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MORPHOLOGICAL PHYLOGENETIC ANALYSIS OF GENUS *XANTHOPIMPLA* SAUSSURE 1892 (HYMENOPTERA: ICHNEUMONIDAE: PIMPLINAE) FROM MALAYSIA

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ABSTRACT

A phylogenetic analysis based on morphological data was done for 31 species of *Xanthopimpla* Saussure 1892 (Hymenoptera: Ichneumonidae: Pimplinae) from Malaysia by using PAUP*4.0. A total of 83 characters were analysed using Maximum Parsimony heuristic search with 100 replications and 2 outgroups namely *Theronia pseudozebra pseudozebra* Gupta 1962 and *Echthromorpha agrestorius notulatoria* Fabricius 1804. Resulted tree are less resolved and supported with length of tree value 351, Consistency index 0.3105, Retention index 0.4622, Rescale Consistency index 0.1435 and Homoplasy index 0.6895. Outcome forms monophyletic branches amongst ingroups. *Xanthopimpla leviuscula* deviated out from the clade due to the absence of notaulus with 62% bootstrap value. Conflict arose when resulted cladogram compared with group-classification by

Townes & Chiu (1970). To simplify, *X. melanacantha melanacantha* + *X. corynoceros corynoceros* clade approves classifications into *Rhopaloceros*-group by Townes & Chiu (1970) by 57%. However, cladogram opposes classification of *X. decurtata detruncata*, *X. polyspila* and *X. flaviceps* into *Terebatrix*-groups as they did not belong to the same clade. Therefore, further phylogenetic studies should be conducted by using molecular data to form more reliable phylogenetic tree.

Keywords: Phylogenetic, *Xanthopimpla*, Malaysia, Pimplini, Pimplinae, Hymenoptera

ABSTRAK

Analisis filogenetik telah dijalankan untuk 31 spesies *Xanthopimpla* Saussure 1892 (Hymenoptera: Ichneumonidae: Pimplinae) dari Malaysia berdasarkan data morfologi menggunakan perisian PAUP*4.0. Sebanyak 83 ciri telah dianalisis dengan menggunakan kriteria pencarian heuristik Parsimoni Maksimum bereplikasikan 100 kali dan dua kumpulan luar iaitu *Theronia pseudozebra pseudozebra* Gupta 1962 dan *Echthromorpha agrestorius notulatoria* Fabricius 1804. Output menghasilkan pohon yang kurang terurai dan tersokong dengan nilai pokok 351, indeks kekonsistenan 0.3105, indeks retensi 0.4622, indeks kekonsistenan pengskalaansemula 0.1435 dan indeks homoplasy 0.6895. Ahli kumpulan dalam menghasilkan klad monofiletik. *Xanthopimpla leviuscula* mencapah keluar dari pohon ekoran daripada ketidakhadiran notaulus dan disokong dengan nilai butstrap sebanyak 62%. Konflik timbul apabila kladogram yang terhasil dibandingkan dengan pengklasifikasian oleh Townes & Chiu (1970). Klad *X. melanacantha melanacantha*, *X. corynoceros corynoceros* menyokong pengkategorian kumpulan Rhopaloceros oleh Townes & Chiu (1970) dan disokong dengan nilai butstrap sebanyak 57%. Namun begitu, pencapahan *X. decurtata detruncata*, *X. polyspila* and *X. flaviceps* menyangkal pengklasifikasian kumpulan Terebatrix

kerana pencapahan ahli-ahlinya. Kesimpulannya, kajian filogenetik lanjutan perlu dilakukan dengan menggunakan data molekul untuk menghasilkan pohon yang lebih meyakinkan.

Kata kunci: Filogenetik, *Xanthopimpla*, Malaysia, Pimplini, Hymenoptera

INTRODUCTION

Fitton et al. (1988), divided Pimplinae divided into 7 tribes namely the Ephialtini, Polispinctini, Rhyssini, Poemeniini, Pimplini, Delomeristini and Diacritini without any general agreement (Example: Compare Finlayson 1967 and Townes 1969). Ephialtini might be the most primitive group and nearly forms paraphyletic grade-group because some of the tribes originated from it. Delomeristini is polyphyletic but could not be separated and its' genera could be joined together unambiguously to other tribes. The other five tribes form holophyletic group though Polysphinctini instable depends on its definition either at larval or adult level (Townes 1969; Gupta & Tikar 1978).

Xanthopimpla belongs to the tribe Pimplini which also contain the other two genera namely the *Theronia* (*Theronia pseudozebra pseudozebra* Gupta 1962 chosen as outgroup) and *Echthromorpha* (*Echthromorpha agrestorius notulatoria* Fabricius 1804 chosen as outgroup). Pimplini's shared characters include sharp rugae or transverse wrinkles on mesoscutum, convex clypeal basal, epicnemial carina that present atleast ventrally, indistinct suture on posterior margin of 3rd to 5th tergites (Gauld & Fitton 1984; Gupta & Tikar 1976). Wheeler (1990) and Graham et al. (2002) listed possible problems arose if outgroup is far or different as they will result in less reliable phylogenetic information, less divergences amongst outgroups and produce tree with lower resolution ingroups. For instances, genus *Aedes*

and *Drosophila* are dipterans nominated as outgroups for hymenopterans does not shows relative monophyly relationship (Derr et al. 1992). Nixon & Carpenter (1993), Maddison et al. (1992), Smith (1994) and Swofford et al. (1996) discussed that outgroups may relative group but able to produce tree with shorter clade if they share almost similar relation among ingroups (Nur Azura 2006).

Basically, genus *Echthromorpha* Holmgren 1868 possess epicnemial carina, long clypeus with narrow and convex marginally with twisted mandible that uncovers labrum when closed. *Echthromorpha* have mesopleuron suture with angled median, malar space which is longer or almost the same width as mandible width, complete occipital carina and mesopleuron without deep or striped groove. In addition, *Echthromorpha* have larger and lobate basal in female, simple femur, forewing with 3r-m vein that encloses rhombic areolet and subpetiole. *Echthromorpha* recorded for having marginal cells with smoky spots distally, hind wing without or with shorter first Cu1 abcissa, and smooth gaster. Ovipositor of *Echthromorpha* is curved, protrudes and extends apex of gaster until almost same length with hind tibia (Gauld & Fitton 1984). Specifically, *Echthromorpha agrestorius notulatoria* Fabricius 1804 possess convex anterior margin of eye, infuscate forewing, antenna with 31-34 segments, female with 5 segmented maxillary palp, while male with 4 (Yu et al. 2005).

Genus *Theronia* Holmgren 1859 have elliptical propodeum spiracle, dorsal propodeum with distinct latero-longitudinal and latero-median carina, complete occipital carina, slightly convex clypeus, untwisted mandible, epicnemial carina present ventrally and mesopleural suture with angled medially. *Theronia* also have oval spiracle, female without lobate basal tarsal claws, forewing with 3r-m vein enclosing rhombic areolet and hind wing with first Cu1 abcissa about 0.3x length of cu-a. In

addition, *Theronia* also have slender first tergite, smooth tergite 2 till tergite 5, and ovipositor that extends till apex of gaster and 1.2-1.9x length of hind tibia (Gauld & Fitton 1984). *Theronia pseudozebra pseudozebra* Gupta 1962 specifically have 2 rounded protruberance in middle of clypeus, mandibular tooth with similar length, carinated frons between socket and antenna, with notaulus, and submetapleural carina shaped like protruding widened tooth on median coxa. Moreover, they also have shorter epomia that does not extend center of pronotum, longer ovipositor, parallel upper and lower ovipositor valves, cylindrical ovipositor apex, closed areolet and does not have media dorsal carina (Amanda et al. 2011). Therefore, both genus were used as outgroups for heuristic phylogenetic analysis by using Phylogenetic Analysis Using Parsimony (PAUP) software with matrix data of 83 morphological characters.

MATERIALS AND METHODS

Morphological data of *Xanthopimpla* species extracted to design matrix data to build heuristic phylogenetic tree by using software PAUP*Ver.4.0. A total of 83 characters, with 2 outgroups namely *Theronia pseudozebra pseudozebra* Gupta 1962 and *Echthromorpha agrestorius notulatoria* Fabricius 1804 were used to generate phylogenetic tree by parsimonial method. Table 1 lists out morphological characters chosen for phylogenetic analysis inputs. Obtained phylogenetic tree was used to explain relationship amongst ingroups.

RESULT AND DISCUSSION

Figure 1 shows maximum parsimony phylogenetic tree supported with bootstrap value and 100 replications. The phylogenetic trees yielded with Length of tree value 351, Consistency index 0.3105, Retention index 0.4622, Rescale Consistency index 0.1435 and Homoplasy index 0.6895.

The tree resulted from this parsimony analysis are less resolved due to high homoplasy which could interfere tree inferens and impacted measurement of clade support (Brandley et al. 2009). The tree shows *Theronia pseudozebra pseudozebra* and *Echthromorpha agrestoria notulatoria* shares synapomorphic characters which causes them to diverged out of *Xanthopimpla* clade with 95% bootstrap value. Both outgroups have scutellum flanges which does not reaches apex of scutellum while ingroups of genus *Xanthopimpla* have scutellum flanges that reaches apex of scutellum.

The tree shows ingroups of *Xanthopimpla* forms monophyletic group due to the presence of malar space that is shorter than basal mandibular width, forewing with cu-a vein opposite basal vein Rs&M (Gauld & Fitton 1984) and divided clypeus with transverse median suture (Townes & Chiu 1970). *Xanthopimpla leviuscula* are the species that diverged out earlier in the clade due to the absence of notaulus and it is supported by 62% bootstrap value.

Townes & Chiu (1970) categorizes *Xanthopimpla* species into 20 group by referring to collective morphological characters. However, the resulted tree did not approve it. For instances, Townes & Chiu (1970) classified *X. conica*, *X. decurtata detruncata*, *X. polyspila*, *X. flaviceps* and *X. diplonyx* into *Terebatrix*-group. Conversely, member of *Terebatrix*-group is diverged and not contained in as same clade. This maybe because of apomorphic characters possess by each member though all of them have scutellum flange that reaches apex of scutellum.

On the other hand, different situation was reflected in the case of *X. corynoceros corynoceros* and *X. melanacantha melanacantha* where Townes & Chiu (1970) classifies them under *Rhopaloceros*-group and the clade itself support the inferens by sorting them into same clade with bootstrap value

57%. These are due to the synapomorphic characters such as opened areolet of forewing and absence of second intercubitus vein.

Xanthopimpla conica and *X. diplonyx* are belongs to the same group, specifically *Terebatrix*- group for having lateral scutellum flanges that extends till apex of scutellum (Townes & Chiu 1970) and eyes that diverge anteriorly. However, *X. conica* has conical scutellum (Townes & Chiu 1970) while scutellum of *X. diplonyx* is not conical. In addition, presence of lateral longitudinal carina about or less than 0.5x length of outer margin of first lateral area and absence of apical spine on hind tibia distinguish *X. conica* from *X. diplonyx* with apical spine on hind tibia and presence of lateral longitudinal carina more than 0.5x length of outer margin of first lateral area.

This analysis manages to gather *X. alternans, X. pasohensis, X. jacobsoni jacobsoni* and *X. decurtata detruncata* into Clade I as they share common characters such as short notaulus, less convex scutellum, widened basal of hair on inner side of hind tarsus, discoidella vein that reaches margin of forewing, 5-9 distal hamuli on hind wing, presence of mesoscutal crest, dorsolateral and mediandorsal carina. *Xanthopimpla alternans* and *X. pasohensis* forms same clade because of closed or partially closed areolet in forewing and presence of second intercubitus vein but later separates because *X. alternans* has second recurrent vein before or in the middle of areolet angle while *X. pasohensis* have it near or at the outer end of areolet.

Xanthopimpla flavolineata and *X. mucronata* forms same clade for having lateral flanges of scutellum that extends till apex of scutellum (Townes & Chiu 1970) and absence of dorsolateral carina on first tergite. *Xanthopimpla flavolineata* separates for having curved, widened basal and blackened hair apical on inner hind tarsal claw (Townes & Chiu 1970) and eyes that diverged anteriorly while *X. mucronata* have straight, narrowed basal and

not blackened apical of hair on inner hind tarsal claw (Townes & Chiu 1970) with parallel eyes.

Xanthopimpla konowi, *X. regina*, *X. gampsura*, *X. clivulus clivulus*, *X. fastigiata fastigiata* and *X. punctata* manages to form clade II due to presence of simplesiomorphic characters specifically presence of tooth on upper ovipositor valve and at once diverges from clade *X. falvolineata* + *X. mucronata* which have tooth on upper ovipositor valve. *X. konowi* and *X. regina* possess tubercle, closed second lateral area of propodeum, pair of spots on second to fourth tergite, and spot on subdorsal of hind and front of hind femur (Townes & Chiu 1970) thus, roots them into the same clade. Besides that, the clade further supported by the acquisition of synapomorphic characters such as more convex face and clypeus with vertical short ridges between toruli and clypeal fovea. Consequently, supports the classification of them into *Regina*-group as proposed by Townes & Chiu (1970). Yet, more convex scutellum and outer profile of mesopleuron and pyramidal scutellum separates *X. konowi* from *X. regina* with less convex scutellum and outer profile of mesopleuron and scutellum without pyramidal shape.

Xanthopimpla stemmator and *X. tricapus impressa* belongs to the same clade due to the presences of curved, widened basal and blackened apical of hair on inner hind tarsal claw other than closed or partially closed areolet on forewing and complete or partially complete intercubitus vein (Townes & Chiu 1970). Later, these 2 species form monophyletic clade as *X. stemmator* possess second recurrent vein before or in the middle of areolet angle (Townes & Chiu 1970) and rounded lower angle of anterior pronotum whereas *X. tricapus impressa* have second recurrent vein near or outer angle of areolet (Townes & Chiu 1970) and tapered lower angle of anterior pronotum.

Xanthopimpla despinosa despinosa and *X. walshae walshae* belongs to the same clade because their propodeum areola not completely surrounded by carina with straight, narrow and not blackened apical of hair on inner hind tarsal claw (Townes & Chiu 1970). However, *X. despinosa despinosa* diverges latter as its apical transverse propodeum carina is absent or partially present with confluenting areola and petiolar area. Meanwhile, *X. walshae walshae* have complete apical transverse propodeum carina with separate areola and petiolar area.

Clade III is separated from clade *X. despinosa despinosa*, *X. walshae walshae* because these 2 species do not have any tooth on upper ovipositor valve but *X. despinosa leipephelis*, *X. honorata honorata*, *X. flaviceps* dan *X. ansata ansata have* 1 to 6 tooth on upper valve of ovipositor. *Xanthopimpla despinosa leipephelis* and *Xanthopimpla honorata honorata* forms same clade as suggested by Townes & Chiu (1970) due to the shared characters such as absences or partially present apical transverse carina with overlapping areola and petiolar area. Townes & Chiu (1970) classify them together under *Occidentalis*-group for shared synapomorphic characters such as straight, narrow, and not blackened apical of hair on inner hind tarsal claw.

Xanthopimpla despinosa leipephelis and *X. honorata honorata* forms monophyletic branch due to the apomorphism. For example, *X. despinosa leipephelis* have shallow notaulus, very convex scutellum, 7 distal hamuli on fore wing, transverse apical carina that is absent or present as stub with spots on 6^{th} and 8^{th} tergite. On the other hand, *X. honorata honorata* have deep notaulus, less convex scutellum, 6 distal hamuli, apical transverse carina lacks medially and without spots on 6^{th} and 8^{th} tergite. Key constructed by Townes & Chiu (1970) supports the divergences of both species as *X. despinosa leipephelis* has 0-3 preapical bristles on hind tibia whereas *X. honorata honorata* has 4-7.

Table 1 List of selected characters of *Xanthopimpla* for matrix data

	Characters	'0'	'1'	'2'
1	Shape of clypeus	less convex	convex/more convex	flat
2	Elongated clypeus	yes	no	
3	Two rounded node in the middle of clypeus	yes	no	
4	Face with ridge between toruli and clypeal fovea	no	yes	
5	Shape of face	more convex/convex	less convex	flat
6	Compressed/deep/concave frons	yes	no	
7	Carinated frons between socket and antenna.	yes	no	
8	Mandible wider than malar space	yes	no	
9	Frons with protrudings lump below median ocellus	no	yes	
10	Width of malar space (mm)	>0.20	0.05-0.12	0.13-0.20
11	Occelocular distance (mm)	0.42-0.52	0.31-0.41	0.20-0.30
12	Number of antenna segments	28-33	34-39	40-45
13	Tapered mandible	yes	no	
14	Twisted mandible	yes	no	
15	Labrum exposed when mandible closed	yes	no	
16	Submarginal carina	present	absent	
17	Complete submarginal carina	yes	no/partially present	
18	Pronotum with black spots	no	yes	
19	Mesoscutum crest	absent	present	
20	Size of mesoscutum crest	absent	small	moderate/large
21	Notaulus	absent	present	

22	Size of notaulus	absent	short	long
23	Depth of notaulus	absent	shallow	deep
24	Notaulus extends half of mesoscutum	absent	yes	no
25	Notaulus reaches line connects centre of tegula.	absent	yes	no
26	Notaulus reaches in front of line connects centre of tegula.	absent	yes	no
27	Notaulus extends beyond line that connects centre of tegula	absent	yes	no
28	Scutellum shape	convex	less convex	more convex
29	Other shapes of scutellum	not pyramidal/cone	pyramidal	cone
30	Scutellum flanges extends till apex of scutellum	no	yes	
31	Sternaulus	absent	not distinct	distinct
32	Postpectal carina	simple	complex	
33	Presence of notch on centre of postpectal carina	present	absent	
34	Axillary trough of mesonotum with black spots	no	yes	
35	Presence of submetapleural carina	absent	present	
36	Completeness of submetapleural carina	complete	incomplete	absent
37	Shapes of submetapleural carina tooth-like with lump between middle coxa	yes	no	
38	Short epomia	yes	no	
39	Epomia not extends centre of pronotum	yes	no	
40	Presence of tubercle	absent	no	
41	Presence of apophysis	absent	no	
42	Presence of costula	absent	no	
43	Presence of areola	absent	no	
44	Wrinkles on basal transverse carina	yes	no	

45	Presences and completeness of apical transverse carina	absent	present, complete	present, incomplete
46	Presence of lateral longitudinal carina	absent	present	
47	Length of upper lateral longitudinal carina 0.5x length of outer margin of first lateral area	no or <0.5x	=0.5x	>0.5x
48	Areola overlaps with second lateral area	yes	no	
49	Areola overlaps with petiolar area	yes	no	
50	Wrinkles on petiolar area	no	yes	
51	Pleural area of propodeum divided	no	yes	
52	Shape of ovipositor tip	straight	curved	
53	Ovipositor apex cylindrical shape	yes	no	
54	Upper ovipositor valve with tooth	yes	no	
55	Ovipositor tip blunt/ flat	no	yes	
56	Lower ovipositor valve with tooth	yes	no	
57	Number of upper ovipositor valve	0	1-6	7-12
58	Number of lower ovipositor valve	0	1-6	7-12
59	Number of apical spine	0	1-6	7-12
60	Number of preapical spine	0	1-6	7-12
61	First hind tarsal segment with spine	yes	no	
62	Base of apical hair on inner part of hind tarsal claw	widened	narrowed	
63	Shape of apical hair on inner part of hind tarsal claw	curved	straight	
64	Colour of apical hair on inner part of hind tarsal claw	black	not black	
65	Tibia longer than ovipositor sheath	no	yes	
66	Areolate on forewing	closed	partially closed	open
67	Second vein (cu-a) cuneate/ distal with first vena (Rs-m)	yes	no	

68	Position of second recurrent vein on areolet of forewing	before/in the middle	on/outer end of angle	
69	Position of nervulus on basal vein of forewing	distal	opposite	not opposite
70	Discoidella vein reaches margin of forewing	reaches	not reaches	
71	Braciella vein reaches margin of forewing	reaches	not reaches	
72	Cubitus vein reaches margin of forewing	not reaches	reaches	
73	Subdiscoides vein reaches margin of forewing	reaches	not reaches	
74	Number of distal hamuli on hind wing	10-15	5-9	
75	Smoke spot on right distal margin of forewing	yes	no	
76	Presence of dorsolateral carina	present	absent	
77	Presence of mediandorsal carina	present	absent	
78	Length of dorsolateral carina	partially present/ short	long	absent
79	Length of mediandorsal carina	absent	partially present/ short	long
80	Dorsolateral carina extends spiracles	yes	no	absent
81	Mediandorsal carina extends spiracles	yes	no	absent
82	Dorsolateral carina reaches apex	yes	no	absent
83	Mediandorsal carina reaches apex	yes	no	absent

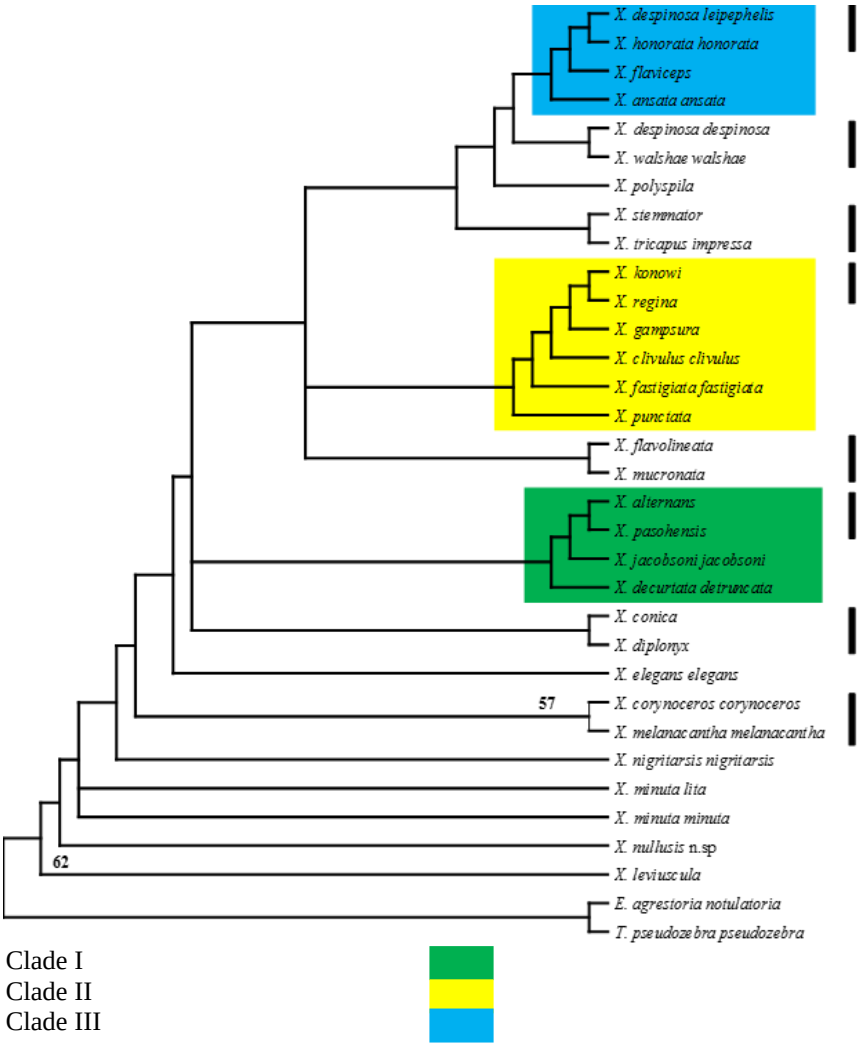

Figure 2 Maximum Parsimony Consensus tree (50% bootstrap majority rule) from morphological data. Clade value on node shows percentage of confidence.

CONCLUSION

Phylogenetic analysis using morphological data resulted in less resolved and supported tree though, though manage to give information on species divergences and supported by bootstrap value. Parsimonial analysis resulted in higher homoplasy maybe due to the tree inferens and disturbances in rate of measuring supported clade (Brandley et al. 2009). This may be due to all used 83 characters are less significant or not an apomorphic characters which yielded in a weak tree. Homoplasy present in all phylogenetic analysis but analysis method used to minimize rate or value of homoplasy and explicit evolution models such as Maximum Likelihood and Bayesian are used to overcome its effects (Felsenstein 1978, 2004; Barandley et al. 2009). Therefore, selection of exact distinguishable characters is very important in running phylogenetic analysis by using morphological data so that more resolved and supported tree could be obtained. Moreover, molecular phylogenetic analysis could be performed to supports the findings further.

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