



FIRST RECORD OF *POLISTES WATTII* CAMERON, 1900 (HYMENOPTERA: VESPIDAE) FROM DISTRICT BHIMBER, AZAD KASHMIR

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ABSTRACT

Wasps are important component of our ecosystem as they act as bio-control agents, pests, pollinators, and predators of caterpillars. Members of genus *Polistes* are distributed worldwide. In current study we did molecular identification and phylogenetic analysis of paper wasps collected from District Bhimber, Azad Kashmir. On the basis of morphological characterization the specimens were identified as *Polistes wattii*. Molecular identification on the basis of SS rDNA showed 99% homology with other species of genus *Polistes*. Gene bank data shows that the SS rDNA of *Polistes wattii* has not been reported previously. Phylogenetic analysis clearly separated the *Polistes wattii* from other species of genus *Polistes* available in the Gene bank.

Keywords: *Polistes wattii*, SS rDNA, wasps, molecular identification, phylogenetic analysis.

INTRODUCTION

Wasps (Hymenoptera: Vespidae) are commonly known as potter wasps, hornets, yellow jackets or paper wasps. They are the main predators of the larvae of insect pests that feed on a variety of agricultural crops (Das and Gupta, 1989). Vespidae is divided into six subfamilies i.e., Eumeninae, Euparagiinae, Mesarinae, Polistinae, Stenogastrinae and Vespinae (Carpenter, 1982; Pickett and Carpenter, 2010). These wasps are solitary, social and eusocial. There are 900 species of Polistinae (paper wasps) all over the world representing 25 genera (Pickett and Carpenter, 2010). Genus *Polistes* is the most abundant among social vespids and found all around the world (Lawison *et al.*, 1961; Gillaspay, 1979; Kishore *et al.*, 2012). About 218 species of genus *Polistes* has been described in tropical and subtropical regions (Pickett and Carpenter, 2010). They are also important pollinators. Morphological identifications are not only time consuming but also challenging due to several reasons (Evans *et al.*, 2007; Huang *et al.*, 2007; Tahir *et al.*, 2018). Therefore, modern taxonomists are using molecular methods to resolve the problems associated with traditional morphological method. Use of molecular methods has greatly accelerated the pace of species identification and their phylogenetic relationships. SS rDNA sequence is highly conserved and commonly used for phylogenetic analysis of wasp (Hines *et al.*, 2007; Brady *et al.*, 2006; Mallatt and Giribet, 2006). Aim

of current study was to identify paper wasps collected from Bhimber, Azad Kashmir on the basis of morphometric characters and analyze its phylogenetic relationship using SS rDNA.

MATERIALS AND METHODS

Wasps were collected in May 2017 from Bhimber, Azad Jammu and Kashmir (32.882527, 74.232256) by using hand net. All life stages (i.e. larvae, pupae and adults) were collected. Samples were brought to Department of Zoology, GC University Lahore for further studies. The information regarding site of collection, date of collection, collector name and other notes of importance were recorded. Keys provided by Siddiqui *et al.* (2015) and Carpenter and Nguyen (2003) were consulted for species level identification. Molecular characterization of wasp specimens was accomplished by partial amplification of SS rDNA. For this, genomic DNA was extracted from part of legs of wasps by using Thermo Scientific Gene JET Genomic DNA Purification kit following instructions provided by manufacturers (Crary, 2012). The SS rDNA was amplified using rc18A and 18N primers (Wiegmann *et al.*, 2000). The PCR mixture consisted of 15 μ l of 2X Amp Master TM taq, 1 μ l of forward primer (10pmol/ μ l), 1 μ l of reverse primer (10pmol/ μ l), 2 μ l of genomic DNA (80ng/ μ l) and 11 μ l of molecular grade water. Total volume of reaction mixture was 30 μ l. PCR reaction

conditions include the initial DNA denaturation at 94°C for 5 min followed by 35 cycles each with denaturation at 94°C for 50 seconds, annealing at 43°C for 55 seconds and elongation at 72°C for 50 seconds. Final elongation step was set at 72 °C for 10 min. Amplified PCR product was sent for sequencing to 1st BASE Malaysia. Quality of sequencing was analyzed using Chromas Lite software. Neighbor joining tree was constructed using MEGA 6 software.

RESULTS

Morphological characterization

All collected wasps belonged to family Vespidae. Adults showed prominent subfamily characters including simple claws (Fig. 1a), absence of parategula (Fig. 1b) and hind wing with jugal lobe (Fig. 1c) (Siddiqui *et al.*, 2015). Genus level characters include funnel shaped first metasomal segment that rounded smoothly from the lateral sides (Fig. 1d) and propodeum with orifice which is dorsally narrowed (Carpenter & Nugyen, 2003). Species level identification showed that isolated wasp belongs to *Polistes wattii* Cameron, 1900. Species level characters were the presence of fine holes on the clypeus which was totally punctate (Fig. 1e), transverse striations of propodeum were weak (Fig. 1f), metapleuron marked with scattered fine punctures (Fig. 1g), ocellus enclosed with black mark (Fig. 1h), mesonotum brownish without distinct yellow stripes (Fig. 1i), metasoma brown and mixed with yellow colour (Siddiqui *et al.*, 2015).

DNA sequence analysis

A sequence of 770bp of SS rDNA was recovered. In the Neighbour joining tree the sequence of *Polistes wattii* was clearly separated from other reported sequences indicating a different species. The gene sequence of 18S of *Polistes wattii* was submitted in the GeneBank database under the accession number MF347998. It is evident from the tree that *Polistes wattii* has closest evolutionary relationships with *Polistes fuscatus*. Both species showed 99% homology in the amplified region of 18SrRNA gene (Fig. 2).

DISCUSSION

The current study was designed to identify yellow wasp from Bhimber, Azad Kashmir for the first time. Phylogenetics consists of application of various methods to check the historical relationships among different taxa (Ward, 2010). Wasps are important insects due to their predatory or parasitic behavior because they act as buffer in the ecosystems where they live. Due to their feeding habit they also serve as pollinators of a large number of flowers (Barthelemy, 2010). Member of genus *Polistes* also act as bio-control agents *i.e.*, *Polistes olivaceus*. Locally present *Polistes* sp. help in

pollination. *Polistes wattii*, *Polistes rothneyi carletoni* and *Polistes olivaceus* are commonly found in Pakistan but these species are also found in other areas of the world like Iraq, India, China, Oman, Iran, Saudi Arabia, Mauritius, Afghanistan, Indonesia, Australia, New Caledonia, Chile, Myanmar, Cambodia, Malaysia, Egypt, Philippines, Madagascar, Singapore, Japan, Sri Lanka, Tanzania, Fiji, Thailand, Laos, Tonga, Vietnam, Hawaii, Australia and New Zealand (Siddiqui *et al.*, 2015). The morphological data indicate that subfamily Polistinae and Vespinae are closely related to each other because both have resemblance in many characters such as presence of ocelli, slender body, simple claws, absence of parategula, pointed clypeus etc. The traits of life and social organization depict that Politinae and Vespinae must have common ancestors (Hunt, 2006). The six subfamily cladogram given by Carpenter (1982) and Carpenter & Nugyen (2003) indicate that both subfamilies share a common ancestor which is Stenogastrinae.

Genus *Polistes* differ from other genera of subfamily Polestinae on basis of morphology. In members of genus *Eumenes* coloration of wasps varies from black to brown with yellow, orange, red or white markings. Their metasoma appears to be "bulbous" with the first metasomal segment narrow and elongated while the first metasomal segment of *Polistes* sp. are slender and form funnel shape structure. The morphological characters of *Polistes wattii* resemble with *Polistes olivaceus* having yellow colored body, large eyes, antennal segments, short scape etc. but *Polistes olivaceus* differ from *Polistes wattii* in having black markings on the thorax and mesopleuron (Siddiqui *et al.*, 2015).

The identification through morphological characters works well for distinct species but may be ambiguous for cryptic and sibling species (Anker *et al.*, 2007; Tavares and Baker, 2008). Very little work has been reported on the molecular characterization of local wasp species. Molecular identification is not only an easy approach for non-taxonomists but also explore the underlying genetic diversity of local wasps. Ward and Brady (2003) have reported the relationship of primitive ants of genera *Myrmecia* and *Nothomyrmecia* on the basis of SS rDNA. Nucleotide sequence analysis of *Polistes wattii* shows 99% homology with *Polistes fuscatus* and *polistes snelleni*. In phylogenetic analysis it appears as a close homologue of other members of genus *Polistes*.

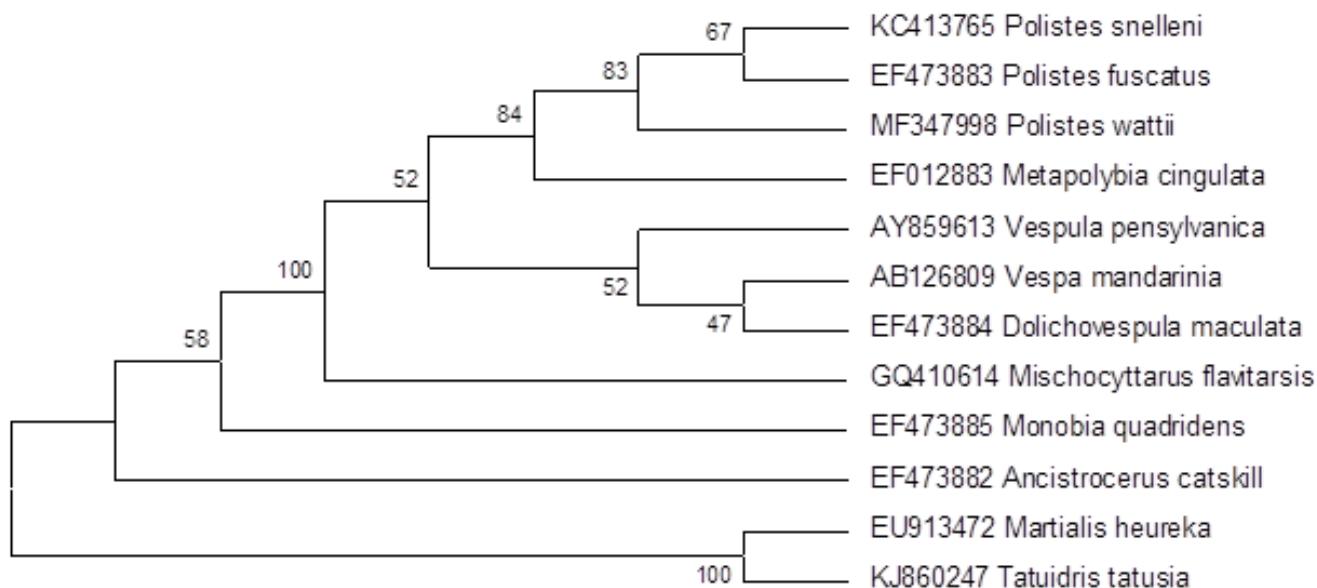
Authors' contributions

MTZ designed the study and helped in collection of samples, RF performed experimental work and HMT helped in drafting, data analysis and editing.



Fig. 1.

Morphological characterization of *Polites wattii*. a) Claws of adult, b) Parategula absent in thorax region, c) Hind wings having jugal lobe, d) Propodeal orifice dorsally narrowed and first metasomal segment is funnel shaped, e) head showing deep punctures in clypeus, f) Propodeum showing weak striations, g) Metapleuron have deep punctures, h) Prominent ocelli enclosed in black marks and mesonotum brown in colour , i) Metasoma yellow and brown in color.

**Fig. 2**

Phylogenetic tree of *Polistes* species using SS rDNA based on Neighbour-Joining method using MEGA6 (Tamura *et al.*, 2013). *P. wattii* shows a close homology with *P. fuscatus*

REFERENCES

- Anker, A., C. Hurt and N. Knowlton, 2007. Revision of the *Alpheus nuttingi* (Schmitt) species complex (Crustacea: Decapoda: Alpheidae), with description of a new species from the tropical eastern Pacific. Zootaxa, 1577: 41-60.
- Barthelemy, C., 2010. A provisional identification guide to the social vespids of Hong Kong (Hymenoptera: Vespidae). Pp. 1-132.
- Brady, S.G., T.R. Schultz, B.L. Fisher and P.S. Ward, 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Natl. Acad. Sci., 103(48): 18172-18177.
- Carpenter, J.M., 1982. The phylogenetic relationships and natural classification of the Vespoidea (Hymenoptera). Syst. Entomol., 7(1): 11-38.
- Carpenter, J.M. and L.P.T. Nguyen, 2003. Keys to the genera of social wasps of South-east Asia (Hymenoptera: Vespidae). Entomol. Sci., 6(3): 183-192.
- Crary, M.J., 2012. Genetic variability and its relationship with *Acanthamoeba pathogenesis*. Columbus, Ohio; The Ohio state University.
- Das, B.P. and V.K. Gupta, 1989. The social wasps of India and the adjacent countries (Hymenoptera: Vespidae): an illustrated account of the vespid fauna of the Indian subregion. Oriental Insects, 23(1):389.
- Evans, K.M., A.H. Wortley and D.G. Mann, 2007. An assessment of potential diatom "barcode" genes (cox1, rbcL, 18S and ITS rDNA) and their effectiveness in determining relationships in Sellaphora (Bacillariophyta). Protist., 158(3): 349-364.
- Gillaspy, J.E., 1979. Management of *Polistes* wasps for caterpillar predation. Southwestern Entomol., 4:334-350.
- Hines, H. M., J.H. Hunt, T.K. O'Connor, J.J. Gillespie and S.A. Cameron, 2007. Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proc. Natl. Acad. Sci., 104(9): 3295-3299.
- Huang, J., Q. Xu, Z.J. Sun, G.L. Tang and Z.Y. Su, 2007. Identifying earthworms through DNA barcodes. Pedobiol., 51(4): 301-309.
- Hunt, J.H., 2006. Evolution of castes in *Polistes*. In *Annales Zoologici Fennici* (pp. 407-422). Finnish Zoological and Botanical Publishing Board. 407-422p.
- Kishore, L., K.M. Shareef and P.G. Kumar, 2012. New record of *Polistes (Polistella) strigosus* Bequaert (Hymenoptera: Vespidae: Polistinae) from South India. Biol. Forum-An Int. J., 4(2): 8-9.
- Lawson, F.R., R.L. Rabb, F.E. Guthrie and T.G. Bowery, 1961. Studies of an integrated control system for hornworms on tobacco. J. Econ. Entomol., 54(1): 93-97.
- Mallatt, J. and G. Giribet, 2006. Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. Mol. Phylogeny. Evol., 40(3): 772-794.
- Pickett, K.M. and J.M. Carpenter, 2010. Simultaneous analysis and the origin of eusociality in the Vespidae (Insecta: Hymenoptera). Arthropod Syst. Phylog., 68(1): 3-33.

- Siddiqui, J.A., I. Bodlah, J.M. Carpenter, M. Naeem, M., Ahmad and M.A. Bodlah, 2015. Vespidae (Hymenoptera) of the Pothwar region of Punjab, Pakistan. Zootaxa, 3914(5): 501-524.
- Tahir, H.M., A. Noor, S. Mehmood, S.M. Sherawat and M.A. Qazi, 2018. Evaluating the accuracy of morphological identification of insect pests of rice crops using DNA barcoding, Mitochondrial DNA Part B, 3:2, 1220-1224.
- Ward, P.S. and S.G. Brady, 2003. Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). Inver. Syst., 17(3): 361-386.
- Wiegmann, B.M., C. Mitter, J.C. Regier, T.P. Friedlander, D.M. Wagner and E.S. Nielsen, 2000. Nuclear genes resolve Mesozoic-aged divergences in the insect order Lepidoptera. Mol. Phylogenetic Evol., 242-259.