# Undiscovered diversity of dipteran parasitoids infecting mongolian orthopterans

Javzansuren Munkhbat, Catherine Moulia, Roger Frutos, Badamdorj Bayartogtokh, Marco Perriat-Sanguinet, Laurent Gavotte

Available Online at ESci Journals
International Journal of Entomological Research
ISSN: 2310-3906 (Online), 2310-5119 (Print)
http://www.escijournals.net/IJER

# UNDISCOVERED DIVERSITY OF DIPTERAN PARASITOIDS INFECTING MONGOLIAN ORTHOPTERANS

**[ab]Javzansuren Munkhbat*, [a]Catherine Moulia, [c]Roger Frutos, [b]Badamdorj Bayartogtokh, [a]Marco Perriat-Sanguinet, [a]Laurent Gavotte**

*[a] Institut des Sciences de l'Evolution, UMR-CNRS 5554, Université de Montpellier, Montpellier, France.*

*[b] Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar 210646, Mongolia.*

*[C] UM2, CPBS, UMR 5236, CNRS-UM1-UM2, 1919 route de Mende, 34293 Montpellier Cedex 5, France.*

**A B S T R A C T**

Diversity of parasitoids species remains questioning in several ecosystems poorly surveyed. The present study is focused on dipteran parasitoids species infecting orthopteran in forest-steppe ecosystem of Mongolia. Among a total of 420 hosts from four species, 20 were found to be infected and 200 larvae of dipteran parasitoids recovered. Genetic studies on a subset of larvae shows the presence of up to seven taxa whose none is represented in databases (although 13 species were previously reported in Mongolia), indicating a large and unexplored diversity of dipteran parasitoid in Mongolia. Further studies will be focused in establishing of this diversity and the determination of the species.

**Keywords**: Dipteran parasitoid, diversity, CO1, Orthoptera, Mongolia.

## INTRODUCTION

Dipteran parasitoids include an estimated 16,000 species, representing approximately 20% of the total number of recorded dipteran species (Feener and Brown, 1997). It was hypothesized that parasite life-style has evolved over 100 times in the Diptera order, since parasitoid species were recognized in 21 families of Diptera (Eggleton and Belshaw, 1992). The parasitoids clearly affect the life history traits of their hosts, strongly impacting their fecundity (Laws and Joern, 2012). These parasites not only appear as main factors of the functioning and evolutionary dynamic of the ecosystems, but also as an efficient agricultural auxiliaries and their use as pest control theorized a long time ago (Parry, 2008). However, the ecological and agricultural importance of those parasitoids their geographical distributions and their biodiversity in numerous ecosystems are still poorly or not studied, as in Mongolia.

Mongolian ecosystems are mainly forest steppe and steppes, among which humans activities are largely dominated by animal breeding and agriculture. Today, 173 species of orthopteran insects were reported in Mongolia (Chogsomzhav, 1989). In 1978, Rohdendorf and Verves recorded 53 taxa of Sarcophagidae in Mongolia. Among them, 13 taxa were noted as parasitoids of orthopteran species: 12 species infecting Acrididae family and one Tettigoniidae family species. However, as usual for parasitoid species for which we lack genetic data, the species determination remains problematic on morphological or host specificity traits. In this study, we present preliminary survey of dipteran parasitoids infecting four orthopteran species in Mongolia. To assess the parasitoid status of Dipteran, larvae present in orthopteran adults caught in the field were studied, and their species tentatively assessed by CO1 mitochondrial gene sequencing.

## MATERIAL AND METHODS

**Study area:** The study site is located 100 km west of Ulaanbaatar city (47$^0$50'N, 106$^0$00'E), in the 600km$^2$ Hustai National Park (HNP) in the forest and steppe ecosystems (Bayarsaikhan *et al*., 2009). Four different locations were surveyed and each characterized by

* Corresponding Author:
Email: munkhbat_j@num.edu.mn

different habitats namely meadow, steppe, forest and mountain steppe.

**Field collection of Orthopteran and Dipteran parasitoids:** A total of 420 orthopteran individuals representing four species (*Acryptera microptera* (Fischer von Waldheim, 1833), *Calliptamus abbreviatus* Ikonnikov, 1913, *Gampsocleis sedakovii* (Fischer von Waldheim, 1846) and *Deracanthella verrucosa* (Fischer von Waldheim, 1846) from two families (Acrididae and Tettigoniidae) were collected. The field trapping campaign (based on line transect method using a sweep net catching) was performed during warm season, in August 2012. All individual were stored in the absolute ethanol at -80$^0$C. Morphological determination of the orthopteran species was based on the femur length measurement as an index of host body size using calipers (Miura and Ohsaki, 2008). After that each individual was dissected under binocular in the laboratory. Among them 20 individuals were found to be infected by dipteran larvae, after that 200 parasitoid larvae were collected (Table 1) and used for genetic studies.

**Parasitological analyses:** Parasitological Parameters (prevalence and mean intensity) of both host species and habitats were calculated, and compared by using a Fisher's exact test.

**Parasitoid genetic analyses:** The mitochondrial Cytochrome Oxydase1 gene (CO1), previously used for phylogenetic analysis of parasitoid Diptera (McDonagh and Stevens, 2011), was used to characterize the individuals. One parasitoid per infected host was analyzed. Total DNA was extracted from muscle of the anterior part of larvae, using the Chelex-based DNA extraction method (Casquet et al., 2012). A 500-bp fragment of CO1 was amplified and sequenced. PCR was performed in an Eppendorf Mastercycler™ in 25µl final volume including 5µL of 5 x Colorless Reaction buffer (Promega™, USA), 0.5mM of MgCl2, 0.2mM of dNTPs, 10pmol/µL each of primer FOR-COI (5'-GGAGGATTTGGAAAATTGATTAGTTC-3') and primer REV-COI (5'-CCCGGTAAAATTAAAATATAAACTTC-3'), 0.25 U of Taq DNA polymerase, 15.25µL of purified distilled water and 3µL of template DNA. PCR conditions were the following: hot-start at 94$^0$C for 3 minutes, denaturation at 94$^0$C for 45 seconds, annealing at 53$^0$C for 45 seconds and extension 72$^0$C for 1 minute. 35 cycles were applied with a final extension 72$^0$C for 10 minutes and cooling down 10$^0$C for 10 minutes. PCR products were sequenced in an ABI 3730XL Analyzer (96 capillary type) using the ABI Prism BigDyeTM Terminator Cycle Sequencing (BIOFIDAL, Lyon, France). Sequence alignments were conducted with Muscle, in SeaView 4.2.6 (Gouy et al., 2010). JModelTest (Posada, 2009) was used to select the optimal evolution model by evaluation of the selected parameters using the Akaike Information Criterion (AIC). This approach indicated GTR+G as the most suitable evolutionary model for the complete data set of sequences. Under the selected models, the Maximum Likelihood (ML) trees were built with phyML (version 3.0). The robustness of nodes was assessed with 500 bootstrap replicates.

**RESULTS AND DISCUSSION**

Prevalence of dipteran parasitoid infestation: After checking the 420 orthopteran individuals collected, 200 larvae of parasitoid dipterans were recovered from 20 individuals (Table 1), displaying a prevalence of 4.8%. Data acquired in other ecosystems (Middle East and North America) showed a large range of dipteran parasitoid prevalences from 1 to 90% (Hostetter, 2000; Elsayed and Sayd, 2014). Our results are compatible with these previous reports but remain low. Absence of other studies in the region or the studied species in other geographical areas do not allow more meaningful comparisons. No significant differences between host species ($p > 0.05$, Fisher's exact test) or between habitats (mountain steppe, steppe and meadow) ($p > 0.05$, Fisher's exact test) were detected except for the forest ecosystems where no dipterans parasitoids were recovered from the 28 orthopteran caught in this location. Among the four host species studied, *G. sedakovii* harbor a significantly higher mean of infection intensity (41.5 larvae per host) compared to the other species (1.67 to 2.44 larvae per host) (Table 1). The number of parasitoids per host vary along to different parameters as i) the parasitoid foraging behavior, that can avoid to depose new larvae in a previously infected host (Hamelin et al., 2007) ii) the size of host limiting the available resources for each larvae (Harvey, 2005). The pattern described in this study could then indicate that parasitoid species infecting *A. microptera*, *C. abbreviatus* and *D. verrucosa* are solitary species infecting only healthy host with very few larvae in order to exploit the whole host resources for their development, whereas at least one species infecting *G. sedakovii* use an indirect exploitation strategy of the host being superparasite (Feener and Brown, 1997; Coupland and Baker, 2004).

Table 1. Prevalence of dipteran parasitoids in mongolian orthopteran hosts, according to the different studied habitats. N–number of hosts, n-inf.–number of infected hosts, Pr. %- prevalence (percentage), Int–mean intensity. MS-mountain steppe, St-steppe, M-meadow, F-forest.

| Species | Habitats | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N/n-inf. | Pr% | Int. | MS | Pr% | St | Pr% | M | Pr % | F | Pr% |
| **Acrididae** *A. microptera* | 109/9 | 8.3 | 2.44 | 65 | 4.6 | 42 | 14.3 | - | - | 2 | - |
| *C. abbreviatus* | 104/4 | 3.8 | 1.75 | 54 | 5.6 | 50 | 2 | - | - | - | - |
| **Tettigoniidae** *G. sedakovii* | 142/4 | 2.8 | 41.5 | 37 | 2.7 | 43 | - | 36 | 8.3 | 26 | - |
| *D. verrucosa* | 65/3 | 4.6 | 1.67 | 8 | - | 57 | 5.3 | - | - | - | - |
| Total | 420/20 | 4.8 | 10 | 164 | 4.3 | 192 | 5.2 | 36 | 8.3 | 28 | - |

**Genetic analysis:** DNA obtained from one individual and from each parasitized host was extracted to perform a PCR and sequence the CO1 gene (Figure. 1). Among the 20 individuals tested, 14 sequences were recovered. Phylogenetic analysis of CO1 sequences suggests the existence of 5 clades (maybe 7 since two of which being potentially dividedin two subclades). A homology research realized with BLAST tool (Mount, 2007) indicates that the sequences of each clade could be related to known families: Muscidae for clade 1, Anthomyiidae for clade 2 and Sarcophagidae for clades 3, 4 and 5. The closest species identified in GeneBank were *Blaesoxipha plinthopiga* (KF030489.1; 92% of homology), *Pegomya ulmaria* (JX438048.1; 91% of homology), *Chrysomya chani* (FJ195377.1; 91% of homology), *Sarcophaga* sp. (KC617862.1; 91% of homology), *Lespesia aletiae* (JQ574709.1; 90% of homology), *Spologina narina* (HM891768.1; 90% of homology), *Sarcophagidae* sp. (JN965186.1; 90% of homology), *Sarcophaga praedatrix* (JN965091.1; 90% of homology), *Sarcophaga siciliana* (JQ582045.1; 87% of homology) and *Sarcophaga villisterna* (JN965161.1; 87% of homology). However, none of these species display a sufficiently high homology with the larvae tested here to allow a species assignation. A mean of 91% (SD: 2%) similarity (with a maximum of 92%) is observed. We highlight at least five taxa (maybe 7 including sub-taxa 3b and 4b) of dipteran parasites among 14 larvae successfully sequenced. This very high variety from a small sampling indicates potentially wide species diversity. Three dipteran families are represented with one taxon related to Muscidae and Anthomyiidae families, and three taxa related to Sarcophagidae. None of the recovered CO1 sequences correspond to the species present in GenBank database and the closest data of each sequence present an identity from 87 to 92%. We can then hypothesize that the species we found in Mongolia are previously known and described species but not sequenced yet or we are in presence of undescribed species from poorly studied ecosystems. For example 2265 sequences of Sarcophagidae CO1 gene are stored in GenBank (http://www.ncbi.nlm.nih.gov/genbank) for an average or 2000 to 2500 species reported. Considering that a single species could be over represented (e.g. 57 sequences of CO1 gene are reported for the single species *Sarcophaga iota*), the sequencing coverage of known Sarcophagidae is not complete.

**Host and host-habitat selection:** The genetic tree showed that the host species A. microptera was infected by three clades corresponding to two families, whereas *C. abbreviatus* and *G. sedakovii* were infected by two clades corresponding to two families, and *D. verrucosa* was infected by only one clade of one family (Fig.1). Concerning the host range of parasites, only the clade 1 is determined as infecting more than one species (*C. abbreviatus* and *G. sedakovii*), all others were found in only one host species. Mountain steppe habitat seems to present more clade diversity (clade 2, 3, 3b, 4b and 5) than other two habitats, respectively housing three clades (1, 4 and 5) for steppe and one clade for meadow (Figure. 1). These results seem to indicate differences in parasitoid distribution between both host species and habitats. But our samples are limited (one parasite tested per host) and the whole diversity of species infecting one host might not have been investigated. However, as mentioned above, 173 orthopteran species are reported from Mongolia (Chogsomzhav, 1989). The preliminary results presented in this paper focusing on only 4 orthopteran species suggest the potential existence of hundreds of dipteran parasitoid species

infecting orthopteran insects in Mongolia, at least much more than the 13 actually reported (Rohdendorf and Verves, 1978). A large part of them were probably not described yet and the diversity of this group remains largely unknown. Further studies must be focused in screening of this undiscovered diversity of parasitoids and the identification of potentially new biological pest control.

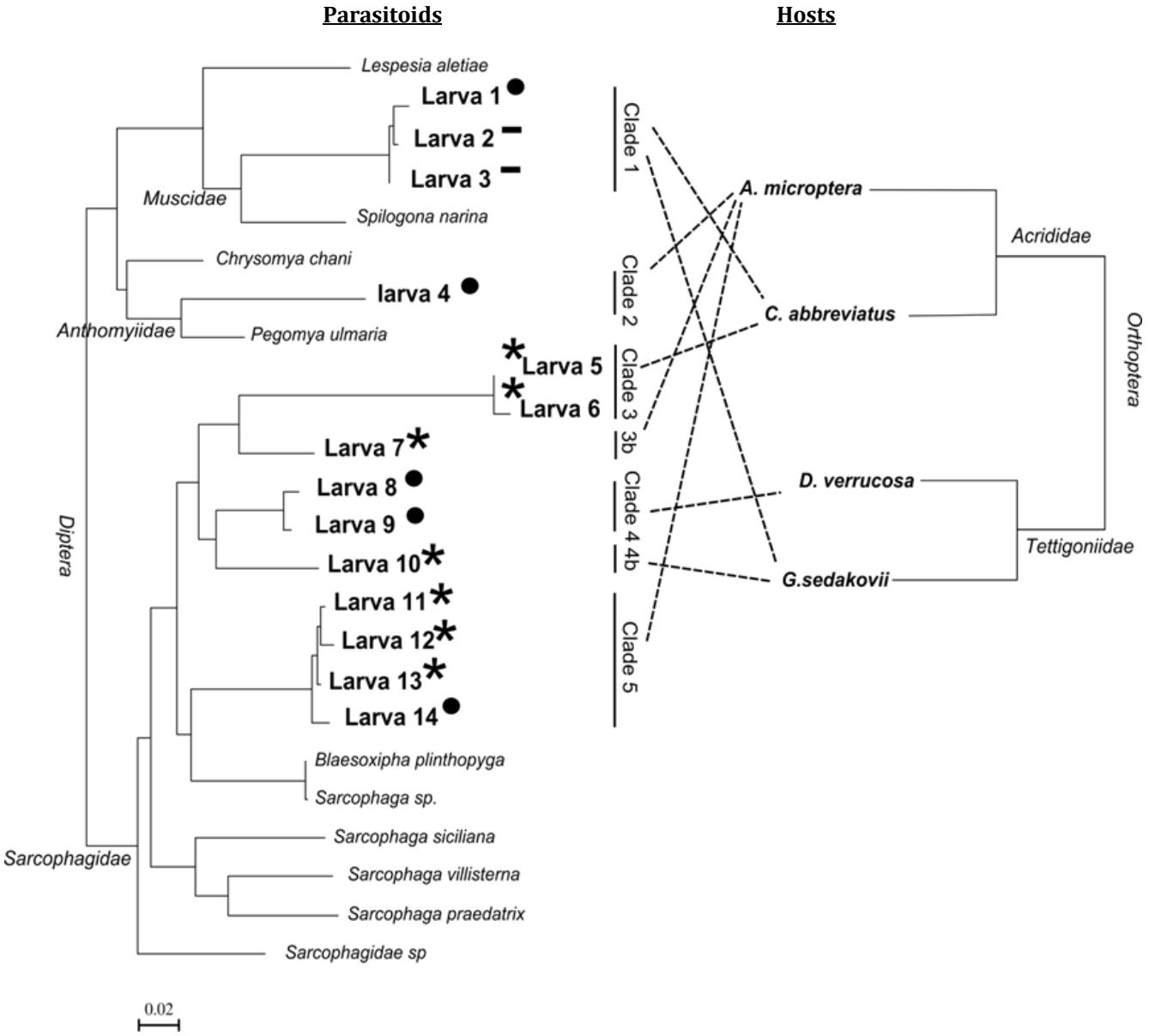


Figure 1. Phylogenetic tree of Mongolian parasitoids based on a mitochondrial COI gene related to host-species and host-habitats. Star indicates mountain steppe, round point indicates steppe and lash indicates meadow. Black dotted line indicates relationship. The horizontal axes present rate of substitution per site. GenBank accession numbers: KP075010 to KP075023.

**ACKNOWLEDGMENT**

Javzansuren Munkhbat was partly supported by Erasmus Mundus Project MAHEVA (Man Health Environment Biodiversity Asia) fellowship. We would like to thank Hustai National Park's administration for supporting field surveys.

**REFERENCES**

Bayarsaikhan U., Boldgiv, B., Kim, K.-R., Park, K.-A. and D. Lee. 2009. Change detection and classification of land cover at Hustai National Park in Mongolia. International Journal of Applied Earth Observation and Geoinformation, 11(4): 273–280.

Casquet J., Thebaud, C. and R. G. Gillespie. 2012. Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. Molecular Ecology Resources, 12(1): 136–41.

Chogsomzhav L. 1989. Compostion and distribution of fauna of the Orthopteroidea in the Mongolian People's Republic. Insect of Mongolia, 10: 84–96.

Coupland J. B. and G. M. Barker. 2004. Diptera as predators and parasitoids of terrestrial gastropods, with emphasis on Phoridae, Calliphoridae, Sarcophagidae, Muscidae and Fanniidae. In: Barker, G.M. (Ed.), Natural enemies of terrestrial molluscs. CABI Publishing, Wallingford, pp. 85–158.

Eggleton P. and R. Belshaw. 1992. Insect Parasitoids: An Evolutionary Overview. Philosophical Transactions of the Royal Society B: Biological Sciences. 337:1-20.

Elsayed G. and S. M. Sayed. 2014. A First Record of *Blaesoxipha rufipes* (Diptera: Sarcophagidae) Parasitizing *Poekilocerus bufonius* (Orthoptera: Pyrgomorphidae) in Saudi Arabia, Entomological News, 124(1):33-37.

Feener D. H. and B. V. Brown. 1997. Diptera as parasitoids. Annual Review of Entomology. 42: 73–97.

Gouy M., Guindon, S. and O. Gascuel. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution. 27:221-224.

Hamelin F., Bernhard, P. and E. Wajnberg. 2007. Superparasitism as a differential game. Theoretical Population Biology, 72(3): 366–78.

Harvey J. A. 2005. Factors affecting the evolution of development strategies in parasitoid wasps: The importance of functional constraints and incorporating complexity. Entomologia Experimentalis et Applicata. 117:1-13.

Hostetter D. L. 2000. Natural Enemies Attacking Grasshopper Nymphs and Adults. In G. L. Cunningham and M. W. Sampson (Eds.), Grasshopper Integrated Pest Management User Handbook (pp. I.8–1–I.8–7).

Laws A. N. and A. Joern. 2012. Variable effects of dipteran parasitoids and management treatment on grasshopper fecundity in a tallgrass prairie. Bulletin of Entomological Research, 102: 123–130.

Mcdonagh L. M. and J. R. Stevens. 2011. The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1α: insights into the evolution of dipteran parasitism. Parasitology, 138: 1760–1777.

Miura K and N. Ohsaki. 2007. Host characteristics related to host use by the flesh fly, *Blaesoxipha japonensis* (Diptera: Sarcophagidae), a parasitoid of adult grasshoppers. Appl. Entomol. Zool. 42 (4): 651–658.

Mount D. W. 2007. Using the Basic Local Alignment Search Tool (BLAST). Cold Spring Harbor Protocols, (7). pdb top 17.

Parry D. 2008. Beyond Pandora's Box: quantitatively evaluating non-target effects of parasitoids in classical biological control. Journal Biological Invasions. 11 (1): 47-58.

Posada D. 2009. Selection of models of DNA evolution with jModelTest. Methods in Molecular Biology (Clifton, N.J.). 537: 93–112.

Rohdendorf B. and Y.G. Verves. 1978. Sarcophaginae (Diptera, Sarcophagidae) from Mongolia. Annales Historico-Naturales Musei Nationalis Hungarici. 70: 241–258.