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Research Article

Molecular characterisation of some Indian anthocorid predators

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ABSTRACT: Anthocorid predators widely used as biocontrol agents were characterised for genetic diversity and phylogeny. *Cardiastethus exiguus* Poppius, *Xylocoris flavipes* Reuter and *Blaptostethus pallescens* Poppius were characterised using Cytochrome oxidase I (COI) marker to elucidate the diversity and phylogeny. The sequencing of the COI region at 650bp was compared with sequence of *Orius majusculus* (Reuter), available at NCBI by BLASTn. The cluster analysis based on dendogram revealed *B. pallescens* and *O. majusculus* to be more closely related, while *X. flavipes* and *C. exiguus* were distinct. The usefulness of characterisation and phylogenetic relationship among the anthocorid populations for their effective utilisation in pest management is discussed.

KEY WORDS: Anthocorids, COI, PCR, phylogeny, predators

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INTRODUCTION

Anthocorid bugs (Anthocoridae:Hemiptera) commonly referred as flower bugs or pirate bugs consists of 400-600 species in 80-100 genera, distributed worldwide (David Horton, 2008, Nasser and Abdurahiman, 2004 and Sohrabi *et al.,* 2013). The predatory bugs are commonly used as biocontrol agents. Nymphs and adults are predatory on thrips, aphids, psyllids, scale insects, small caterpillars and mites (Lattin and Stanton, 1992, Saulich and Musolin, 2009, Ballal *et al*., 2012) in many crop ecosystems such as corn, alfalfa, cotton, coconut, sweet pepper, cucumber, forest crops, green house crops and ornamentals (Brower and Muller, 1990, Chambers *et al.,* 1993., Lorenzana *et al*., 2010, Nian wan Yang, *et al.,* 2014) and granaries (Sing and Arbogast, 2008 and Kaur and Jaspal, 2011). *Blaptostethus pallescens* and *Anthocoris muraleedharani* predate on cotton mealybug and consumed 141 crawlers while *B. pallescens* could predate on 35 crawlers (Ballal *et.al.,* 2012)

The molecular diversity of the common predatory anthocorids in India have been less studied and poorly understood. The internal transcribed spacer 1 (ITS-1) and its flanking rDNA genes and molecular markers were utilized to characterize the species earlier (Honda *et al.,* 1998, Hinomoto *et al.,* 2004., Muraji *et al.,* 2004 and Sayed *et al.,* 2012). Accurate identification of natural enemies, understanding their population dynamics, separation of strains, evaluation of genetic diversity, phylogeny, predator –prey association can be studied with molecular diagnostic tools (Unruh and Woolley, 1999, Menalled *et al*., 2004 and Greenstone, 2006). In the present study, the anthocorid predators *Cardiastethus exiguus* Poppius, *Xylocoris flavipes* Reuter and *Blaptostethus pallescens* Poppius were characterised using Cytochrome oxidase I (COI) marker to elucidate the diversity and phylogeny. In India, *C. exiguus* is an important predator of coconut blackheaded caterpillar, *Opisina arenosella* Walker (Ballal *et al*., 2003a and Lyla *et al*., 2006) while, *B. pallescens* was recorded as predating on spider mites and moth pests in storage (Ballal *et al.,* 2003b., Kaur and Virk, 2011). *X. flavipes* was recorded as an important predator of moth pests in storage (Ballal *et al*., 2013).

MATERIALS AND METHODS

Mass rearing of the predator

In vitro rearing of the anthocorid predators was earlier reported by Ballal *et al*., (2003a), Gupta and Ballal (2009)., Ballal *et al*., (2012), Sigsgaard and Esbjerg (1997), Schmidt et.al., (1995) and Sobhy *et al*., (2014). The anthocorid predators considered for study were obtained from mass production laboratory, at ICAR-NBAIR, where the predators were maintained on UV irradiated eggs of the rice moth *Corcyra cephalonica* Stainton. The nymphs and adults were utilised for molecular characterisation. Three populations of anthocorid predators were used for the study *viz., C. ex-*

iguus (National Accession No. NBAIR- MP-ANT-01), *B. pallescens* (NAN. NBAIR- MP-ANT-04) and *X. flavipes* (NAN. NBAIR- MP-ANT-05).

Characterisation of the predators

The protocol prescribed by Muraji *et al*., (2004) was followed. The individual adult predators were hyomogenised in 100 µl lysis buffer (200mM Tris HCl; pH 8.0, 70mM EDTA; 2M Sodium chloride; 20mM Sodium metabisulphite), (5% Chelex-100) and 4 µl of proteinase K (20 µg/ µl) in 1.5 ml tube. The solution was vortexed for 45 seconds and incubated for 6 hours at 56 °C in a waterbath followed by 95 °C for 10 minutes and then centrifuged at 10,000 rpm for 10 minutes. DNA in the supernatant was measured in Spectrophotometer (Beckman DU-640) at 280nm and then used for PCR.

Five adults of each population were utilised for molecular characterisation and there were three replicates.

PCR amplification of the CO1 gene of mitochondrial DNA

PCR was carried out in 0.2 ml microcentrifuge tubes. Amplification of the CO1 gene region was done with forward primer 5^1 ------3^1 and reverse primer 5^1 ------3^1. PCR was performed in 50 µl reaction volumes using 5µl of 10X Taq assay buffer, 1 µl dNTP's each in 10 Mm concentration, 1 µl forward and reverse primer (10 pmol/l), 0.25 µl Taq polymerase (1U/µl) and 5 µl of template DNA. The cycling conditions for initial denaturation was 94°C for 3 minutes followed by denaturation at 94°C for 1 minute by 30 cycles followed by annealing at 45 °C for 1 minute and extension at 72 °C for 5 minutes with final extension at 72 °C for 10 minutes. PCR product was electrophoresed on a 1.8% low melting point agarose gel in TAE (40mM Tris mM acetic acid) and the gel was stained with ethidium bromide. Molecular weight ladder of 50 bp was used as a marker along with the sample. The PCR product was purified from gel with spin column prescribed in the kit.

DNA sequencing of the PCR product

DNA fragments were extracted from the gel using the Qiagen kit. PCR product of the region was sequenced directly with the corresponding PCR primers on both strands and the partial sequence for CO1 gene sequencing was determined using a ABI PRISM 310 BigDye Terminator cycle sequencing kit.

Multiple Sequence Alignment (MSA)

MSA provides for alignment of more sequences to infer the evolutionary relationship between them. Pairwise sequence alignment tools were used to identify the regions of similarity for structural, functional and evolutionary relationships. CLUSTAL W and BLASTn were used.

RESULTS AND DISCUSSION

Molecular analyses conducted in this study allowed discrimination among the common anthocorid predators, based on the different sizes of the amplified DNA fragments that characterize each species. The sequencing of the COI region at 650bp (Table 1) was compared with sequences of NCBI by BLASTn. Similarities or dissimilarities were determined by Pair wise alignment and multiple alignments using Clustal W (Table 2). The CO1 region is useful for inferring phylogenetic relationships among the populations and a tool to generate barcode. The differences in the length and sequence of the flanking region fragments would identify genetic variability. COI revealed variability among the different predators (Fig. 1). Variability in anthocorid predators was earlier reported by Honda *et al*., 1998, Hinomoto *et al*., 2004 and Muraji *et al*., 2004. Relative relationship of different anthocorid predators was also indicated by several workers (Saitou *et al*., 1987., Schuh *et al.,* 1991 and Jung *et al*., 2010). The cluster analysis based on dendogram revealed *B. pallescens* and *O. majusculus* to be closely related, while *X. flavipes* and *C. exiguus* were distinct (Fig. 2). Similar observations were made by in the species of *Orius,* using the internal transcribed spacer 1 (ITS1) and its flanking rDNA genes to infer the relationship Gomez *et al.,* 2013 and Sayed *et al.,* (2013). Phylogenetic classifications can be of value for predicting life history data for groups with incompletely known biology and determine whether or not environmental-associated variations contribute to the fitness attributes and behavioural approaches of the organism. In biological control programmes, divergence among cryptic species is essential to understand the effect of local mate competition for the co-existence of the species and their ability to bring down insect populations. Intra guild predation, predator prey population dynamics and trophic interactions could be well studied based on the observations made, for effective use of these anthocorids in pest management.

Table 1. COI gene sequence for anthocorid predators

Sl. No.	Anthocorid	Sequence
1	*Xylocoris flavipes*	TATATTTTATATTCGGAATATGAGCAGGAATAGTAGGAACATCATTAATTGAATTATTCGAATTGAACTAGGACAACCAGGAGCATTCATTGGAGGATCAAATTTATAATGTAGTAGTCACAGCACACGCATTCATTATAATTTTTTTATAGTTATACCAATTATAATTGGAGGATTCGGAAACTGATTAGTGCCATTAATAATTGGAGCTCCTGATATGGCATTCCCTCGAATAAATAATATAAGATTTTGACTTTTACCTCCTTCATTAACTCTATTAATTGCCAGATCAACTGTAGAAAGAGGAGCGGGTACAGGATGAACTGTTTATCCTCCCCTATCAGCTAATATTGCACATAGAGGAGCATCAGTAGATTTAGCAATTTTCTCACTACACCTAGCAGGTGTGTCATCAATTCTAGGTGCAATCAATTTTATTTCAACAATCATTAATATACGACCTGAGGGGATAACAGCTGAACGTATTCCTTTATTCGTTTGATCAGTAGGAATTACTGCATTACTATTATTAATTTCATTACCAGTATTAGCTGGTGCCATCACAATACTACTAACAGATCGAAATTTTAATACATCCTTCTTTGACCCGAAGGAATAACAGCAGAGCGAATTCCTTTATTTGTATGATCAGTAGGAATTACTGCACTATTACTATTATTATCACTACCAGTATTAGCAGGAGCTATCACAATACTTTTAACAGATCGAAACTTTAACACATCATTCTTTGACCCAGTAGGAGGGGGGG
2	*Blaptostethus pallescens*	ACGCAGGAGGATTTGGAAATTGATTAGTTCCTTTAATAATTGGAGCACCAGATATAGCATTTCCTCGAATAAACAATATAAGTTTTTGATTATTACCTCCATCAATTACATTATTAATCAGTAGATCTTTAGTAGAAAGAGGAGTAGGTACAGGATGAACTGTATATCCTCCATTATCTAGAAATATTGCTCATAGAGGAGCATCAGTAGACTTAGCAATTTTTTCATTACATCTAGCAGGAGTATCTTCAATTTTAGGAGCTATTAATTTTATTTCAACAATTATTAATATACGACCAGAAGGTATAACATCTGAACGAATTCCTTTATTTGTATGATCTGAAGGAATTACAGCTTTATTACTACTTTTATCTCTACCAGTATTAGCTGGAGCTATTACTATATTGTTAACAGATCGAAATTTTAATACTACTTTCTTTGACCCAGCAGGAGGAGGAGAGATCCAATTGGATACAGGACGTGGAGATGATACCGAACGGATTAA
3	*Cardiastethus exiguus*	TGGGAGGATTTGAAATTGATTAGTTCCTTTAATAATTGGAGCACCAGACATAGCATTTCCTCGAATAAACAATATAAGTTTTTGATTATTACCTCCATCAATTACATTATTAATCAGTAGATCTTTAGTAGAAAGAGGAGTAGGTACAGGATGAACTGTATATCCTCCATTATCTAGAAATATTGCTCATAGAGGAGCATCAGTAGACTTAGCAATTTTTTCATTACATCTAGCAGGAGTATCTTCAATTTTAGGAGCTATTAATTTTATTTCAACAATTATTAATATACGACCAGAAGGTATAACATCTGAACGAATTCCTTTATTTGTATGATCTGTAGGAATTACAGCTTTATTACTACTTTTATCTTTACCAGTATTAGCTGGAGCTATTACTATATTGTTAACAGATCGAAATTTTAATACTACTTTCTTTGACCCAGCAGGAGGAGGAGATCCAATTGGATACAGAACGTGGAGTTGATACTGAACGGAATT
4	*Orius majusculus*	ATATGAGCAGGAATATTAGGAACATCATTAAGATGAATTATTCGAATTGAACTAGGACAACCAGGATCATTCATTGGAGATGATCAAATTTATAATGTTGTAGTTACAGCACATGCATTTATTATAATTTTTTTTATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATAATTGGAGCACCAGATATAGCATTCCCACGAATAAATAATATAAGATTTTGACTTTTACCTCCATCAATTACATTACTTATTATAAGATCTATAGTTGAAAGAGGAGCAGGAACTGGATGAACAGTATATCCACCTCTTTCAGCTAATATTGCCCATAGAGGAGCATCAGTAGATTTAGCAATCTTTTCTTTACATTTAGCAGGTGTTTCATCAATTCTAGGAGCAATTAATTTTATTTCAACAATTATTAATATACGACCTGAAGGTATAACTGCAGAACGTATTCCATTATTTGTTTGATCAGTTGGAATTACTGCACTACTACTATTATTATCATTACCAGTATTAGCAGGAGCAATTACAATATTACTAACAGATCGTAATTTTAATACATCATTTTTTGACCCGGTAGGGGGAGGGGACCCAA

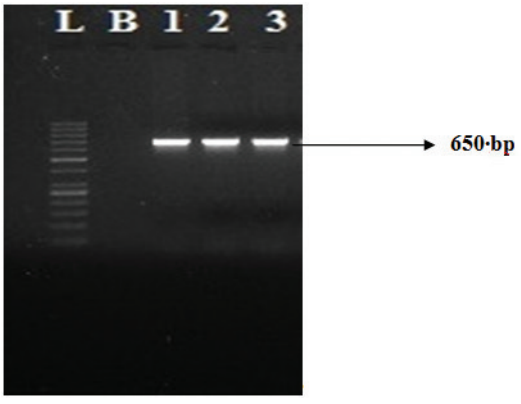

Fig. 1. Amplification of COI for anthocorid predators. (1- *Xylocoris flavipes*, 2- *Blaptostethus pallescens* and 3- *Cardiastethus exiguus*) (L- Ladder 650 bp, B- Blank)

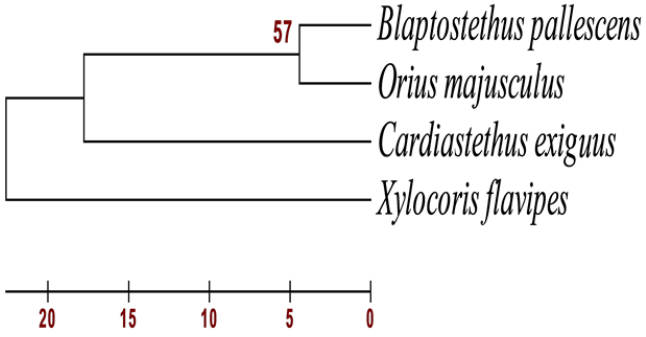

Fig. 2. UPGMA cluster analysis-based dendrogram depicting genetic relationships among different anthocorid predators (Generated by Boot strap test Phylogeny using N-J MEGA 4 SOFTWARE)

Table 2. Multiple Sequence Alignment for anthocorid predators

1	TCGGAAACTGATTAGTGCCATTAATAATTGGAGCTCCTGATATGGCATTCCCTCGAATAA	240
4	TTGGAAATTGATTAGTACCTTTAATAATTGGAGCACCAGATATAGCATTCCCACGAATAA	223
2	TTGGAAATTGATTAGTTCCTTTAATAATTGGAGCACCAGATATAGCATTTCCTCGAATAA	72
3	TTG-AAATTGATTAGTTCCTTTAATAATTGGAGCACCAGACATAGCATTTCCTCGAATAA	68
1	ATAATATAAGATTTTGACTTTTACCTCCTTCATTAACTCTATTAATTGCCAGATCAACTG	300
4	ATAATATAAGATTTTGACTTTTACCTCCATCAATTACATTACTTATTATAAGATCTATAG	283
2	ACAATATAAGTTTTTGATTATTACCTCCATCAATTACATTATTAATCAGTAGATCTTTAG	132
3	ACAATATAAGTTTTTGATTATTACCTCCATCAATTACATTATTAATCAGTAGATCTTTAG	128
1	TAGAAAGAGGAGCGGGTACAGGATGAACTGTTTATCCTCCCCTATCAGCTAATATTGCAC	360
4	TTGAAAGAGGAGCAGGAACTGGATGAACAGTATATCCACCTCTTTCAGCTAATATTGCCC	343
2	TAGAAAGAGGAGTAGGTACAGGATGAACTGTATATCCTCCATTATCTAGAAATATTGCTC	192
3	TAGAAAGAGGAGTAGGTACAGGATGAACTGTATATCCTCCATTATCTAGAAATATTGCTC	188
1	ATAGAGGAGCATCAGTAGATTTAGCAATTTTCTCACTACACCTAGCAGGTGTGTCATCAA	420
4	ATAGAGGAGCATCAGTAGATTTAGCAATCTTTTCTTTACATTTAGCAGGTGTTTCATCAA	403
2	ATAGAGGAGCATCAGTAGACTTAGCAATTTTTTCATTACATCTAGCAGGAGTATCTTCAA	252
3	ATAGAGGAGCATCAGTAGACTTAGCAATTTTTTCATTACATCTAGCAGGAGTATCTTCAA	248
1	TTCTAGGTGCAATCAATTTTATTTCAACAATCATTAATATACGACCTGAGGGGATAACAG	480
4	TTCTAGGAGCAATTAATTTTATTTCAACAATTATTAATATACGACCTGAAGGTATAACTG	463
2	TTTTAGGAGCTATTAATTTTATTTCAACAATTATTAATATACGACCAGAAGGTATAACAT	312
3	TTTTAGGAGCTATTAATTTTATTTCAACAATTATTAATATACGACCAGAAGGTATAACAT	308
1	CTGAACGTATTCCTTTATTCGTTTGATCAGTAGGAATTACTGCATTACTATTATTAATTT	540
4	CAGAACGTATTCCATTATTTGTTTGATCAGTTGGAATTACTGCACTACTACTATTATTAT	523
2	CTGAACGAATTCCTTTATTTGTATGATCTGAAGGAATTACAGCTTTATTACTACTTTTAT	372
3	CTGAACGAATTCCTTTATTTGTATGATCTGTAGGAATTACAGCTTTATTACTACTTTTAT	368
1	CATTACCAGTATTAGCTGGTGCCATCACAATACTACTAACAGATCGAAATTTTAATACAT	600
4	CATTACCAGTATTAGCAGGAGCAATTACAATATTACTAACAGATCGTAATTTTAATACAT	583
2	CTCTACCAGTATTAGCTGGAGCTATTACTATATTGTTAACAGATCGAAATTTTAATACTA	432
3	CTTTACCAGTATTAGCTGGAGCTATTACTATATTGTTAACAGATCGAAATTTTAATACTA	428

(1. *Xylocoris flavipes*, 2. *Blaptostethus pallescens*, 3. *Cardiastethus exiguus* and 4. *Orius majusculus*).

CONCLUSION

Cardiastethus exiguus Poppius, *Xylocoris flavipes* Reuter and *Blaptostethus pallescens* Poppius were characterised using Cytochrome oxidase I (COI) marker to elucidate the diversity and phylogeny.

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REFERENCES

Ballal CR, Singh SP, Poorani J, Tripti G. 2003a. Feasibility of mass multiplication and utilization of *Cardiastethus exiguus* Poppius, a potential anthocorid predator of *Opisina arenosella* Walker (Lepidoptera : Oecophoridae). pp. 29-33. In PL Tandon, CR Ballal, SK Jalali (Eds) 354 pp.

Ballal CR, Singh SP, Poorani J, Gupta T. 2003b. Biology and rearing requirement of an anthocorid predator *Blaptostethus pallescens* Poppius (Heteroptera: Anthcoridae). *J Biol Control.* **17**: 29–33.

Ballal CR, Tripti G, Sunil J. 2012. Predatory Potential of Two Indigenous Anthocorid Predators on *Phenacoccus solenopsis* Tinsley and *Paracoccus marginatus* Williams and Granara De Willink. *J Biol Control.* **26**: 18–22.

Ballal CR, Gupta T, Joshi S. 2013. Production and evaluation of the warehouse pirate bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae). *Proc the Int Congress on Insect Sci. 2013,* 14th to 17th February, 2013. GKVK, Bangalore.

Brower JH, Mullen MA. 1990. Effects of *Xylocoris flavipes* (Hemiptera: Anthocoridae) releases on moth populations in experimental peanut storages. *J Entomol Sci*. **25**: 268–276.

Chambers RJ, Long S, Helyer NL. 1993. Effectiveness of *Orius laevigatus* (Hem.: Anthocoridae) for the control

of *Frankliniella occidentalis* on cucumber and pepper in the UK. *Biocontl Sci Tech.* **3**: 295–307.

David HR. 2008. Minute pirate bugs (Hemiptera: Anthocoridae). *Encycl Entomol.* pp 2402–2412.

Gomez-Polo, Alomar P, Castane C, Riudavets J, Agusti N. 2013. Identification of *Orius* spp. (Hemiptera: Anthocoridae) in vegetable crops using molecular techniques. *Biol Control*. **67**: 440–445.

Gupta T, Chandish BR. 2009. Protocols for the commercial production of *Orius tantillus (*Motschulsky) (Hemiptera: Anthocoridae). *J Biol Control*. **23**: 385–391.

Greenstone MH. 2006. Molecular methods for assessing insect parasitism. *Bull Entomol Res.* **96**: 1–13.

Hinomoto N, Muraji M, Noda T, Shimizu T, Kawasaki K. 2004. Identification of five *Orius* species in Japan by multiplex polymerase chain reaction. *Biol Contl*. **31**: 276–279.

Honda JY, Nakashima Y, Yanase T, Kawarabata T, Hirose Y. 1998. Use of the internal transcribed spacer (ITS-1) region to infer *Orius* (Hemiptera: Anthocoridae) species phylogeny. *Appl Entomol Zool*. **33**: 567–571.

Jung S, Kim H, Yamada K, Lee S. 2010. Molecular phylogeny and evolutionary habitat transition of the flower bugs (Heteroptera: Anthocoridae). *Mol Phyl Evol.* **57**: 1173–1183.

Kaur R, Jaspal SV. 2011. Role of *Blaptostethus pallescens* Poppius and *Xylocoris flavipes* (Reuter) in the suppression of *Corcyra cephalonica* Stainton in stored rice grain. *J Biol Control.* **25**: 329–332.

Lattin JD, Stanton NL. 1992. A review of the species of Anthocoridae (Hemiptera: Heteroptera) found on *Pinus contorta*. *J New York Entom Soc*. **101**: 95–107.

Lorenzana A, De Mendoza AH, Seco MV, Casquero PA. 2010. Population development of *Phorodon humuli* and predators (*Orius* spp.) within hop cones: Influence of aphid density on hop quality. *Crop Prot*. **29**: 832–837.

Lyla KR, Pathummal BS, Chandish BR. 2006. Field evaluation of anthocorid predator, *Cardiastethus exiguus* Poppius against *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) in Kerala. *J Biol Control* **20**: 229–231.

Menalled FD, Landis DA, Dyer L. 2004. Research and extension supporting ecologically based IPM systems. *J Crop Imp*. **11**: 153–174.

Muraji M , Kawasaki K, Shimizu T, Noda T. 2004. Discrimination among Japanese species of the *Orius* flower bugs (Heteroptera: Anthocoridae) based on PCR-RFLP of the nuclear and mitochondrial DNAs. *Japan Agri Res Qty*. **38**(2): 91–95.

Nasser M, Abdurahiman UC. 2004. Anthocorid predators and their biocontrol potential In *Indian insect predators in Biological Control.* pp: 91–114. (Ed.) Sahayaraj, K. Daya Publishing House, New Delhi.

Nian-Wan Y, Lian-Sheng, Zang SW, Jian-Ying, Hong-Xing GX, Fang-Hao FZW. 2014. Biological pest management by predators and parasitoids in the greenhouse vegetables in China. *Biol Control.* **68**: 92–102.

Schmidt, J. 1994. Pirate bugs plunder greenhousepests. *Agric Food Res Ontario*, **17**: 12-15.

Sigsgaard L, Esbjerg, P. 1997. Cage experiments on *Orius tantillus* predation of *Helicoverpa armigera Entomol Exp et Appli*, **82**: 311-318.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* **4**: 406–425.

Saulich AK, Musolin DL. 2009. Seasonal development and ecology of anthocorids (Heteroptera, Anthocoridae). *Entomol Review.* **88**: 257–291.

Sayed SM, Montaser MM. 2012. Preliminary molecular characterization and utilization of native *Orius* sp. (Hemiptera: Anthocoridae) for controlling aphids infesting Taif rose. *Arch Phytopath Pl Prot.* **45**: 373–380.

Sayed SM, Metwally MM, Elsayed G, Sayed Amer AM. 2013. Preliminary Molecular Identification of a predatory bug *Orius albidipennis* collected from ornamental plants. *J Insect Sci.* **13**: 11–13.

Sigsgaard L, Esbjerg P. 1997. Cage experiments on *Orius tantillus* predation of *Helicoverpa armigera*. *Entomol Expt Appl.* **82**(3): 311–318.

Schuh RT, Stys P. 1991. Phylogenetic analysis of cimicomorphan family relationships (Heteroptera). *J New York Ent Soc.* **99**: 298–350.

Schmidt JM, Richards PC, Nadel H, Ferguson G. 1995. A rearing method for the production of large numbers of the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *Can Entomol*. **127**: 445–447.

Sing SE, Arbogast RT. 2008. Optimal *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) density and time of introduction for suppression of bruchid progeny in stored legumes. *Environ Ent.* **37**: 131–142.

Sobhy IS, Amany M, Abdul-Hamid, Awad AS, Ahmed A, Shoukry, Nasser SM, Stuart RR, Reitz. 2014. Life history traits of *Blaptostethus pallescens* (Hemiptera: Anthocoridae), a candidate for use in augmentative biological control in Egypt. *Appl Entomol Zool.* **49**: 315–324.

Sohrabi F, Annie E, Parviz S, Moosa S, Mohammad SM. 2013. Intraguild predation by the generalist predator *Orius majusculus* on the parasitoid *Encarsia formosa*. *Biol Control.* **58**: 65–72.

Unruh TR, Woolley JB. 1999. Molecular methods in classical biological control. p57-85. In Fisher TW, Bellows TS, Caltagirone LE, Dahlsten DL, Huffaker CB, Gordh G. (Eds). *Handbook of biological control*: *Principles and applications of biological control*. Academic Press, 1046 pp.