2, *Te*. LON3 and *Te*. LON4. Morphological characterization and host relationships of each are from Johnson (1984a). Outgroup taxa include four genera of Scelioninae – six species of *Gryon*, two species of *Scelio* Latreille, and one species each of *Oxyteleia* Kieffer and *Probaryconus* Kieffer – and one species of Teleasinae, *Trimorus caraborum* (Riley). Material studied was freshly collected from around the world.

After nondestructive DNA extraction the voucher specimens were deposited in the C. A. Triplehorn Insect Collection (OSUC). Supplementary File S1 lists the specimens, voucher information and GenBank accession numbers for markers included in the analyses.

DNA extraction, amplification, and sequencing

Fresh specimens were sorted from bulk material, preserved in 95% ethanol, and maintained in the freezer at −15°C. Nondestructive DNA extraction was performed using the DNeasy extraction protocol (Qiagen, Germantown, MD; cat. num. 69506) as modified by C.D. Zhu and J.S. Noyes (unpublished data): Individual specimens were initially softened in 70% ethanol at room temperature for 1 day. Vortexing in step 2 of the manufacturer's protocol was modified by mixing the reaction gently and incubating at 55°C for 24 h. The mixture was stored at −20°C for 24 h. The intact specimen was then removed from the tube and prepared for standard mounting. The reaction was incubated for 10 min at 70°C after addition of Buffer AL. Then 200 μL of cold ethanol (96–100%) was added to the supernatant. Finally, in Step 7 the Buffer AE was warmed to 55–70°C before addition.

Four genes were targeted: *18S* (positions 398 to 142) and the D2–D3 regions of *28S* rDNA (nucleotide positions D2-3365 to D3-4413), the mitochondrial protein-coding gene cytochrome oxidase I (*COI*), and the F2 copy of the nuclear protein-coding gene elongation factor 1-alpha (*EF-1α*). Primers for PCR amplification were drawn from Whiting *et al.* (1997: ai, bi for *18S*; D23F, 28Sb for *28S*), Giribet *et al.* (1996: 18S-5R for *18S*), Simon *et al.* (1994: 1F, 1R for COI), Folmer *et al.* (1994: HCO2198, LCO1490 for *COI*), Danforth *et al.* (1999: F2F, Cho10 for *EF-1α*), Heraty *et al.* (2011: F2F8, For3, F2R6 for *EF-1α*) and Simon *et al.* (2010: F7, F9 for *EF-1α*). Amplification was carried out via PCR following the protocols of Murphy *et al.* (2007) and Klompen (2000).

Nested PCR optimizations were employed to amplify the F2 copy of *EF-1α*. This approach is relatively successful in reducing or eliminating unwanted products, although it dramatically increases sensitivity to contamination. The initial PCR primers were F2F and Cho10 (Danforth *et al.*, 1999) followed by a combination of other primers from Heraty *et al.* (2011): F2F8 [5′–CAA RTA TGC NTG GGY ATT GGY AAG–3′], F2R6 [5′–TTG WGC RGT GAA GTC AGC NGC–3′], and Simon *et al.* (2010): EF-7 [5′–AAC AAR ATG GAY TCN ACN GAR CCN CC–3′] and EF-9 [5′–CCN ACN GGB ACH GTT CCR ATA CC–3′]. The choice of second round primers depended on the taxa and product results. Thermocycle conditions of F2F-Cho10, F2F8-F2R6 and For3-Cho10 followed Klompen (2000) except that annealing temperatures of 54°C, 54°C and 58°C were used (respectively). The amplification profile for EF-7 and EF-9 followed Simon *et al.* (2010). We excluded the F1 copy from the analysis because of the difficulty of amplification and the often incomplete products (as in Heraty *et al.*, 2011). Contaminants were detected using negative controls in all rounds

of PCR, as well as comparisons of the product sequences with each other and with a range of published arthropod sequences. PCR products were purified either using the QIAquick PCR purification kit (Qiagen) protocol or done prior to sequencing by Beckman Coulter Genomics (Danvers, MA). Products were sequenced in both directions and assembled using Sequencher v4.0 (Gene Codes Corporation, Ann Arbor, MI).

Sequence alignment

Sequences of *COI* (approximately 857 bp) were aligned initially using MUSCLE (Edgar, 2004) with default settings and then adjusted by eye. The total of 1124 bp of EF-1α were aligned by eye against a reference taxon (*Archaeoteleia mellea* – GenBank:GQ410731.1) and reported intron positions (Danforth & Ji, 1998; Heraty *et al.*, 2011). Alignments of both protein-coding genes were translated to amino acids to verify the homology using Mesquite (Maddison & Maddison, 2011). Secondary structural alignments were implemented for ribosomal RNA sequences of *18S* and *28S* for a total of 1783 bp. The by-eye alignment conventions followed Kjer (1995) with slight modifications (Gillespie, 2004). The core alignments follow published secondary structure models of Hymenoptera (as in Gillespie *et al.*, 2005; Murphy *et al.*, 2007; Heraty *et al.*, 2011). Ambiguous homology positions were defined across all taxa based on the structural criteria of Kjer (1995). These ambiguous regions were excluded from the final analyses. The final matrix alignment for analyses contains 3767 bp (*COI*: 857, *18S*: 1003, *28S*: 782, *EF-1α*: 1125). The alignment summary, gene partitions, nucleotide composition and percentage of parsimony-informative sites are presented in online supporting documentation.

Data coding and partitioning

Regier & Zwick (2011) discussed the role of heterogeneity in base composition and associated synonymous nucleotide change in protein-coding genes in reducing the performance of phylogenetic analyses, and they proposed mechanisms to attempt to compensate for this effect. Here we employ three strategies to reduce the effect of synonymous changes. The third codon position (nt3) is typically the source of most synonymous change and, therefore, one option is to eliminate (or completely degenerate) these data from the analysis. Additionally, synonymous changes in the first codon position are possible for sequences coding for arginine and leucine. The DEGEN approach (Regier & Zwick, 2011) degenerates synonymous nucleotide changes in both the first and third codon positions. The Perl code for DEGEN coding was modified to use the invertebrate mitochondrial genetic code for *COI*. The third strategy is to segregate data from nt3 in a separate partition.

We employed three partitioning schemes: (i) use of a single partition for data from all four markers; (ii) four partitions, separating data from *18S*, *28S*, *COI* and *EF-1α*; and (iii) six partitions for data from *18S*, *28S*, *COI* nt1+2, *EF-1α* nt1+2, *COI* nt3 and *EF-1α* nt3. Combining the coding and partitioning strategies resulted in seven datasets for analysis: (1–3) no coding, 1, 4 and 6 partitions; (4–5) nt3 excluded, 1 and 4 partitions; (6–7) DEGEN coding, 1 and 4 partitions.

Phylogenetic analyses

Three phylogenetic estimation strategies were implemented to infer relationships: maximum parsimony, maximum likelihood and Bayesian approaches. Maximum parsimony analyses were conducted in TNT v1.1 (Goloboff *et al.*, 2008). Heuristic tree search algorithms were implemented using the New Technology Search by default setting with some modifications followed by tree bisection-reconnection (TBR) branch swapping. Some parameters were adjusted: using a sectorial search, treating gaps as missing data, tree drifting of four cycles, tree fusing of five rounds and best score hit of 20–25 times. The branch support was estimated using the Parsimony Jackknife (JK; Farris *et al.*, 1996) executed with the setting 36% probability removal, 'emulate JAC' resampling, 1000 replications, 'random addition sequences' = 1, and 'hold trees' = 2 (Freudenstein *et al.*, 2004; Klompen *et al.*, 2007). The parsimony jackknife was selected over the alternative, bootstrapping, because it is less sensitive to missing and invariant characters (Farris *et al.*, 1996; Freudenstein *et al.*, 2004).

Maximum likelihood analyses were executed using RAxML v7.2.6 (Stamatakis, 2006; Stamatakis *et al.*, 2008). By using GTRGAMMA for the model of nucleotide substitution (-m), individual α-shape parameters, GTR-rates and empirical base frequencies were estimated and optimized for each partition. RAxML analyses were implemented using a rapid bootstrap and search for the best-scoring ML tree in one single program run. Heuristic hill-climbing tree searches were performed generated from 1000 distinct randomized maximum parsimony starting trees and computed for 1000 repetitions. An additional ten runs were made using random starting seeds: all resulted in the same topology and likelihood scores.

Bayesian analyses were performed using the parallel (MPI) version of MrBayes v3.2.1 (Ronquist *et al.*, 2012) and the computations were conducted using the CIPRES Science Gateway (www.phylo.org). The evolutionary models used for each data partition were select by comparison of the Akaike Information Criterion (AICc) as calculated in jModelTest v0.1.1 (Posada, 2008), shown in File S2. The Markov Chain Monte Carlo (MCMC) parameters for combined data were as follows: ngen = 40 000 000 printfreq = 1000 samplefreq = 1000 nchains = 4 savebrlens = yes stoprule = yes stopval = 0.01. For each individual marker analysis, the MCMC was designed as ngen = 20 000 000 printfreq = 1000 samplefreq = 1000 nchains = 4 savebrlens = yes stoprule = yes stopval = 0.01.

Results

Data properties

Total DNA sequences of 1185–3322 bp (mean = 2253.5, median = 2242.5) from the four molecular markers were

successfully obtained for 78 taxa. Amplified PCR products varied in length: COI: 565–681 bp (with one short sequence of 195 bp), 18S: 549–974 bp, 28S: 595–802 bp, EF-1α: 277–1123 bp. Sequence characteristics of the four gene partitions are summarized in File S2. Parsimony analyses of the individual gene partitions showed a consistent amount of homoplasy in the data: *18S* (150 trees, 192 steps, CI = 0.21, RI = 0.15); *28S* (16 trees, 1025 steps, CI = 0.22, RI = 0.14); *COI* (80 trees, 3746 steps, CI = 0.16, RI = 0.18); and *EF-1α* (17 trees, 1873 steps, CI = 0.29, RI = 0.27) (trees not shown).

Relationships

The results are largely congruent among the three inference techniques. The parsimony results are, in general, less well-resolved and less well-supported. The level of support for clades discussed below in different analyses are presented in File S3. Figure 2 illustrates the results of the maximum likelihood analysis of the single partition DEGEN matrix.

The representatives of the two genera of the *Psix* group – *Psix* and *Paratelenomus* – are individually monophyletic, and the two genera are also consistently grouped together in all analyses (parsimony jackknife support 99–100%, maximum likelihood bootstraps 100%, Bayesian posterior support 100%). Although *Paratelenomus* is represented by a single species, the samples span its geographic distribution from Ghana to Thailand. Together, *Psix*, *Paratelenomus* and *Gryon* are monophyletic (bootstraps from maximum likelihood analyses varying from 68 to 75%, Bayesian posterior probability 99%).

All of the remaining Telenominae – species of *Telenomus*, *Trissolcus*, *Eumicrosoma* and *Phanuromyia* – together form a monophyletic group (bootstraps 85–98%, Bayesian posterior probability 98–100%). The two species of *Phanuromyia* are monophyletic (jackknife 50–53%, bootstraps 59–89%, Bayesian posterior probabilities 89–99%), and the *Telenomus crassiclava* species group is monophyletic in most analyses (jackknife 80–82%, bootstraps 68–90%, Bayesian posterior probabilities 84–98%). *Phanuromyia* is consistently the sister to the cluster of *Telenomus crassiclava* group species (bootstraps 59–89%, Bayesian posterior probabilities 89–95%). The remaining Telenominae – that is, the species of *Trissolcus*, *Telenomus* (not including the *crassiclava* group) and *Eumicrosoma* – are monophyletic (bootstraps 54–93%, Bayesian posterior probabilities 84–98%). The species of the *Telenomus californicus* complex, the large group within which the parasitoids of the eggs of Lepidoptera and Diptera are found, group together in all analyses. This group, represented by eight species, is not supported as a distinct clade because of the inclusion of one species of the *floridanus* group (*Telenomus* FLO3) and, sometimes, *Te. sechellensis* Kieffer (of the *podisi* group; Bayesian analysis, DEGEN coding). Representatives of the *tabanivorus* group, parasitoids of the eggs of horse flies, emerge as a monophyletic group within the clade including lepidopteran parasitoids (bootstraps 74–87%, Bayesian posterior probabilities 70–99%). The *Te. longicornis* (bootstraps 59–96%, Bayesian posterior probabilities 99–100%) and *Te. laricis* (bootstraps 95–100%, Bayesian posterior probabilities 100%) species groups were recovered as monophyletic groups in all coding and partitioning schemes.

Telenomus (*Aholcus) dalmanni* (Ratzeburg) and *Platytelenomus* always group with the species of the *Te. californicus* complex. The position of *Eumicrosoma* is unstable: it emerged either with the *californicus* complex (most analyses) or as sister to *Trissolcus ogyges* Dodd (Bayesian analysis, DEGEN coding, four partitions).

Neither *Telenomus* nor *Trissolcus* are resolved as monophyletic groups, even with the exclusion of the *crassiclava* species group from the former. Representatives of the *thyantae* group of *Trissolcus* emerge near the base of the *Telenomus* + *Trissolcus* + *Eumicrosoma* clade, but none of the three species groups of *Trissolcus* are monophyletic. The species assigned to the *podisi* and *floridanus* groups of *Telenomus* do not group together. The *floridanus* group is particularly dispersed, with one species, *Te*. FLO1, consistently placed at the base of the *Telenomus* + *Trissolcus* + *Eumicrosoma* clade (but with low support values).

Discussion

Scope of Telenominae

Telenominae has been one of the best-defined groups within the superfamily Platygastroidea since the subfamily was first formally recognized by C.G. Thomson (1860). It has been characterized by the combination of the following characters: metasoma with wide laterotergites loosely attached to the sterna; the absence of laterosternites on the metasoma; the second metasomal segment is significantly longer than any other; and the number of antennomeres in the female is reduced (10 or 11) in comparison to the male (12). Kozlov's (1970) inclusion of Tiphodytini and Aradophagini was based solely on the broad laterotergites in these two (then) monobasic tribes, but these species possess laterosternites, and the antenna is 12-segmented in both sexes (Masner, 1972; Masner & Huggert, 1979). Masner's (1976) restriction of the concept of the subfamily to the nominate tribe restored a clear definition of the taxon.

We believe that the results presented here strongly indicate that the subfamily, even in this restricted definition, is not monophyletic. We did not anticipate this outcome because the genera of the *Psix* group have the four synapomorphies of Telenominae. However, the position of these genera within the subfamily has been problematic. They all share a distinctive and plausibly apomorphic character: the central keel on the frons forks ventrally to pass on either side of the antennal insertions. Additionally, they all have strongly developed fanlike striae on the frons arising near the anterior mandibular articulation (very rarely present and only weakly developed in a few other telenomines), have a different structure of the metascutellum (the dorsellar lip of Johnson & Masner, 1985), and lack differentiated sublateral setae on the first metasomal

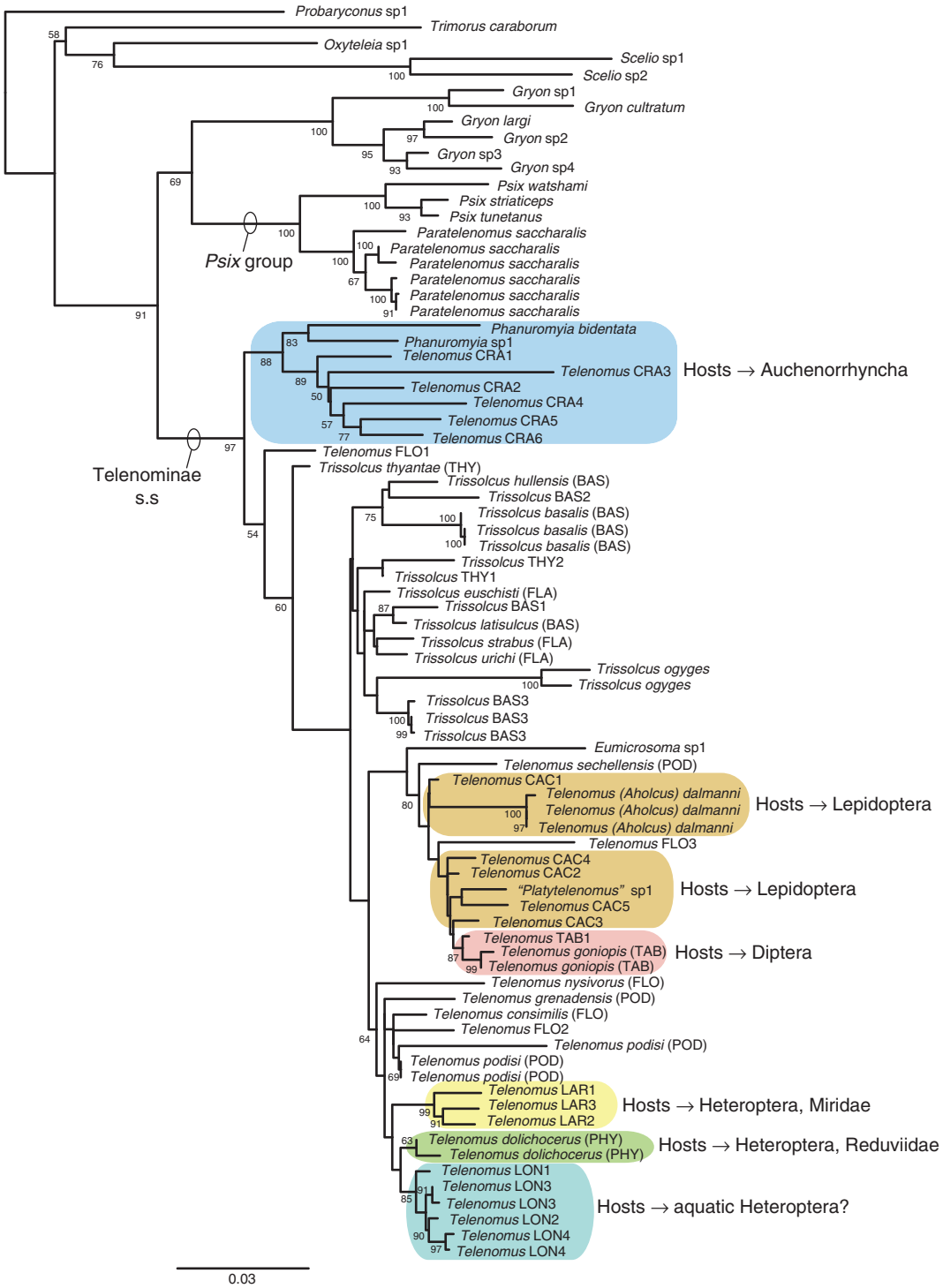

Fig. 2. Relationships of Telenominae derived from RAxML analysis of combined *COI*, *18S*, *28S* and *EF-1α* sequences with 1000 bootstrap replicates, DEGEN coding, single partition. Bootstrap values above 50% indicated on branches. The three-letter abbreviations for species of *Telenomus* and *Trissolcus* indicate the species-group to which it belongs: BAS, *basalis*; CAC, *californicus*; CRA, *crassiclava*; FLA, *flavipes*; FLO, *floridanus*; LAR, *laricis*; LON, *longicornis*; PHY, *phymatae*; POD, *podisi*; THY, *thyantae*. The position of the branches subtending the *Psix* group of genera and the Telenominae s.s. are indicated. Hosts of species in unshaded are various terrestrial Heteroptera, primarily Pentatomoidea and Coreoidea. Shaded areas highlight clades that have shifted to new groups of hosts.

tergite. Mikó *et al.* (2007) discovered that the two groups differ in that the pleural apodeme of the mesothorax is fused with the anterior margin of the speculum in *Telenomus* and *Trissolcus*, whereas in *Psix* and *Paratelenomus* the apodeme extends to the mesopleural articulation. Finally, the *Psix* group of genera lack two previously unreported, apparent synapomorphies of the other telenomines. First, the metapostnotum is delimited posteriorly by a deep sulcus that arises anteriorly from the propodeal spiracle and continues posteriorly and medially beneath the metascutellum (see, e.g., figs 18, 48 in Johnson, 1984a). Reexamination of newly collected specimens of *Mudigere* confirms this character, contradicting the statement in Johnson (1988a). Second, the labrum is large, visible externally, articulated with the external ventral margin of the narrow clypeus (i.e. not on the internal surface within the buccal cavity), and is almost always separated from the clypeus by a distinct suture.

The molecular data not only reaffirm the unity of the *Psix* group, but indicate that they are more closely related to *Gryon* than to other telenomines. Thus, the defining characters of the subfamily must be reinterpreted as having evolved independently in the *Psix* group of genera and in the Telenominae s.s. Most of these features are also found in other lineages within the superfamily. The reduction in number of female antennomeres is also found in the Gryonini genus *Maruzza* Mineo, and commonly occurs in other parts of the subfamily Scelioninae. Wide laterotergites are characteristic of species of *Tiphodytes* and *Aradophagus*, as well as males of *Baeus* Haliday. The size dominance of the second metasomal segment is also found among some species of *Gryon*, in more distantly related genera such as *Yunkara* Galloway and *Baeus*, as well as in the subfamilies Platygastrinae and Sceliotrachelinae. The last remaining character is the loss of laterosternites in the metasoma. This is, we believe, one strong piece of morphological evidence supporting the monophyly of Telenominae *sensu* Masner because it occurs so rarely elsewhere in Platygastroidea. However, it stands in stark contrast to the molecular evidence we present here.

We conclude that the taxonomic concept of Telenominae must be modified. Two options are available, consistent with our desire that any formally recognized taxon be monophyletic. One is to expand the concept of Telenominae to include the genus *Gryon* and, by implication, closely related genera. The second is to exclude *Psix* and *Paratelenomus* – and by extension the closely related *Mudigere* and *Nirupama* – from the subfamily. We prefer the latter option. From a practical point of view, to include *Gryon* in Telenominae would put us in the position of having no morphological characters by which to recognize the subfamily. Furthermore, the design of this study may be inadequate to support such a conclusion. The analyses lead to the conclusion that the *Psix* group is more closely related to species of *Gryon* than to other species of Telenominae, but do not necessarily demonstrate that *Gryon*, out of all other genera of Platygastroidea, is the group to which it most closely related overall. We therefore prefer the option of a further contraction and refinement of the subfamily Telenominae. In this concept the subfamily can be defined on the basis of the presence of broad laterotergites, absence of laterosternites, sexual heterogeneity in antennomere number, as well as the presence of externally demarcated metapostnotum and labrum. We consider the *Psix* group of genera to belong to the subfamily Scelioninae.

Phanuromyia

Within Telenominae s.s. the data support the merging of *Phanuromyia* together with the species of the *crassiclava* group of *Telenomus. Phanuromyia* languished in obscurity for nearly 90 years after it was originally described (Dodd, 1914a) because it had been defined only on the basis of the exserted ovipositor (a highly variable character even among individuals of the same species) and the type species, *P. rufobasalis*, had not been studied. Johnson & Musetti (2003) offered additional characters (claval formula, head shape, absence of a hyperoccipital carina, orientation of the malar sulcus and size of the gena) that could be used to recognize and define *Phanuromyia*, specifically to distinguish it from the *crassiclava* group. Mineo (2006) subsequently rejected this on the grounds that these characters '... are generally used to advocate a species group of either *Telenomus* or other scelionid wasps.' We cannot adopt the implication that some characters are *a priori* acceptable to define genera whereas others are only of value in defining a group of species. The critical issues are whether a new taxon is at least plausibly monophyletic and what effect the recognition of the new taxon would have on the monophyly of other recognized genera. *Phanuromyia*, now in an expanded concept to include the *crassiclava* group, is demonstrated in these analyses to be a monophyletic group, and its recognition does not result in other existing genera becoming paraphyletic. To follow Mineo's position of synonymizing *Phanuromyia* with *Telenomus*, while maintaining monophyly, would require the inclusion of all of the genus *Trissolcus* as well as any related genera. We believe that the requirement of monophyly is the only objective criterion by which to choose among alternative classifications. As such, it seems that the best option at this point is to treat *Phanuromyia* as a valid genus. We could treat the *crassiclava* group as an independent genus, but with this level of taxon sampling, it seems more prudent to recognize only a single genus.

The *Telenomus aradi* group of Kozlov & Kononova (1983) is essentially the same as the *crassiclava* group of Johnson (1984a) and, therefore, should also be subsumed within *Phanuromyia*. The expansion of the generic concept of *Phanuromyia* to include species of the *crassiclava* and *aradi* groups results in the following generic transfers: *P. afficis* (Kozlov & Kononova) **comb.n.**, *P. amazonica* (Cameron) **comb.n.**, *P. aradi* (Kozlov) **comb.n.**, *P. aspera* (Kozlov & Kononova) **comb.n.**, *P. caucasica* (Kozlov & Kononova) **comb.n.**, *P. clavata* (Kozlov & Kononova) **comb.n.**, *P. corticata* (Kozlov & Kononova) **comb.n.**, *P. crassiclava* (Nixon) **comb.n.**, *P. cyane* (Kozlov & Lê) **comb.n.**, *P. flaviventris* (Kozlov & Kononova) **comb.n.**, *P. impressa* (Ashmead) **comb.n.**, *P. infuscatipes* (Ashmead) **comb.n.**,

P. jugoslavica (Szabó) **comb.n.**, *P. longiceps* (Kozlov) **comb.n.**, *P. longistriata* (Kozlov) **comb.n.**, *P. longiventris* (Cameron) **comb.n.**, *P. maculipennis* (Ashmead) **comb.n.**, *P. marshakovi* (Kozlov & Kononova) **comb.n.**, *P. meridiana* (Kozlov & Kononova) **comb.n.**, *P. minima* (Kozlov) **comb.n.**, *P. minuscula* (Kozlov & Kononova) **comb.n.**, *P. nioba* (Kozlov & Kononova) **comb.n.**, *P. picta* (Kozlov) **comb.n.**, *P. propingua* (Kozlov & Kononova) **comb.n.**, *P. proxima* (Kozlov & Kononova) **comb.n.**, *P. rubella* (Kozlov & Kononova) **comb.n.**, *P. sphingis* (Ashmead), **comb.n.**, *P. taurus* (Johnson) **comb.n.** and *P. tuberculus* (Kozlov & Kononova) **comb.n.**. According to Article 59.4 of the International Code of Zoological Nomenclature the replacement names *Telenomus russianicus* Özdikmen and *T. moldovianus* Özdikmen, proposed for *T. impressus* Kononova and *T. minimus* Kozlov respectively by Özdikmen (2011) for junior homonyms in *Telenomus*, are abandoned in favour of the original epithets (**syn.n, stat.n.**).

Most species of *Phanuromyia* may be morphologically recognized among Telenominae s.s. by the presence of a well-developed sternaulus on the mesepisternum. Typically this is indicated as a line of deep foveae (the episternal foveae of Johnson, 1984a), but sometimes only as a crease in the sclerite. In this genus the sternaulus arises anteriorly near the dorsal apex of the acetabular carina and continues obliquely dorsally toward the mesepisternal pit. The great majority of species may also be recognized as *Phanuromyia* by the convex frons (frontal depression limited to a small area just dorsal of the antennal insertions), the presence of two parallel dorsoventral lines of setae below the median ocellus, eyes glabrous or with very short setation, second metasomal tergite with shallowly incised reticulate sculpture posterior to the basal longitudinal costae.

Status of Aholcus

Whereas females over 90% of telenomine species have 11-segmented antennae, 70 species of *Telenomus* have been described in which this number is reduced to 10. Kieffer (1913) created the genus *Aholcus* for a single species from Kenya, *A. monticola* Kieffer. The same year, Dodd (1913) erected the genus *Neotelenomus* for five Australian species. In both cases, the only character differing from *Telenomus* was the number of female antennomeres. Nixon (1935) synonymized *Aholcus* with *Telenomus*, but later reversed his finding and treated it as a valid subgenus (Nixon, 1937). In the latter paper he synonymized *Neotelenomus* with *Telenomus (Aholcus)*. Since then, *Aholcus* has been used as a valid name, both as a subgenus and, occasionally, as a genus in 20 publications (including Nixon, 1937) for 31 newly described species. Johnson (1984a) suggested that *Aholcus* may be polyphyletic and in subsequent papers has not adopted the rudimentary subgeneric classification (currently there are only two subgenera recognized).

Aholcus is represented in our study by the common Holarctic species *Telenomus dalmanni* (Ratzeburg), a parasitoid of the eggs of the rusty tussock moth, *Orgyia antiqua* (Linnaeus) (Lepidoptera: Lymantriidae). In all of the data coding, partitioning and analysis schemes employed, this species consistently clusters with those of the *californicus* complex of *Telenomus*. Our taxon sample did not include *T. monticola* (Kieffer), so our results do not directly indicate how this species – the type species of *Aholcus* – is related to other telenomines. We can conclude, though, that recognition of a genus defined solely on the basis of the reduced number of antennomeres in the female sex would make *Telenomus* paraphyletic. Furthermore, the male genitalia of *T. dalmanni* (see Johnson, 1984a) are very distinctive, having a spectacularly long aedeagal lobe. This feature is not characteristic of all *Aholcus* species (see illustrations in Nixon, 1935, 1937), but is found in some species of *Telenomus* s.s. in which the females may have either 10- or 11-segmented antennae (e.g., Johnson & Bin, 1982). This strongly suggests that the reduction in antennomere number has occurred in parallel among different parts of *Telenomus* in the broad sense and that *Aholcus* is a polyphyletic taxon. Although we have no molecular data for *Telenomus monticola* itself, its relationship to the lepidopteran parasitoids is corroborated by a previously unpublished morphological character. Specifically, the ventral portion of the occipital carina is deflected anteriorly around the posterior articulation of the mandible and is visible laterally (see fig. 31 in Johnson, 1984a). In conclusion, we consider *Aholcus* Kieffer, 1913 to be a junior synonym of *Telenomus* Haliday (**syn.n**), 1833 and reject its use as a subgenus.

Status of Platytelenomus

The name *Platytelenomus* is less commonly encountered than *Aholcus*, but its history is similar in many respects. The genus was originally described by Dodd (1914b) for an Australian species in which the body is strongly flattened dorsoventrally. Johnson (1988b) proposed that the same name had been used for two distinct groups of species: one, represented by Dodd's type species, *P. planus*, has elongate clavomeres and corresponds to the *floridanus* group of *Telenomus*; the second has transverse clavomeres and parasitizes the flattened eggs of grass-feeding Lepidoptera (hence the flattened body). The species used in this study, on the basis of morphology, falls in the latter group. Consistent with Johnson (1988b), this species clusters with the other moth parasitoids of the *californicus* complex, and not with the species of the *floridanus* group. Therefore, we reaffirm the conclusion of Johnson (1988b) and consider *Platytelenomus* Dodd, 1914 to be a junior synonym of *Telenomus* Haliday, 1833.

Position of Eumicrosoma

This name encompasses a small group of species found worldwide that parasitize the eggs of blissine bugs. The genus is quite distinctively defined by its dorsoventrally depressed body, elongate lanceolate wings, elongate marginal and

truncate postmarginal vein in the forewing, and opisthognathous mandibles. Johnson (1984a) noted the similarity between *Eumicrosoma* and the *floridanus* group of *Telenomus*, and implied that the former was merely a morphologically extreme form of the latter. Our results do not support the hypothesis that *Eumicrosoma* is most closely related to species of the *floridanus* group. The position of *Eumicrosoma* is unstable: the genus arises near the *californicus* complex in some analyses or with the Australian species *Trissolcus ogyges* (Dodd) in others. In fact, the *floridanus* group itself fails to emerge as a monophyletic group; the only species with a stable position in the phylogeny – with low support – is *Telenomus* FLO1 at the base of the clade of *Trissolcus* + *Telenomus* + *Eumicrosoma*. In view of the lack of strong support for resolution in this part of the inferred phylogeny, we prefer to adopt a conservative approach and continue to treat *Eumicrosoma* as a separate, valid genus.

The speciose genera: Telenomus and Trissolcus

The genera that today go by the names *Telenomus* and *Trissolcus* were first distinguished by C.G. Thomson (1860) as *Phanurus* Thomson and *Telenomus*, respectively. Most species were recognized by comparison of the sculpture of the frons: absent in *Telenomus*, present in *Trissolcus*; eye setation: long in *Telenomus*, short or absent in *Trissolcus*; development of notauli: almost always absent in *Telenomus*, commonly found in *Trissolcus*; shape of the second metasomal tergite: longer than wide in *Telenomus*, wider than long in *Trissolcus*; and number of female clavomeres: most *Telenomus* with five or fewer, *Trissolcus* with six. In general, species of *Trissolcus* are robust and stout-bodied, whereas many *Telenomus* species are elongate and gracile. None of these is without exception, but in concert they leave few species that cannot be unambiguously placed under one name or the other. The monophyly of these taxa, however, has never been demonstrated. The morphological diversity among species that has made it so difficult to unambiguously diagnose genera is also reflected in the attempts to delimit groups of species within them. We began this study not with the expectation that either *Telenomus* or *Trissolcus* would turn out to be monophyletic, but, rather, with the hope that the relationships among the species groups would provide the scaffold upon which to build a better understanding of the phylogeny of the subfamily.

Our data fail to settle decisively the question of the monophyly of either of the two core genera of the subfamily, *Trissolcus* and *Telenomus*. Further, though, none of the three species groups of *Trissolcus* were recovered in these analyses. At least one of these, the *flavipes* group, is very homogeneous and distinctive in morphology and, therefore, seems likely on those grounds to be monophyletic. The basal position of two of the species of the *thyantae* group is consistent with our expectations based on structure – for example, presence of notauli, presence of sublateral setae on the first metasomal tergite, and presence of setae on the posteroventral metapleuron.

We were able to recover some of the expected structure among the *Telenomus* species represented. Particularly, the placement of the *crassiclava* group distinctly apart from other *Telenomus* is significant. Among the remaining groups, the *podisi* and *floridanus* groups were not found to be monophyletic. As discussed in the section on *Eumicrosoma*, species of the *floridanus* group are scattered throughout the phylogeny. The only Old World species of the *podisi* group in the study, *Te. sechellensis* Kieffer, is always more closely related to the *californicus* complex than with others of its putative group. This result is corroborated by morphological evidence, specifically the same character of the occipital carina described above for *Te. monticola*. This feature is found in all Old World *Telenomus* parasitoids of stink bugs as well as in all species of the *californicus* complex, and it is lacking in all New World members of the *podisi* group. Consistent with the hypotheses in Johnson (1984a) the species of the *californicus* complex grouped together in a single clade, from which emerges the *tabanivorus* species group. These are all united by the structure of the metascutellum (evenly produced, coarsely sculptured dorsally, longitudinally striate ventrally) and the lower course of the occipital carina. The *laricis* and *longicornis* groups are also monophyletic, and most species are fairly easily recognized on the basis of morphology. The *laricis* group is characterized by the greatly enlarged gena and elongate body; those of the *longicornis* group have a strongly pigmented basal vein on the forewing, distinctive male genitalia with an elongate, spatulate aedeagal lobe, and a distinctively convex mesosoma.

Host relationships

Biological data have been acquired for 16 of the 51 ingroup species (31%). Thus, our assumptions of the host relationships and specificity should be viewed with some caution. Nevertheless, the number of host records for telenomines overall is very substantial. With that caveat in mind, it appears that the combined clade of *Gryon*, the *Psix* group, and Telenominae s.s. evolved from an ancestral species that probably parasitized the eggs of terrestrial Heteroptera. Hosts in the Pentatomoidea, Coreoidea, Reduvioidea and Lygaeoidea are shared among these three taxa. *Paratelenomus* has only been recorded as a parasitoid of turtle bugs (Pentatomoidea: Plataspidae), whereas species of *Psix* have been reared from a number of species of Pentatomoidea as well as Coreoidea. For the basal groups of Telenominae s.s., with the exception of *Phanuromyia*, the most common families of hosts are the stink bugs and their allies in Pentatomoidea, particularly the Pentatomidae and Scutelleridae. This is true of all three of the species groups of *Trissolcus*, as well as the *Telenomus podisi* group (represented by *Te. sechellensis, Te. grenadensis* and *Te. podisi*). Species of the *floridanus* group of *Telenomus*, parasites of Lygaeidae, also arise at or near the base of the sister group of *Phanuromyia*. These include *Te. nysivorus, Te. consimilis* and three unidentified species. The single species of *Eumicrosoma* in this study is from Thailand and its host is as yet unknown.

However, three species of the genus, *E. beneficum* Gahan, *E. bicolor* (Ashmead) and *E. blissae* (Maki) are all well known as parasitoids of chinch bug eggs (*Blissus, Ischnodemus*: Heteroptera: Lygaeidae). *Gryon* is a very large genus of Scelioninae (331 valid species). A few species have been recorded as having been reared from eggs of Lepidoptera, but the vast majority parasitizes the same groups of Heteroptera as these telenomines (with the exception of Blissidae).

The parasitism of the eggs of auchenorrhynchous bugs – planthoppers of the families Fulgoridae and Issidae are the only hosts so far recorded – appears to have evolved but once, in *Phanuromyia* (interpreted as including the *T. crassiclava* and *aradi* groups). *Telenomus* has often been described as a group composed primarily of parasitoids of the eggs of moths and butterflies (e.g. Masner, 1976). Certainly a good number of species have this biology, and these are commonly encountered. Yet the host relationships of the genus are much more diverse. Parasitism of the eggs of Lepidoptera has only been elsewhere recorded in the Platygastroidea for two species of *Gryon*. Clearly, the shift(s) to this pool of hosts was evolutionarily significant, at least for diversification within *Telenomus*. On the basis of our results this shift occurred once or twice, depending on the interpretation of the position and putative host relationships of *Telenomus* FLO3 within the clade corresponding to the *Te. californicus* complex. Then, from a lepidopteran parasitoid, there was a subsequent shift in the *Te. tabanivorus* group to attack the eggs of some groups of horse flies (Diptera: Tabanidae) and soldier flies (Diptera: Stratiomyiidae). In a separate lineage are the *laricis* group of *Telenomus*; one representative of the *phymatae* group, *Te. dolichocerus* (Ashmead); and the *longicornis* group of *Telenomus*. The first of these, the *laricis* group, has few host records, but these are limited to the plant bugs (Miridae), including the economically important tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). *Telenomus dolichocerus* is a parasitoid of Reduviidae. The hosts of the *longicornis* group are as yet unknown. However, the species are somehow associated with water: they are commonly found alongside ponds and streams and have even been collected in floating emergence traps. Marshall (2006) asserted that one species in this group parasitizes the eggs of *Rhagovelia obesa* Uhler (Heteroptera: Veliidae), but supporting data have not been published, and we have never seen specimens so labelled. Certainly, given the position of the *longicornis* group, in a clade of heteropteran parasitoids, either Gerridae or Veliidae would be good candidates for hosts.

In summary, the sequence of host shifts that best fits the results presented here begins from a ground-plan host of some group of terrestrial Heteroptera. From there, one clade, *Phanuromyia*, moved on to the Auchenorrhyncha. Its sister group went in two different directions, with one subgroup (the *californicus* complex) shifting first to Lepidoptera and then to Diptera, whereas a second group shifted to Miridae (the *laricis* group), Reduviidae and a group of aquatic or semiaquatic insects, probably Heteroptera.

Although we have discussed host relationships in terms of taxonomic groups, obviously the parasitoids are using other cues in order to locate suitable eggs to parasitize. Host names merely serve as proxies for the real sensory modalities involved, such as long- or short-range chemicals (e.g. Colazza *et al.*, 2007, 2009; Peri *et al.*, 2007; Moraes *et al.*, 2009; Salerno *et al.*, 2009), tactile cues (e.g. Laumann *et al.*, 2007), or possibly vision (Laumann *et al.*, 2011). Determination of the breadth of host range and shifts from one set of hosts to another would seem involve either an overlap in the cues directly or indirectly produced by a host species, or a change in the capacity or responses of parasitoids to these stimuli. Research on the proximate mechanisms of host-finding in telenomines has been focused on species that are parasitoids of important pests such as *Trissolcus basalis*, *Telenomus podisi*, *Te. busseolae* (Gahan) and *Te. remus* Nixon. A phylogenetic hypothesis is sorely needed to be able to frame questions about the evolution of host relationships and to appropriately integrate the findings from these several species. The results presented here are a first step in that direction, providing support for some hypotheses and highlighting those areas in need of more intense future work.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12032

File S1. List of taxa and sequenced markers for phylogenetic analysis of Telenominae: GenBank accession numbers and identifiers for specimens sequenced.

File S2. Alignment summary, gene partitions, nucleotide composition and percentage of parsimony informative sites.

File S3. Support values for selected clades across analyses.

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