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**Short Communication** 

## Phylogenetic Reconstruction of *Ropalida* brevita (Vespidae: Polistinae) using 12S rRNA and Cytochrome Oxidase 1 (*Co1*) Genes

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#### ABSTRACT

A total of 67 samples of *Ropalida brevita* were collected from District Abbottabad, Pakistan during 2011 to 2013. Species was confirmed by taxonomic key, which are belonging to subfamily of Polistinae. Phylogenetic tree were reconstructed using 12 SrRNA (12S) gene showing homology of 73% compared with gene bank data while on the basis of cytochrome oxidase 1 (*Co1*) gene 93% homology were recorded. It was concluded that *Co1* gene is more reliable than 12S rRNA gene for confirmation of *R. brevita* position in the Vespidae family.

Cocial wasps of Indian subcontinent and South-East Asia are an important place for diversity of social wasps (Carpenter, 2003; Kojima et al., 2007). Adult wasps are fascinating, generally predominantly black or brown but are often extensively have yellow or white marking (Goulet and Huber, 1993) which play an important role in the functioning of ecosystems nearly everywhere around the globe *i.e.* they destroy pests of cultivated and ornamental plants (Abbasi et al., 2006; Fateryga, 2009), whereas some are scavengers and even others steal prey of other wasps for their young ones (Richter, 1990). Member of the genus Polistes store nectar in their nests for feeding their larvae (Grinfeld, 1977), some wasps of the subfamilies Eumeninae, Polistinae and Vespinae also consume pollen (Hunt et al., 1991). Recently, Santos et al. (2014) carried out the phylogeny of the paper wasp genus Polistes is investigated using six genes (COI, 12S, 16S, 28S, H3, and EF1-a). The results of this investigation showing the evolutionary hypotheses about the genus: that Polistes first evolved in Southeast Asia and then

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#### Authors' Contributions

MS designed the study and collected the samples. MSK and MAR provided lab facilities and access to the National Insect Museum, Islamabd. HA and JMC provided molecular lab facilities including sequencing and analyzed the data. MS wrote the article.

Key words

Wasps, Phylogenic tree, Co1 gene, 12SrRNA, Ropalida brevita.

dispersal to the New World; while, Siddique *et al.* (2015) reported nineteen (19) species of thethree subfamilies of family Vespidae. Some studies have been carried out on this important family (for example Chaudhry *et al.*, 1966; Das and Gupta, 1989; Gusenleitner, 2006, 2008; Dvorak, 2007); Bodlah, (2012; Faiz, 2012); Mahmood *et al.*, 2012; Shah *et al.*, 2013; Perveen and Shah, 2013). The present research work is based on the molecular study of *Ropalida brevita* species from the genus *Ropalida* of the Subfamily Polistinae of family Vespidae using 12S rRNA gene and Cytochrome 1 (*Co1*) gene of collected from Abbottabad, Pakistan.

#### *Materials and methods*

Wasp collection were carried out during the active season of 2011 to 2013 from district Abbottabad by aerial net from fields, gardens and other vegetation and preserved in 95% ethanol. Specimens were identified up to species level with the help of stereoscope (Labomet CZM4-4X) by running them through keys (*i.e.* Ebrahimi and Carpenter, 2008; Goulet and Huber, 1993). Isolation of DNA from dried wasps was carried out by using Qiagen kit with spin columns with modification in the procedure provided by the company.

The isolated DNA was used as template and 12s and Cytochrome oxidase (*Co1*) primers were used for amplification of target gene. The PCR results were verified on agarose gel. Data was analyzed by software "DNAMAN" version 5.2.2.0.

#### Results and discussion

Collected samples were identified up to species level with help of available literature. Results of the PCR amplified product for 12S rRNA with 430bp whereas *Col*  locus shows 650bp. Homology during the comparison of *Ropalida brevita* species using 12S rRNA gene and Cytochrome oxidase 1 (*Co1*) showing their position in the homology tree was constructed, where it showing its position in subfamily Polistinae 93% on *Co1*, 77% on 12SrRNA locus with clear position of the genera *Ropalidii* from *Polistes* (Fig. 1). The results also show the genetic diversity of *Ropalida brevita* in the tree with 0.116 on 12S rRNA, while 0.067 on *Co1* locus (Fig. 2).

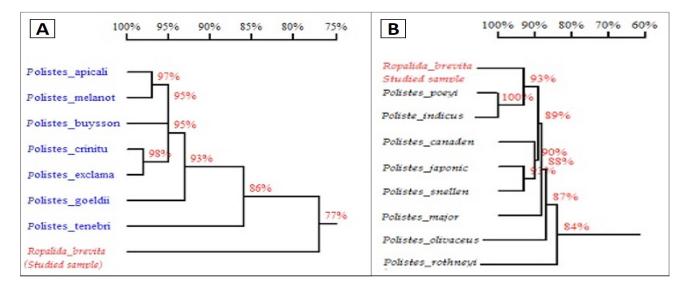


Fig. 1. Homolgy tree based on partial sequences of 12SrRNA locus (A) and cytochrome oxidase (*Co1*) gene (B) by using DNAMAN software version 5.2.2.

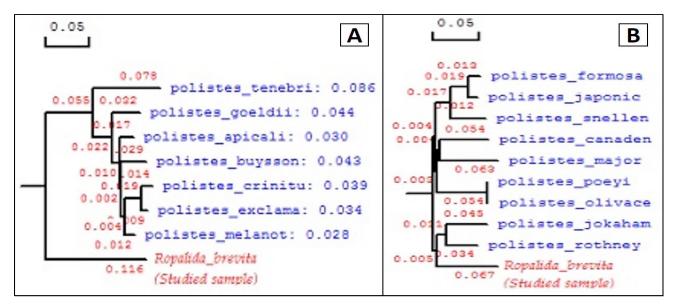


Fig. 2. DNA sequences analysis of *Ropalida brevita* for 12S rRNA locus (A) and cytochrome oxidase (*Co1*) gene (B) aligned with other species of subfamily *Polistinae*.

Ropalida brevita was collected from three localities Ayubia, Havelian and Kakul of Abbottabad district and identified at species level representing first time reported from Abbottabad belonging from genus Ropalidii, Subfamily Polistinae, family Vespidae. The species of subfamily Polistinae mostly found in tropical regions, cosmopolitan in distribution with more than 900 species under 29 genera world-wide (Carpenter and Rasnitsyn, 1990; Dubatolov and Milko, 2004). Genus Polistes Latrielle is the only genus under tribe Polistinii with its 200 species worldwide. It is Palearctic and Afrotropical genus but also occurs in Oriental Region. Polistes gallicus, was reported by Das and Gupta (1989) from Pakistan and India. Gusenleitner (2007) reported this species from Jaglot and Ghizar valley. Later, Aziz (2008) and Mahmood et al. (2012) reported this species from Skardu; while, Faiz (2012) reported this specie from Gilgit (Jaglot, Jalal Abad), Ghizer and Hunza-Nagar. According to Santos et al. (2014), Polistes first evolved in Southeast Asia and 2<sup>nd</sup> that dispersal to the New World occurred only once, and 3<sup>rd</sup> that long-term monogyny evolved as an adaptation to overwintering in a temperate climate. So this study confirms that this oriental region is the origin source from where these species evolved.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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