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Predicting Hosts through Molecular Analysis of Ichneumonid Guts

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ABSTRACT

Ichneumonidae are well-known parasitoids that attack the larvae or pupae of other insects. This study analyzed whether the abdominal DNA of two ichneumonid wasps, *Pimpla disparis* and *Theronia atalantae gestator*, showed the signature of the host species, *Ivela auripes*. Observations confirmed that these two ichneumonids were the representative parasitoid species growing in the larvae of *I. auripes*. In addition, sequence analysis showed that the mitochondrial cytochrome oxidase I gene of the host was amplified completely from the DNA extracted from the gut tissues of the ichneumonids. Even after 96 h of adulthood, the host's DNA traces did not disappear and were amplified in many individuals. These results suggest a constructive first step for establishing of a host information bank for ichneumonids in the future.

Keywords: COI, Hymenoptera, Ichneumonidae, Lepidoptera, Lymantriidae, parasitoid wasps

INTRODUCTION

The order Hymenoptera is one of the largest taxa on Earth. Species in this order either live alone or form elaborately developed eusocial colonies to reproduce and survive in close interaction with other life forms in the ecosystems (Pilgrim et al., 2008; Cardinal and Danforth, 2011). More than 146,000 species in this order have been recorded worldwide (Huber, 2009). In any part of the planet, hymenopteran species occupy important ecological positions with a profound impact on the ecosystems and human life as pollinators, pests and parasites or predators to other insects (Huber, 2009; Vilhelmsen and Turrisi, 2011). In particular, parasitic wasps have attracted considerable attention from scientists in recent years, because of their economic value, such as the potential for biological control applications (Quicke, 2015).

The most representative parasitoid wasps are species belonging to the superfamily Ichneumonoidea, where Ichneumonidae accounts for the highest proportion with 40 subfamilies (Königsmann, 1978; Gauld, 1984). Most species in this family attack the pre-adult staged individuals of holometabolous insects or, occasionally, adult spiders (Quicke, 2015; Takasuka et al., 2018). Parasitoid wasps (including Ichneumonidae) usually grow in the larva stage by feeding their hosts. They seldom prey on insects after reaching adulthood (Rougerie et al., 2011).

The ecological features of ichneumonids could be applied to control some pests that damage crops, orchards, and forests. A complete list of ichneumonid species parasitic to pests must be obtained before such applications, even though the research on the ecological relationship between parasitoid wasps and the hosts is still in its infancy (Godfray, 1994). Recently, molecular analysis was proposed to trace the signature of the hosts using multiple primer sets in genomic DNA extracted from the guts of adult parasitoid wasps (molecular analysis of parasitoid linkages (MAPL); Rougerie et al., 2011). Using MAPL, the hosts of many species can be listed in a short time. On the other hand, it needs to be verified that this method works effectively for all parasitoid wasps, and this type of study has not been attempted on this taxon existing in East Asia.

This study examined whether the abdominal DNA of two ichneumonid wasps, *Pimpla disparis* and *Theronia atalantae gestator*, showed the signature of the host species, *Ivela au-*

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Table 1. Primer sequences used to amplify the mitochondrial COI sequences of parasitoid wasps and the host, *Ivela auripes* in this study

Target	Primer	Primer sequence (5′-3′)	Reference
Parasitoid & Host (F)	LepF1	ATTCAACCAATCATAAAGATATTGG	Hebert et al. (2004)
Parasitoid (R)	LepR1	TAAACTTCTGGATGTCCAAAAAATCA	Hebert et al. (2004)
Host (R)	MLepR1	CCTGTTCCAGCTCCATTTTC	Hajibabaei et al. (2006)

COI, mitochondrial cytochrome oxidase I.

ripes. Only the DNA traces of *I. auripes* should be detected from the gut contents of the two ichneumonid wasps because individuals of these two ichneumonid species were grown inside the pupae of *I. auripes* in the laboratory before becoming adults. In addition, this study investigated whether the DNA traces of *I. auripes* were detected across all growth stages, namely, larval, pupal, and adult stages.

MATERIALS AND METHODS

Collection of specimens

The larvae of *Ivela auripes* usually migrate away from their host plants to become pupae. More than 1,400 individuals of last-instar larvae and pupae were collected from May to June 2015 (Mt. Cheongryangsan, Bongwha-gun, South Korea) and 2020 (Mt. Danseoksan, Gyeongju-si, South Korea). All *I. auripes* individuals collected were placed individually in a labeled petri dish (diameter × height: 55 mm × 15 mm with a mesh hole in the lid). As soon as adults of ichneumonid wasps emerged, they were transferred to a new petri dish (diameter × height: 100 mm × 42.8 mm with a mesh hole in the lid) that was provided with a few drops of dissolved food (3 g of sodium, 11 g of carbohydrates and 11 g of sugars in 100 mL purified water).

Detection of host DNA

Ichneumonid wasps from *I. auripes*. *Pimpla disparis* and *Theronia atalantae gestator* emerged directly from the pupae of *I. auripes* were gathered to determine if the genetic traces of *I. auripes* could be found in gDNA extracted from gut contents of ichneumonid wasps. The ichneumonids were placed in 80% ethanol. The specimens were stored as dry samples immediately after extracting the genomic DNA.

Detection of host DNA through developmental stages. *Ivela auripes* individuals were monitored to check for the genetic signature of the host at various developmental stages of parasitoid wasps. Adults of *P. disparis* (n = 50) that emerged from *I. auripes* were placed randomly in a freezer at −20°C after 0, 24, 48, 72 or 96 h. How long the traces of the host remained after adulthood could be determined by comparing these five treatment groups. In addition, *I. auripes* pupae were dissected and 17 larvae and pupae of *P. disparis* growing were obtained and stored in 80% ethanol. These 17 individuals were also compared with the adults from the five treatment groups separated by time. The genomic DNA was extracted and analyzed from all these 67 individuals.

Genetic analysis. The total genomic DNA was extracted from the body of individuals with wings and legs removed using a QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA). Each PCR was performed in a 30-μL volume consisting of 15 μL of a commercial premixure solution (Solg 2× Taq PCR Pre-Mix: 0.5× Band Doctor with dye, *Taq* DNA polymerase [(5 U/μL)], 10× *Taq* Reaction Buffer [(with 25 mM $MgCl_2$)], 10 mM each dNTP Mix; Solgent, Daejeon, Korea), 1 μL of DNA template, 2 μL of primer set (10 pmol) and 12 μL of DNase free water. Three primer sequences were used to amplify two different products, one for the COI (mitochondrial cytochrome oxidase I) partial sequence of parasitoid wasps (LF/LR; 700 bp) and the other for the COI partial sequence of the host (LF/MLR; 300 bp) (Table 1). The thermocycling profile was composed of an initial predenaturation at 94°C for 5 min, 35 cycles of a denaturation for 1 min at 94°C, annealing for 1 min at 50°C and extension for 1 min at 72°C, and a final extension for 5 min at 72°C. The amplified fragments were cleaned using a PCR purification kit (Solgent) and were sent to Solgent for the commercial sequencing analysis. The sequences were aligned, rechecked through BLAST searching and examined against the inferred reading frame for the corresponding proteins using the ClustalW implemented in MEGA6 (Tamura et al., 2013). A neighbor-joining tree was reconstructed based on the genetic distance calculated using the Kimura-2-parameter (K2P) model using MEGA6. The level of node support was assessed by bootstrapping with 1,000 pseudoreplicates.

RESULTS

Parasitic rate of *Ivela auripes*

Of the 330 pupae of *I. auripes* monitored, 52 (16%) were allegorized as (not parasitic) adults and 154 (46%) died as

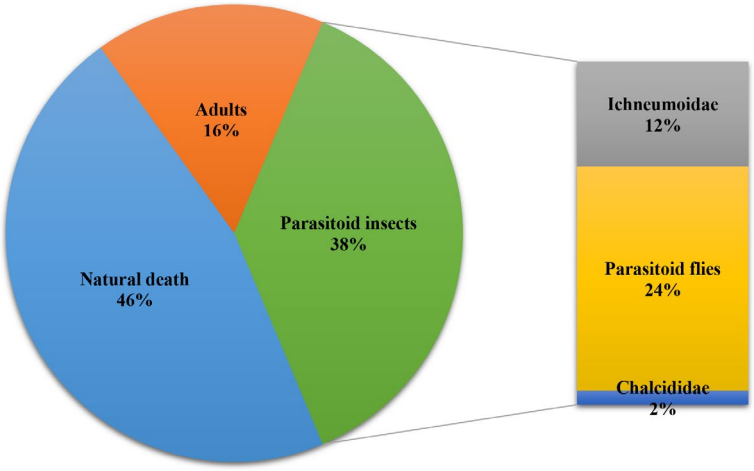

Fig. 1. Relative frequency of growing taxa parasitic to *Ivela auripes* (n=124). The individuals (green) with parasitoids, excluding those that died (blue) or were allegorized as adults (orange), were parasitized by parasitoid flies or (ichneumonid or chalcidid) wasps.

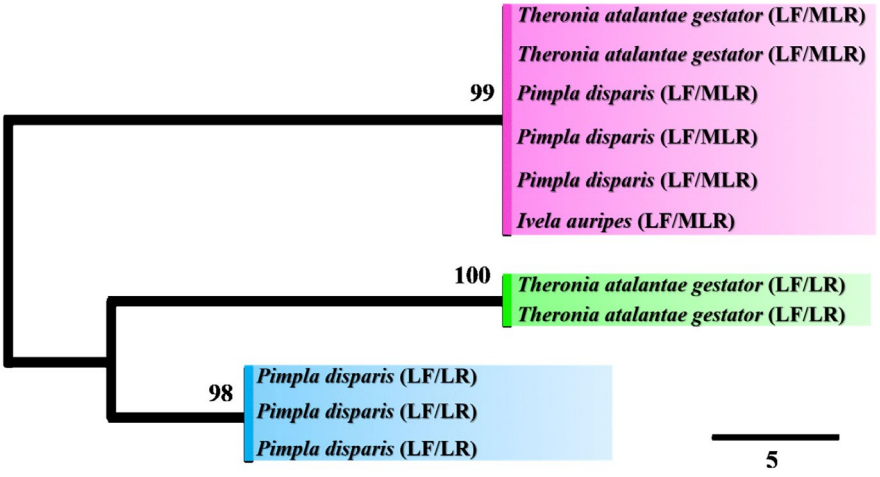

Fig. 2. Phylogenetic clustering pattern using the neighbor-joining algorithm of the sequences shown when gDNA samples of *Ivela auripes*, *Pimpla disparis*, and *Theronia atalantae gestator* were amplified using two pairs of primers (LF/LR and LF/MLR). The scientific names of species included in the clades refer to the gDNA samples used.

pupae. From the remaining 124, 81 parasitoid flies (24%), 38 ichneumonids (12%), and five chalcidid wasps (2%) emerged (Fig. 1).

Detection of host DNA from gut content of reared ichneumonid wasps

The guts of *Pimpla disparis* and *Theronia atalantae* gestator individuals that emerged from *Ivela auripes* were used in genetic analysis to examine the signature of the host DNA. *Ivela auripes* individuals were also analyzed for comparison. Successful amplification was achieved in *I. auripes*, *P. disparis*, and *T. a. gestator* using the LF/MLR primer set, which was designed to detect the host species sequences. All the amplified sequences were the same and were grouped into one clade with very low K2P distance values (Fig. 2; pink), indicating these sequences could be considered those of *I. auripes*. In the case of amplification using the LF/LR primer set, the unique sequences of *P. disparis* (blue) or *T. a. gestator* (green) were amplified and allocated into separate clades (Fig. 2).

Host detection efficiency comparison through the level of growth

The larvae, pupae and adult individuals of various ages (0, 24, 48, 72 and 96 h) (Table 2) were genetically analyzed to determine how long the genetic traces of the host were de-

Table 2. Amplification of host DNA in the different growth stages of *Pimpla disparis*

Target DNA		Larva	Pupa	Adult				
				0 h	24 h	48 h	72 h	96 h
Parasitoid	○	4	6	10	10	10	10	10
(LF/LR)	×	0	1	0	0	0	0	0
	Double	3	3	0	0	0	0	0
Host	○	7	10	9	9	10	9	8
(LF/ML)	×	0	0	0	1	0	1	2
	Double	0	0	1	0	0	0	0

The gene amplification of the host was attempted using two different primer sets (LF/LR and LF/ML) in each of the larvae, pupa, and adult individuals (of various ages; 0, 24, 48, 72 and 96 h).
○, successfully amplified; ×, no amplified; double, two different sequences.

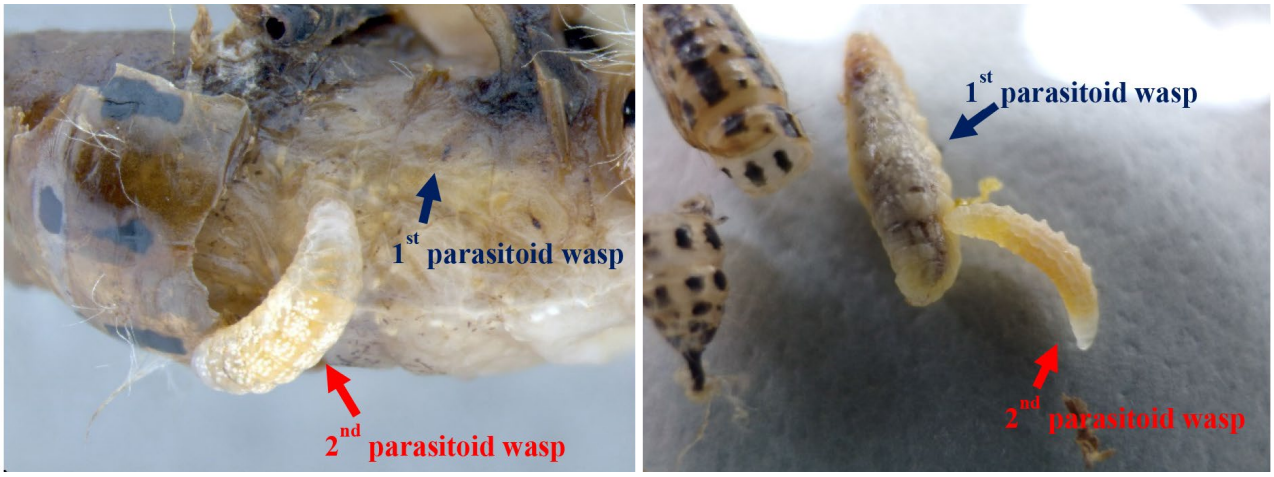

Fig. 3. Cases of hyperparasitism observed during our experiments.

tected after adulthood of *P. disparis*. The amplification of the host DNA was almost complete in all stages of growth (Table 2). In particular, the amplification efficiency did not decrease significantly even if the time after adulthood was long (Table 2). One peculiarity was that there were quite a few cases where two different sequences were amplified in the larvae and pupae when amplification was attempted using the LF/LR primer set (Table 2). Given that the detection of the double sequences was only shown in larval and pupal stages, this is probably because another parasitoid species existed in the body of *P. disparis* (hyperparasitism).

DISCUSSION

There are more than 350,000 parasitoid wasp species worldwide (Gaston, 1991), which means more than seven times the diversity of all vertebrates (Saunders and Ward, 2018). The diversity may be much higher than what is known because new parasitoid wasp species are being reported continuously and more species are predicted to be discovered (Saunders and Ward, 2018). In contrast to the active research aimed at understanding the taxonomic and phylogenetic diversity of parasitoid wasps, their ecological diversity, particularly host specificity, has received less attention (Forbes et al., 2018). The diversity of hosts can be considered a major factor in the speciation of parasitoid wasps considering that taxonomically very similar parasitoid wasp species often lay eggs in different hosts (Hopper et al., 2013). Hence, the failure to understand the host specificity properly implies a poor grasp of the evolutionary factors for the enormous diversity of parasitoid wasps. Extensive information on the host specificity can also open the way to biological control of various insect species that damage agricultural plants (Brodeur, 2012). *Ivela auripes*, which we used as a model host, also harms the sprouts of *Cornus controversa* (Cornaceae), and feeding increases significantly after the third instar larva (Kodani and Togashi, 1992).

Breeding the pests of interest and looking at which parasitoid species are growing in hosts can also be a way to increase information about the host specificity. On the other hand, it is not feasible in terms of time and cost to individually rear host species, which exhibit greater diversity than parasitoid wasps. This breeding method is not acceptable, considering that many parasitoid species lay eggs in multiple species (Lachaud and Pérez-Lachaud, 2012; Zhao et al., 2013; Wyckhuys et al., 2017), and the host specificity of a species also varies geographically (Funk and Bernays, 2001). Furthermore, the possibility of hyperparasitism (Day, 1994) should be considered. If the accuracy can be guaranteed, tracking the signatures of the host(s) remaining in the body of the parasitoid species is the most reliable method. Moreover, it is possible to obtain a large amount of information while encompassing the individual and geographic variations of host specificity.

This analysis showed that several parasitoid species grew in *Ivela auripes* and developed as adults. As is already known, two ichneumonids, *Pimpla disparis* and *Theronia atalantae gestator*, were found to be the main species laying in the larvae of *I. auripes*, based on the frequency shown in the present analysis. In MAPL analysis, the adults of these two species carried the genetic signature of *I. auripes*. Nevertheless, this study was not designed simply to re-validate existing attempts (Rougerie et al., 2011). In the present results, the genetic signature of the host was revealed in the individuals five days after they became adults with no tendency to fade over time. Further research will be needed to determine why the host signature remains intact. This means that the host tissues are more likely to remain in the mid-gut beyond the larval period. In addition, it is less likely to be subjected to decomposition reactions because there is little digestive activity in this mid-gut after pupation.

When the larvae and pupae of *P. disparis* were analyzed using the LF/LR primer set, sequences other than that of this species are frequently found, probably due to hyperparasitism of other species. There are numerous situations in which hyperparasitism occurred during the experiments (Fig. 3). Considering that the LF/LR primer set can be used effectively in most Hymenoptera species, the presence of hyperparasitism can be identified easily by examining the larvae and pupae of ichneumonid species in this way. On the other hand, caution is needed when judging hyperparasitism because epicuticles constituting cocoons may contain the tissues of other insect species.

Overall, the host species of *P. disparis* and *T. a. gestator* could be predicted by amplifying of the host genomic DNA extracted from the gut tissues. The hosts for many ichneumonids are well-known (e.g., Yu et al., 2016). While these data are vast, the reports for some taxa were constructed based solely on observations of only a few cases. Moreover, most reports contain few precise findings of geographic variations or host differences between related species. With the introduction of MAPL, the information might be more extensive and accurate. On the other hand, the construction of a gene information bank of host species that can be identified only by genetic analysis will be needed.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

Brodeur J, 2012. Host specificity in biological control: insights from opportunistic pathogens. Evolutionary Applications, 5:470-480. https://doi.org/10.1111/j.1752-4571.2012.00273.x

Cardinal S, Danforth BN, 2011. The antiquity and evolutionary history of social behavior in bees. PLoS ONE, 6:e21086. https://doi.org/10.1371/journal.pone.0021086

Day WH, 1994. Estimating mortality caused by parasites and diseases of insects: comparisons of the dissection and rearing methods. Environmental Entomology, 23:543-550. https://doi.org/10.1093/ee/23.3.543

Forbes AA, Bagley RK, Beer MA, Hippee AC, Widmayer HA, 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. BMC Ecology, 18:21. https://doi.org/10.1186/s12898-018-0176-x

Funk DJ, Bernays EA, 2001. Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. Ecology, 82:726-739. https://doi.org/10.1890/0012-

9658(2001)082[0726:GVIHSR]2.0.CO;2

Gaston KJ, 1991. The magnitude of global insect species richness. Conservation Biology, 5:283-296. https://doi.org/10.1111/j.1523-1739.1991.tb00140.x

Gauld ID, 1984. An introduction to the Ichneumonidae of Australia. British Museum (Natural History), London, pp. 1-420.

Godfray HCJ, 1994. Parasitoids, behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ, pp. 1-473.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN, 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America, 103:968-971. https://doi.org/10.1073/pnas.0510466103

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM, 2004. Identification of birds through DNA barcodes. PLoS Biology, 2:e312. https://doi.org/10.1371/journal.pbio.0020312

Hopper KR, Prager SM, Heimpel GE, 2013. Is parasitoid acceptance of different host species dynamic? Functional Ecology, 27:1201-1211. https://doi.org/10.1111/1365-2435.12107

Huber JT, 2009. Biodiversity of Hymenoptera. In: Insect biodiversity: science and society (Eds., Foottit RG, Adler PH). Blackwell Publishing, Hoboken, NJ, pp. 303-323. https://doi.org/10.1002/9781444308211.ch12

Kodani J, Togashi K, 1992. Leaf expansion and shoot elongation of *Cornus controversa* Hemsley. Japanese Journal of Ecology, 42:115-123.

Königsmann E, 1978. Das phylogenetische system der Hymenoptera. Teil 3: 'Terebrantes' (Unterordnung Apocrita). Deutsche Entomologische Zeitschrift, 25:1-55. https://doi.org/10.1002/mmnd.19780250102

Lachaud JP, Pérez-Lachaud G, 2012. Diversity of species and behavior of hymenopteran parasitoids of ants: a review, Psyche, 2012:134746. https://doi.org/10.1155/2012/134746

Pilgrim EM, von Dohlen CD, Pitts JP, 2008. Molecular phylogenetics of Vespoidea indicate paraphyly of the superfamily and novel relationships of its component families and subfamilies. Zoologica Scripta, 37:539-560. https://doi.org/10.1111/j.1463-6409.2008.00340.x

Quicke DLJ, 2015. The Braconid and Ichneumonid parasitoid wasps: biology, systematics, evolution and ecology. John Wiley & Sons, New York, pp. 1-681. https://doi.org/10.1002/9781118907085

Rougerie R, Smith MA, Fernandez-Triana J, Lopez-Vaamonde C, Ratnasingham S, Hebert PDN, 2011. Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. Molecular Ecology, 20:179-186. https://doi.org/10.1111/j.1365-294X.2010.04918.x

Saunders TE, Ward DF, 2018. Variation in the diversity and richness of parasitoid wasps based on sampling effort. PeerJ, 6:e4642. https://doi.org/10.7717/peerj.4642

Takasuka K, Fritzén NR, Tanaka Y, Matsumoto R, Maeto K, Shaw MR, 2018. The changing use of the ovipositor in host shifts by ichneumonid ectoparasitoids of spiders (Hymenoptera, Ichneumonidae, Pimplinae). Parasite, 25:17. https://doi.org/10.1051/parasite/2018011

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30:2725-2729. https://doi.org/10.1093/molbev/mst197

Vilhelmsen L, Turrisi GF, 2011. Per arborem ad astra: morphological adaptations to exploiting the woody habitat in the early evolution of Hymenoptera. Arthropod Structure and Development, 40:2-20. https://doi.org/10.1016/j.asd.2010.10.001

Wyckhuys KAG, Graziosi I, Burra DD, Walter AJ, 2017. Phytoplasma infection of a tropical root crop triggers bottom-up cascades by favoring generalist over specialist herbivores. PLoS ONE, 12:e0182766. https://doi.org/10.1371/journal.pone.0182766

Yu DS, Van Achterberg C, Horstmann K, 2016. Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive [Internet]. Dicky Sick Ki Yu, Ottawa, Accessed 1 Jan 2016, <http://www.taxapad.com>

Zhao ZH, Liu JH, He DH, Guan XQ, Liu WH, 2013. Species composition and diversity of parasitoids and hyper-parasitoids in different wheat agro-farming systems. Journal of Insect Science, 13:162. https://doi.org/10.1673/031.013.16201

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