PHYLOGENY AND MORPHOMETRIC VARIATION OF SEVERAL WEEVILS SPECIES (COLEOPTERA: CURCULIONIDAE) FROM MALAYSIA

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ABSTRACT

The phylogeny of 43 species under 20 genera of seven subfamilies of weevils' species (Coleoptera: Curculionidae) from Malaysia were successfully inferred based on 34 morphological characters, of which combined the qualitative and quantitative inputs. The monophyletic clade of the Curculionidae was supported by Neighbor Joining (NJ) and Maximum Parsimony (MP) trees with bootstraps values of 79% and 76%, respectively. Although the arrangement and placement among the 19 species of Dryophthorinae were not fully resolved, however the phylogenies were able to elucidate the relationships of the other curculionids from the Curculioninae, Entiminae, Lixinae and Molytinae subfamilies. Therefore, further multivariate analyses were conducted on 17 selected species of Curculionidae, which has been successful to discriminate the examined species. From the principal component analysis (PCA; eigenvalues of PC1 for cluster 1 = 7.4650; eigenvalues of PC1 for cluster 2 = 5.1874) and canonical variate analysis (CVA; cluster 1 with $p < 0.0001$; cluster 2 with $p < 0.0001$), the diagnostic morphological characters were resulted from the elytron, pronotum, total length of body, and femur length. As a conclusion, the morphometrics has proven to be reliable and informative as another alternative to subfamilies classification and to show the relationships within the examined insect's species. However, it is also recommended that further studies should include more diagnostic and informative characters to represent up to the tribes or genus levels in future.

Keywords: Curculionidae, phylogeny, morphometrics, multivariate, weevils

ABSTRAK

Filogeni bagi 43 spesies kumbang terpilih di bawah 20 genus dan tujuh subfamili (Coleoptera: Curculionidae) dari Malaysia berjaya dibina berdasarkan 34 ciri morfologi yang menggabungkan data kualitatif dan kuantitatif. Klad monofiletik bagi famili Curculionidae adalah menerusi pohon *Neighbor Joining* (NJ) dan Parsimoni Maksimum (MP), dibuktikan dengan nilai butstrap sebanyak 79% dan 76%, masing-masing Walaupun pengkelasan bagi 19 spesies Dryophthorinae adalah tidak selesai sepenuhnya, namun pohon filogeni berjaya menunjukkan hubung kait antara kumbang Curculionidae dari subfamili Curculioninae, Entiminae, Lixinae dan Molytinae. Seterusnya analisis multivariat dijalankan ke atas 17 spesies Curculionidae yang terpilih dan berjaya membezakan spesies yang dikaji. Daripada analisis komponen prinsipal (PCA; nilai eigen bagi PC1 untuk kluster 1 = 7.4650; nilai eigen bagi PC1 untuk kluster 2 = 5.1874) dan analisis variat kanonik (CVA; kluster 1 dengan $p < 0.0001$; kluster 2 dengan $p < 0.0001$), ciri-ciri morfologi yang diagnostik ialah elitron, pronotum, panjang keseluruhan badan dan panjang femur. Kesimpulannya, kajian morfometrik adalah terbukti boleh dipercayai dan bermaklumat sebagai alternatif lain kepada pengkelasan subfamili dan menunjukkan hubung kait antara spesies serangga yang dikaji. Adalah disarankan agar ciri-ciri yang lebih diagnostik dan bermaklumat untuk mewakili peringkat suku atau genus digunakan dalam kajian lanjut pada masa hadapan.

Kata kunci: Curculionidae, filogeni, morfometrik, multivariat, kumbang

INTRODUCTION

Studies on the beetle species, which generally involved only small samples from certain geographical areas from Malaysia, have been conducted by various researchers recently such as Hazmi and Sharifah (2017), Luqman et al. (2018), Marcellinus et al. (2020), Muhaimin et al. (2017), Musthafa et al. (2019), Nor-Izyani et al. (2019) and Nurul-Ain and Arumugam (2019). Despite of many ecological studies published here, phylogenetic studies of Malaysian beetles however are still very much lacking. Among the beetles species, the weevils from the family Curculionidae (Coleoptera) are the most widely recorded insects in the world (Alonso-Zarazaga & Lyal 1999; Anderson 2002; Marvaldi & Lanteri 2005). However, the current classification of these weevil species remains unclear, especially due to the addition of new morphological characteristics on larvae and adults, as well as the implementation of the molecular data in phylogenetic analysis that showing various findings (Marvaldi & Lanteri 2005).

As a result, the imbalance in the characterization of beetle's morphology occurs and thus creates a disagreement in many concepts of species groups, hierarchical positioning and monophyletic status of several subfamilies and families (Alonso-Zarazaga & Lyal 1999; Anderson 2002; Davis 2014; Lawrence et al. 2011; Marvaldi & Lanteri 2005; Morrone 2000). The monophyletic status for Dryophthorinae and Curculioninae is also in no exception, in which still debatable among the taxonomic and systematic experts to date.

The abundance and diversity of beetle species around the world creates many opportunities especially in the field of statistical ecology. Beetles are the best model to examine the cause and the correlation of species diversity in the context of evolution (Lawrence et al. 2011). Through the statistical and computational technology approaches, morphometrics studies have proved to be an effective method for identification of various insects, such as the honey bee (Daly 1992), aphid (Foottit 1992), beetles (Sanmartin & Martin-Piera 1999; Van

Rensburg et al. 2003), flies (Huey et al. 2000), lac insects (Ahmad et al. 2014) and mosquitoes (Jaramillo-O et al. 2015).

Hence, using the morphological approach, this present study aims to evaluate the monophyletic status of the weevils from Curculionidae, as well as to assess the effectiveness of morphological characterization to elucidate the relationships between selected weevil species in Malaysia. Furthermore, the morphometrics studies were also conducted to identify the significant morphological characters in discriminating the examined weevils.

MATERIALS AND METHODS

Data Collection

A total of 43 curculionid species of weevils from Malaysia (Table 1), representing 20 genera from seven subfamilies, namely, Dryohpthorinae, Cryptodermatinae, Curculioninae, Entiminae, Lixinae, Molytinae and Brentinae were examined from insect collections deposited at the Sarawak Museum (SM), Department of Agriculture, Kuala Lumpur (JPKL), Department of Agriculture, Semongok (JPS), Centre for Insect Systematics (UKM) and Insects Reference Collection (UNIMAS).

Table 1 List of 43 curculionid species examined in this study

Subfamily	Species	Repository	Locality	No. of Individuals
Dryophthorinae	*Cosmopolites sordidus*	JPKL, JPS	Serdang, Samarahan	8
	Cyrtotrachelus bipartilus	SM	Limbang, Kinabalu	18
	Cyrtotrachelus longimanus	UNIMAS	Samarahan	1
	Odoiporus longicollis	JPKL, JPS	Bagan Datoh, Shah Alam, Serdang, Samarahan	10
	Ommatolampus haemorrhoidalis	SM	Kuching	9
	Ommatolampus hewitti	SM	Kuching	4
	Omotemnus nanus	SM	Kuching, Miri	3
	Otidognathus myrmidon	JPKL	Raub	1
	Poteriophorus bouringi	SM	Kuching	1
	Poteriophorus fuscovarius	SM	Kuching	3
	Poteriophorus sp.	SM	Kuching	3
	Protocerius colossus	JPKL, SM	Jeram, Serdang, Kuching	11
	Protocerius fervidus	JPKL	Kuala Terengganu	1
	Protocerius purpuratus	SM	Kuching, Kinabalu	7

	Rhynchophorus ferrugineus	UKM	Kuala Terengganu, Pangkalan Chepa, Pulau Gadong, Perlis	45
	Rhynchophorus sp.	SM	Kuching	2
	Rhynchophorus vulneratus	UNIMAS	Kuching, Samarahan, Mukah, Miri, Kinabalu, Serdang, Pulau Gadong, Jeram, Chembong	53
	Sphenocorynus cinereus	JPKL	Selangor	6
	Tetratopus sericans	JPKL	Selangor	4
Cryptodermatinae	*Cryptoderma adumbrata*	SM	Kuching, Miri, Lawas, Kinabalu	11
Curculioninae	*Dyscerus anceps*	JPKL	Perak	1
	Dyscerus lateralis	SM	Kuching	3
	Dyscerus perdix	SM	Kuching	6
	Dyscerus sparsus	SM	Kuching	3
	Dyscerus sparsutus	JPKL	Tanah Rata	1
	Dyscerus sexpunctatus	SM	Kuching, Miri	13
Entiminae	*Dermatoxenus hians*	JPKL	Kuala Selangor	2
	Episomus chlorostigma	JPKL	Klang, Jerangau	2
	Episomus illustris	JPKL	Klang	3
	Episomus nobilis	SM	Kuching, Miri	10
	Episomus sobrinus	JPKL	Perlis	1
	Episomus timidus	SM	Kuching	15
	Hypomeces squamosus	JPKL	Kedah, Perlis, Klang, Kajang, Perak	11
Lixinae	*Lixus confusus*	SM	Kuching	8
	Lixus javanus	JPKL	Raub	2
	Lixus nebulifasciatus	SM	Limbang	1
	Lixus vetula	JPKL	Raub, Kedah	2
	Lixus xanthusi	JPKL	Tanah Rata	3
Molytinae	*Alcidodes crassus*	JPKL	Kepong	8
	Alcidodes tutus	JPKL	Kuala Terengganu	8
	Alcidodes sp.	JPKL	Serdang, Seri M	7

			elaka	
Brentinae	*Diurus* sp.	UNIMAS	Samarahan	1
	Eutrachelus temmincki	UNIMAS	Samarahan	1

Subfamily and Species Identification

The first 21 morphological features were following descriptions made by Anderson (2002), Davis (2014), Marvaldi and Lanteri (2005), and Marvaldi and Morrone (2000) for the identification up to the subfamily's level. Next, 13 subsequent morphological features were made from the size of the weevils' body and measured using a digital caliper (Mitutoyo TM) and calibrated to almost 0.01 mm, following description by Sazali et al. (2018).

Phylogeny Reconstruction

Thirty-four morphological characters were identified and transformed into the polynomial codes of 0, 1, 2 or 3 (Table 2). The data matrix for 43 weevil species was analysed using Mesquite software version 3.31 and stored in nexus file format (.nxs) (Maddison & Maddison 2017) and later analysed using Phylogenetic Analysis using Parsimony (PAUP) version 4b10 (Swofford 2002). The Neighbour-joining (NJ) clustering was performed using the Kimura 2-parameter evolution model (Kimura 1980), whereas the unweighted maximum parsimony (MP) method was conducted using full heuristic search. Two species of Brentinae, namely, *Diurus* sp. and *Eutrachelus temmincki* were used as the outgroups and rooted to both trees, since they are sister family to Curculionidae. The phylogenetic confidence was estimated by bootstrapping (Felsenstein 1985) with 1000 replications of data sets and only bootstrap values greater than 65% were shown at each node of the phylogenies.

Table 2 List of morphological characters and states used and transformed into polynomial codes

No.	Characters	Code	Description
1	Antenna type	0	Straight
		1	Geniculate
2	Scape of antenna	0	Short, not passing front margin of eyes
		1	Short or almost equal the funicle size, passing front margin of eyes
3	Radial sclerite in hind wing	0	Single
		1	Paired with two sclerites
4	Frons	0	Narrow
		1	Broad
5	Snout	0	Very short and wide
		1	Elongated and slendered, some are straight and forward downwards
		2	Elongated and straight
6	Antennal club (segment 9-11)	0	The first 2 or 3 segments loosely articulated
		1	All segments tightly articulated with three articles
		2	All segments tightly articulated with four articles
7	Antennal insertion on snout	0	Base
		1	Middle
		2	Peak
8	Funicle numbers	0	7
		1	6
		2	> 7

9	Scrobe on snout	0	Absent
		1	Present, not passing antennal insertion
		2	Present, passing antennal insertion
10	Eyes shape	0	Rounded
		1	Oval
		2	Oval and narrow vertically
11	Eyes size	0	Small
		1	Big
12	Pronotum size	0	Smaller than elytron
		1	Smaller than elytron anteriorly and getting bigger posteriorly
13	Body shape (lateral view)	0	Elongated
		1	Expanded in middle section
		2	Almost cylindrical
14	Body shape (dorsal view)	0	Elongated and tuberculated
		1	sub-rhomboidal, with pronotum width obviously smaller than body width
		2	sub-rhomboidal, with pronotum width less than body width
		3	Broadly oval
15	Body colouration	0	1 colour
		1	2 colours
		2	Metallic colours
		3	Many colours (polymorphism)
16	Pronotum edging (base)	0	Round or oval
		1	‘V’ shaped (sharped)
		2	Almost flat
17	Pronotum shape (lateral view)	0	Subsphere or dome-shaped
		1	Almost flat or flat
18	Marks on pronotum (dorsal view)	0	Absent
		1	Present, spotted or striped
		2	Present, patterns vary
19	Puncture on elytron (dorsal view)	0	Absent or having fine lines or grooves
		1	Present, with coarse lines or grooves
20	Marks on elytron (dorsal view)	0	Absent
		1	Present, spotted or striped
		2	Present, patterns vary
21	Pygidium	0	Exposed beyond the elytra apex
		1	Covered by elytra
22	Snout length (SL)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
23	Snout width (SW)	0	< 1.0 mm
		1	1.01 – 2.0 mm
		2	< 3.0 mm
24	Pronotum length (PL)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
25	Pronotum width (PW)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
26	Elytron length (EL)	0	< 10.0 mm
		1	10.01 – 15.0 mm
		2	15.01 – 20.0 mm

		3	> 20.0 mm
27	Elytron width (EW)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
28	Total length (TL) of body	0	< 20.0 mm
		1	20.01 – 30.0 mm
		2	30.01 – 40.0 mm
		3	> 40.0 mm
29	Profemur length (F1L)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
30	Protibia length (F1Tb)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
31	Mesofemur length (F2L)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	< 15.0 mm
32	Mesotibia length (F2Tb)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	> 10.0 mm
33	Metafemur length (F3L)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	10.01 – 15.0 mm
		3	> 15.0 mm
34	Metatibia length (F3Tb)	0	< 5.0 mm
		1	5.01 – 10.0 mm
		2	> 10.0 mm

Multivariate Analyses

For the morphometrics analysis, eight morphological characters, namely the pronotum length (PL), pronotum width (PW), elytron length (EL), elytron width (EW), total length (TL) of body, profemur length (F1L), mesofemur length (F2L) and metafemur length (F3L) were measured using a digital caliper (Mitutoyo TM) and calibrated to 0.01 mm.

a. Principal Component Analyses (PCA)

The principal components analyses (PCA) were run using the Minitab version 17.0 software (Minitab Inc. 2013) based on the correlation matrix and only the components with eigen value greater than 1.0 were extracted (Kaiser 1960). The distributions of the weevil species were then plotted using the first two principal components, where the *x*-axis represented the first principal component (PC1) while the *y*-axis represented the second principal component (PC2).

b. Canonical Variate Analyses (CVA)

The canonical variate analyses (CVA) were further conducted to identify the significant and informative morphological characteristics to discriminate the examined species within their respective families. All characters were loaded simultaneously to investigate the integrity of a predetermined species group, using the Wilk's lambda and the squared Euclidean distances that adapt to different variances (Hair et al. 1995). The distributions of weevil species were plotted based on the first two functions, where the *x*-axis represents the first function while the *y*-axis represents the second function. Cross-validation tests were also conducted to assess the capabilities of the predicted model.

RESULTS

Phylogeny Reconstruction

Reconstruction of the phylogenetic trees using the Neighbor-Joining (NJ, Figure 1) and Maximum Parsimony (MP, Figure 2) suggested that both Dryophthorinae and Curculioninae formed their own monophyletic groups, supported with 79% and 76% bootstrap values, respectively. However, the arrangement for some weevil species in Dryophthorinae, namely *Ommatolampus*, *Poteriophorus* and *Rhynchophorus* were mixed.

Based on NJ tree, the curculionids were divided into two major clusters represented by number 1 and 2. Cluster 1 is divided into 2 subgroups namely 1A and 1B and supported with 80% bootstraps value. Cluster 1A comprised of five species, namely, *Cosmopolites sordidus*, *Odoiporus longicollis*, *Ommatolampus hewitti* and *Sphenocorynus cinereus* (Dryophthorinae), and also *Cryptoderma adumbrata* (Cryptodermatinae) supported by 83% of bootstrap value. Meanwhile, cluster 1B gathered the remaining of 15 weevils of Dryophthorinae into two subgroups, where the placement of these species was not supported with convincing bootstrap values of less than 50%. Furthermore, cluster 2 is divided into two subgroups, namely, 2A and 2B, which was supported with 77% bootstrap value. In clusters 2A, the compilation of weevils from Curculioninae and Entiminae was supported by 68% bootstrap value. On the other hand, *Hypomeces squamosus* which was supposedly attached within this cluster was forming as a basal clade to cluster 2B that included weevils of *Alcidodes* (Molytinae) and *Lixus* (Lixinae), supported with 76% bootstrap value.

Based on unweighted MP analysis, the tree length was 163 with a consistency index (CI) of 0.4294 and a retention index (RI) of 0.7657. The grouping and placement of weevils in the subfamily of Dryophthorinae were relatively different from the NJ tree. In general, the Curculionids were divided into two major clusters represented by numbers 1 and 2. Cluster 1C showed no clear separation for the weevils, whereas *C. adumbrata* (Cryptodermatinae) represented by cluster 1D formed as a basal clade to this cluster, supported with 77% of bootstrap value. On top of that, cluster 2 was divided into two subgroups, namely 2C and 2D, supported with 74% bootstrap value. Cluster 2C was representing six *Dyscerus* species (Curculioninae), meanwhile cluster 2D compiled those weevils from Molytinae, Lixinae and Entiminae according to own subfamily, respectively. Three *Alcidodes* species and five *Lixus* species formed the first subgroup, supported with 66% bootstrap value, whereas *Episomus*, *Dermatoxenus hians* and *H. squamosus* formed own subgroups, supported with 72% bootstrap value.

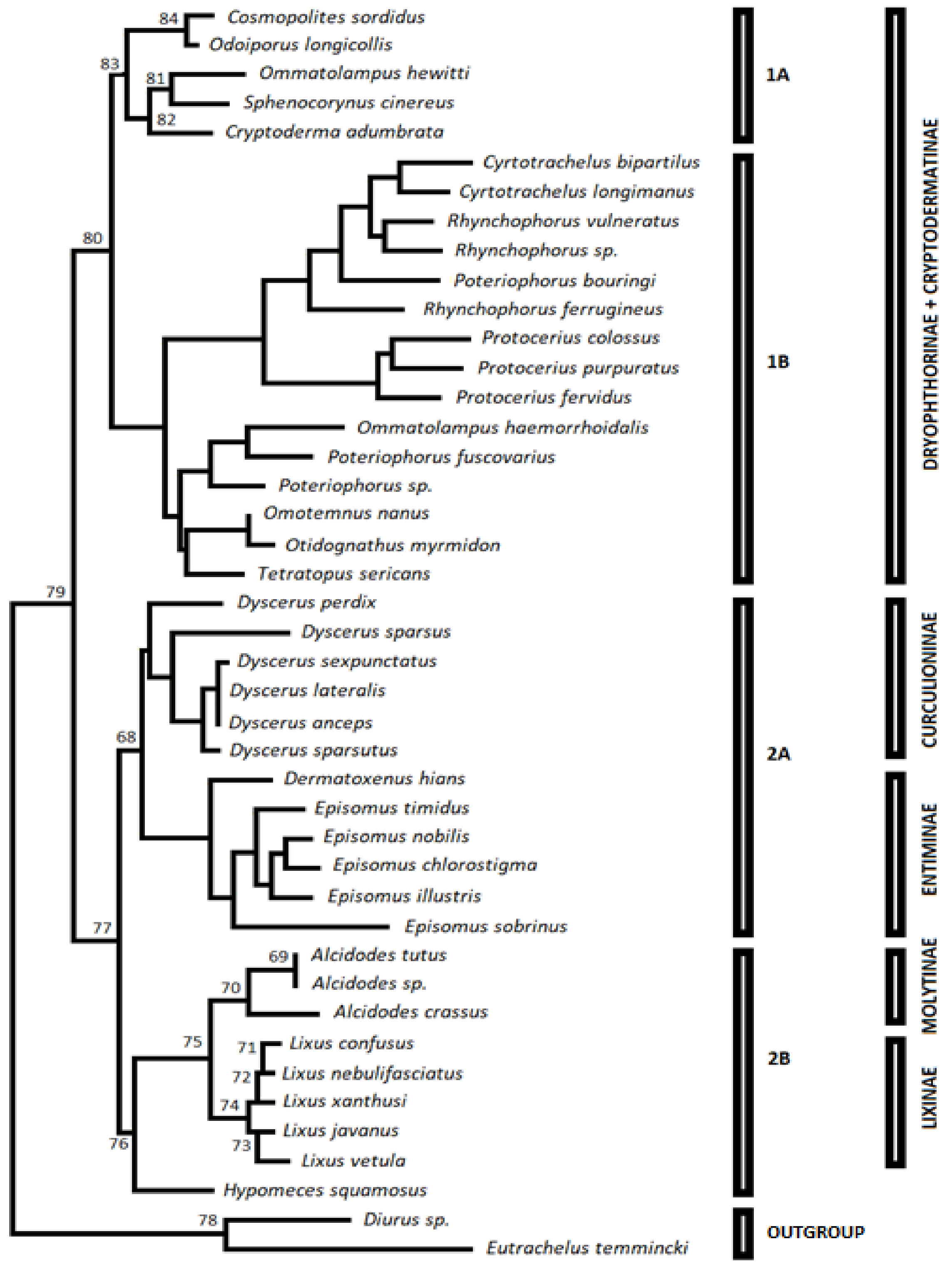

Figure 1 Phylogeny of curculionids based on data matrix using Kimura 2-parameter. Values on the branches represent NJ bootstrap estimates based on 1000 replications

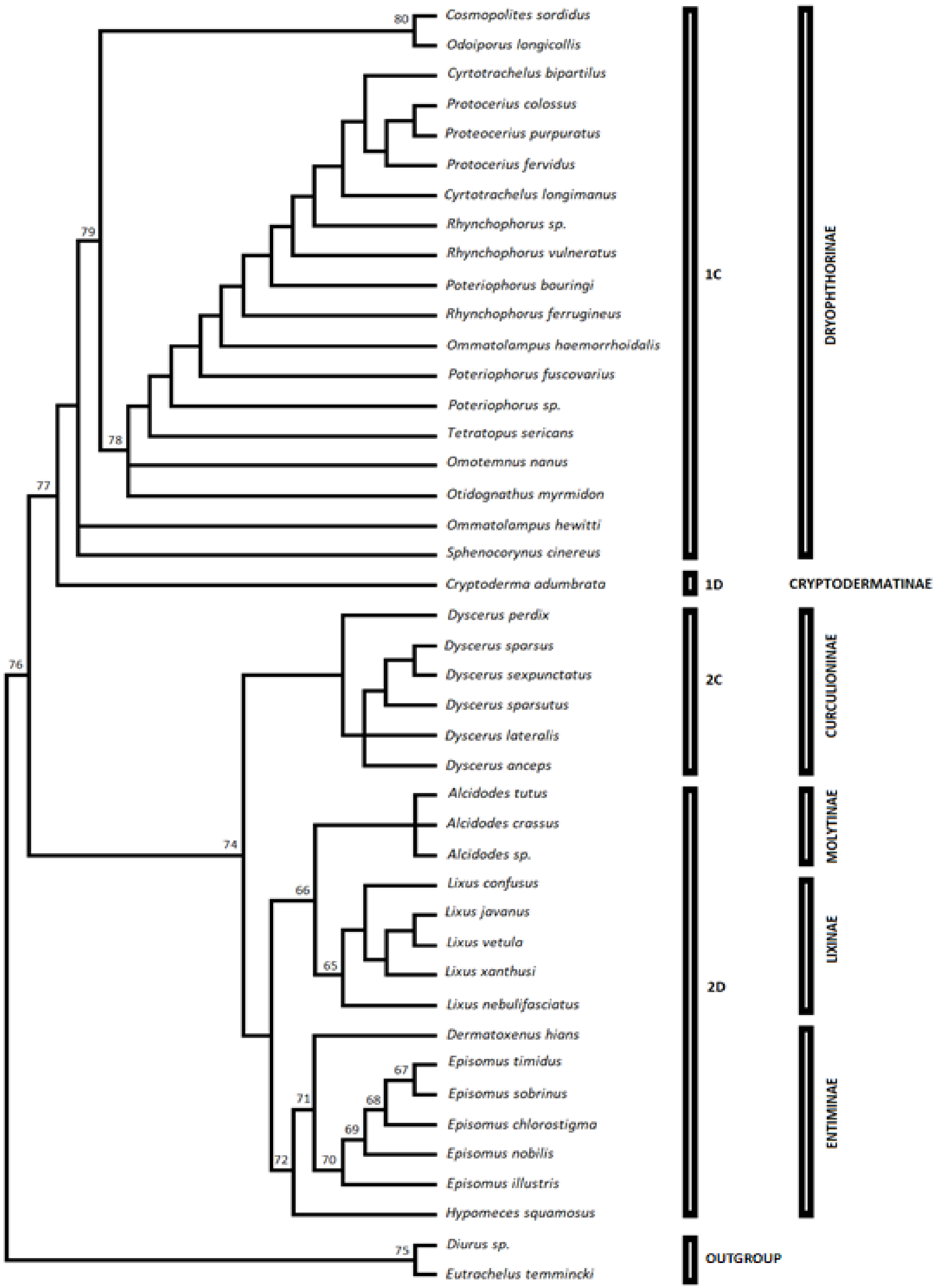

Figure 2 Phylogeny of curculionids based on data matrix using Maximum Parsimony. Values on the branches represent MP bootstrap estimates based on 1000 replications

Multivariate Analyses

a. Principal Component Analyses (PCA)

In Figure 3, based on cluster 1, the first two principal components showed a cumulative variation of 98.5%, in which only the first principal component (PC1) showed an eigen value of 7.4650, resulted from the elytron length (EL, 0.361), pronotum width (PW, 0.360) and metafemur length (F3L, 0.360). The scatter plot showed six clusters in which *Cosmopolites sordidus* and *Odoiporus longicollis* formed a single cluster, while *Ommatolampus haemorrhoidalis* and *Sphenocorynus cinereus* formed separate clusters but close to each other. Moreover, both pairs of *Protocerius colossus* and *P. purpuratus*, and *Rhynchophorus vulneratus* and *R. ferrugineus* formed a separate cluster, respectively. Individual groupings were shown by *Cyrtotrachelus bipartilus* (Dryophthorinae) and *Cryptoderma adumbrata* (Cryptodermatinae).

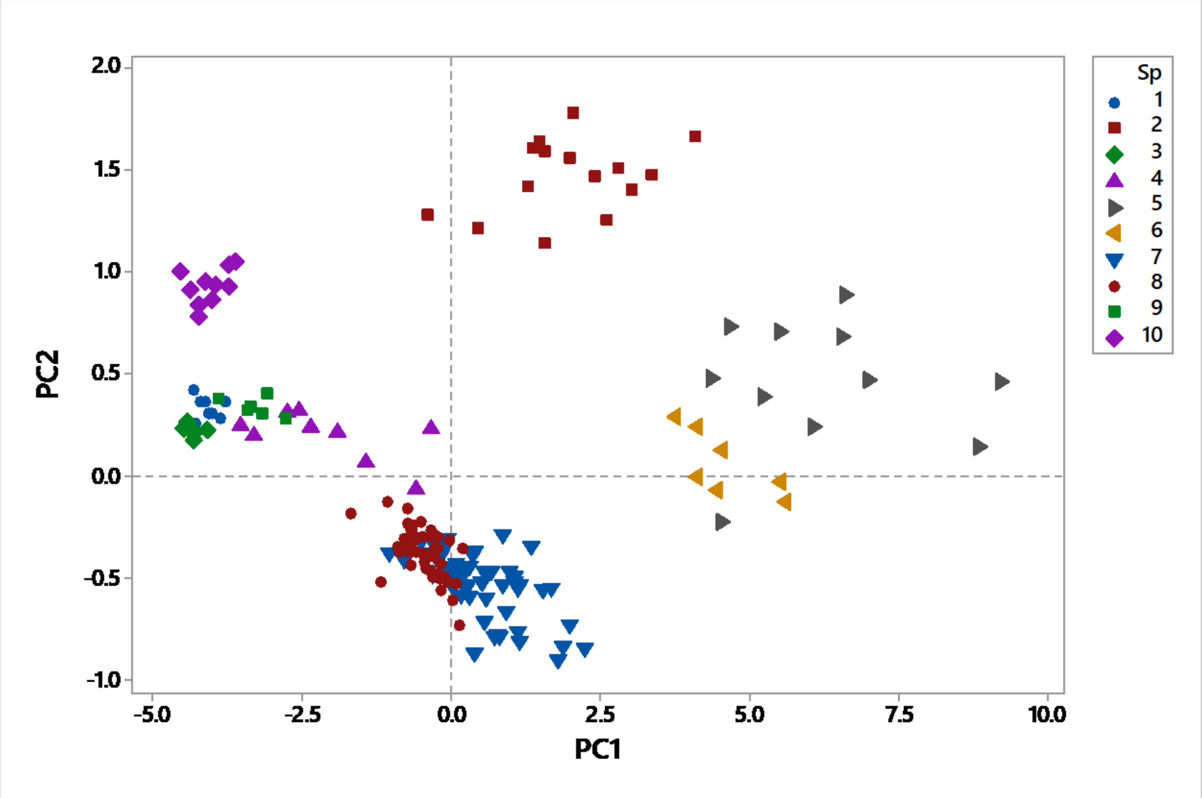

Figure 3 Principal component plot for Dryophthorinae dan Cryptodermatinae. 1: *Cosmopolites sordidus*, 2: *Cyrtotrachelus bipartilus*, 3: *Odoiporus longicollis*, 4: *Ommatolampus haemorrhoidalis*, 5: *Protocerius colossus*, 6: *Protocerius purpuratus*, 7: *Rhynchophorus vulneratus*, 8: *Rhynchophorus ferrugineus*, 9: *Sphenocorynus cinereus*, 10: *Cryptoderma adumbrata*

For cluster 2, a total of seven species from subfamily Curculioninae, Entiminae, Lixinae and Molytinae were analysed and a cumulative variation of 81.7% (Figure 4). The first principal component (PC1) showed an eigen value of 5.1874 with 64.8% variation, meanwhile the second principal component (PC2) showed an eigen value of 1.3552 with 16.9% variation. The highest loadings in the first principal components (PC1) were the elytron width (EW, 0.397) and the mesofemur length (F2L, 0.389), whereas the highest loadings on the second principal component (PC2) were the total length (TL, –0.541) and pronotum width (PW, 0.519). All three species of Entiminae which were *Episomus timidus*, *E. nobilis* and *H. squamosus* and *Lixus confusus* from Lixinae formed a mixed grouping. Besides that, *Dyscerus sexpunctatus* from Curculioninae and *Alcidodes crassus* and *Alcidodes* sp. from Molytinae also formed independent cluster, respectively.

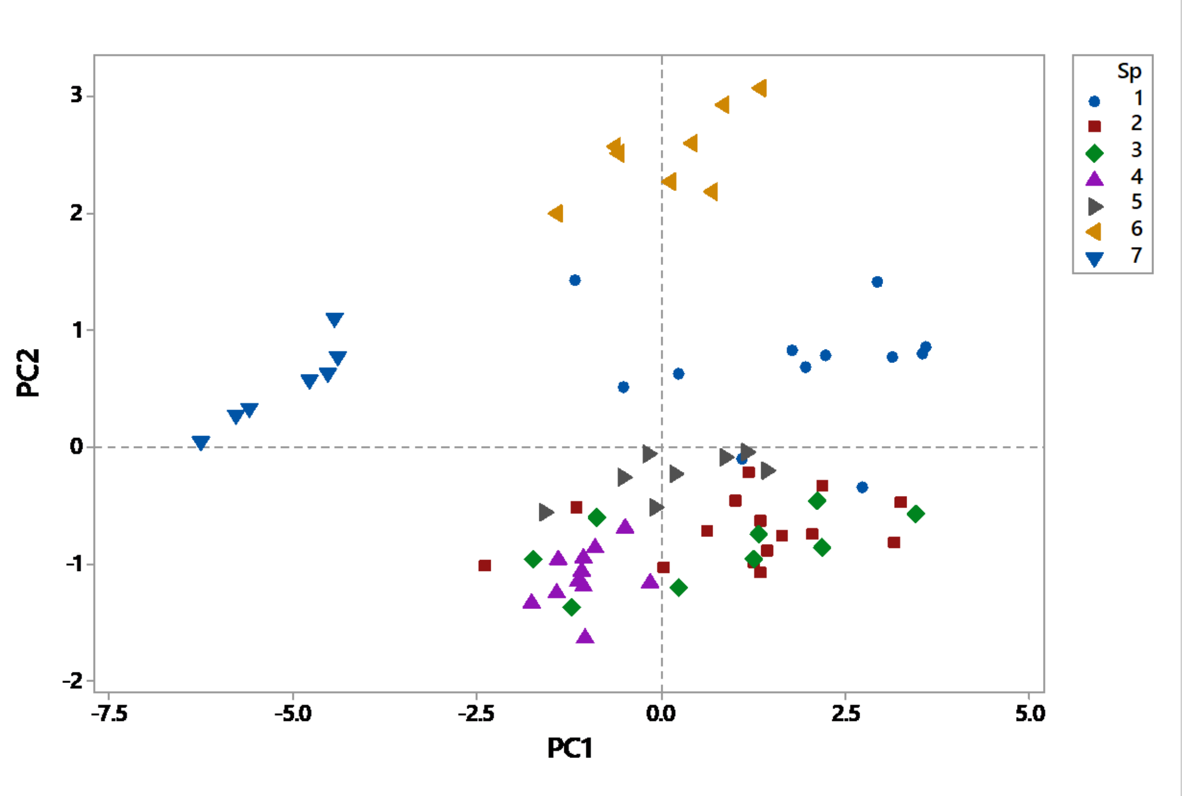

Figure 4 Principal component plot for Curculioninae, Entiminae, Lixinae dan Molytinae. 1: *Dyscerus sexpunctatus*, 2: *Episomus timidus*, 3: *Episomus nobilis*, 4: *Hypomeces squamosus*, 5: *Lixus confusus*, 6: *Alcidodes crassus*, 7: *Alcidodes* sp.

b. Canonical Variate Analyses (CVA)

The canonical variate analysis (CVA) conducted on 10 weevil species in cluster 1 showed that the first eight functions extracted were significant ($p < 0.0001$) with a cumulative variance of 100%. In general, function 1 showed 49.2% variance ($p < 0.0001$), followed by function 2 with 22.3% variance ($p < 0.0001$). The highest loadings in function 1 were the pronotum length (PL, –1.942) and elytron width (EW, –1.538), while in function 2, the highest loadings were shown by the mesofemur length (F2L, –1.942) and metafemur length (F3L, 1.518). Based on the canonical variate plot for the first two functions, each weevil species was found to form independent cluster, except for two *Rhynchophorus* species, which were plotted closer and slightly overlapping (Figure 5). As compared to the principal component analysis (PCA), the canonical variate analysis (CVA) is able to differentiate all beetle species from Dryophthorinae and Cryptodermatinae. Using the cross-validation tests procedure, 97.6% of the original grouped cases were correctly classified, whereas in the cross-validated procedure, 96.5% of the cases were correctly assigned.

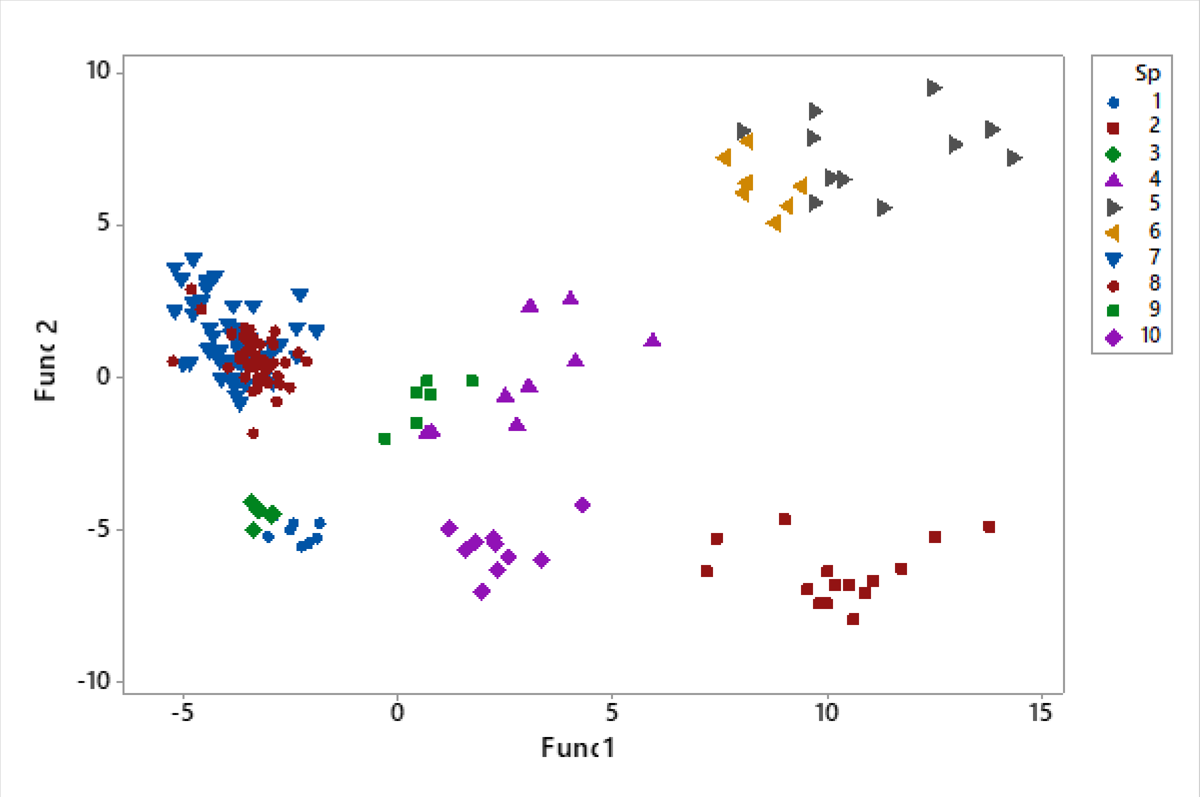

Figure 5 Canonical variate plot for Dryophthorinae and Cryptodermatinae. 1: *Cosmopolites sordidus*, 2: *Cyrtotrachelus bipartilus*, 3: *Odoiporus longicollis*, 4: *Ommatolampus haemorrhoidalis*, 5: *Protocerius colossus*, 6: *Protocerius purpuratus*, 7: *Rhynchophorus vulneratus*, 8: *Rhynchophorus ferrugineus*, 9: *Sphenocorynus cinereus*, 10: *Cryptoderma adumbrata*

In cluster 2, the canonical variate analysis (CVA) conducted on seven species described the first six functions with 100% cumulative variance. In function 1, 53.7% variance ($p < 0.0001$) was explained, while function 2 showed 33% variance ($p < 0.0001$). The highest loadings in function 1 were the total length (TL, 2.013) and elytron width (EW, –1.435), meanwhile in function 2 were the elytron width (EW, 1.401) and total length (TL, –1.401). Moreover, Figure 6 showed that each species was independently clustered and hence are reliable to distinguish the seven species of weevils from four subfamilies of Curculioninae, Entiminae, Lixinae and Molytinae, compared to principal component analysis (PCA). Using the cross-validation tests procedure, 98.6% of the original grouped cases were correctly classified, whereas in the cross-validated test, 91.4% of the cases were correctly assigned.

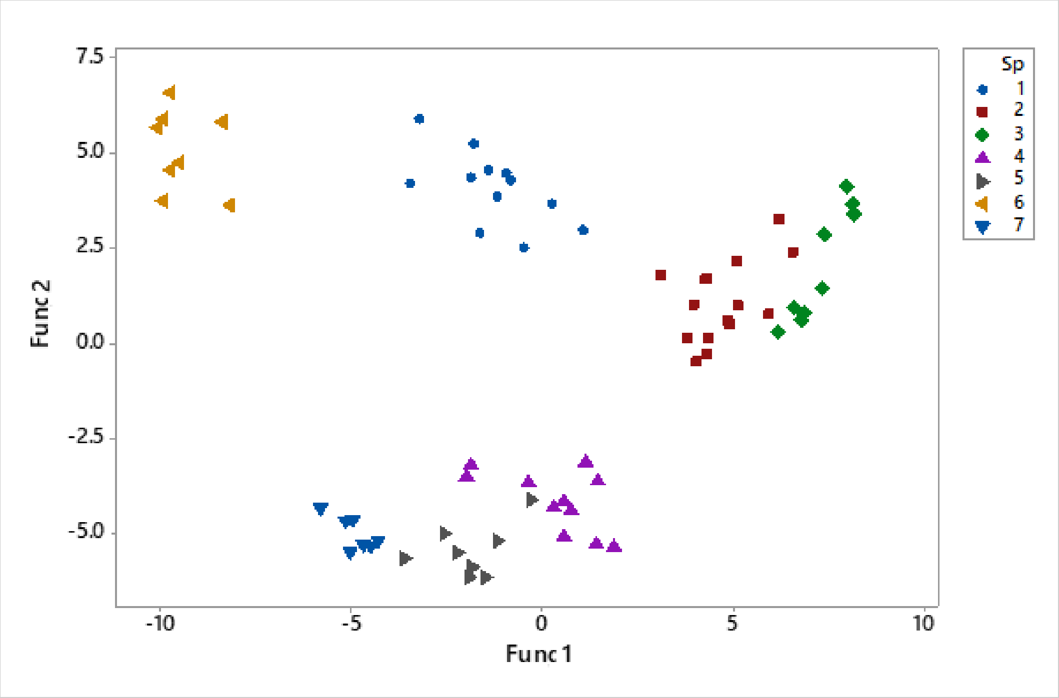

Figure 6 Canonical variate plot for Curculioninae, Entiminae, Lixinae and Molytinae. 1: *Dyscerus sexpunctatus*, 2: *Episomus timidus*, 3: *Episomus nobilis*, 4: *Hypomeces squamosus*, 5: *Lixus confusus*, 6: *Alcidodes crassus*, 7: *Alcidodes* sp.

DISCUSSION

The ability of molecular method is undoubtedly effective in resolving problems related to species identification, taxonomy and systematic. However, the use of morphological method is also not less important because morphological characterization was the earliest method that had been applied by taxonomist and systematist (Wiens 2004). Indeed, the use of morphological data is still relevant and additional data collection should be continued for phylogenetic analysis, and improvements in morphological-based phylogenetic methods should be addressed (Wiens 2004).

Lawrence et al. (2011) also supported the findings by Lopardo et al. (2011) and Seago et al. (2011), that morphological data is very effective in resolving the divergence of beetle species that cannot be solved by the use of molecular data alone. In addition, Davis (2014) also stressed that exploration on more detailed morphological data is required as most phylogenetic studies using molecular data sequences only consider limited taxa sampling and this leads to confusion in the classification of insects' families.

Moreover, the morphometrics techniques is also capable of solving the specific and taxonomic complexities in insects (Ahmad et al. 2014; Jaramillo-O et al. 2015). It is proven effective through the efficiency of univariate and multivariate statistical analyses (Foottit & Sorensen 1992). Additionally, the use of morphological data thus far is still relevant and appropriate as it is capable of solving phylogenetic relationships involving fossils and also useful when combined with the remaining taxa in a phylogenetic study (Wiens 2004). The use of morphological data is environmentally safe as it does not affect the physical condition of voucher and fossil specimens.

Generally, Dryophthorinae can be identified based on their antennal club which is glossy and spongiform at the apex and having six segments in the funicle as the seventh segment is compressed and forms into a club (Anderson 2002; Marvaldi & Morrone 2000; Morrone 2000). In this subfamily, its prementum is not visible in ventral view and have dorsal and ventral dermal lobes separating the tarsal claws (Marvaldi & Morrone 2000; Morrone 2000; Zimmerman 1993). Besides that, the aedeagal pedon has a lateral line or groove, whereas its aedeagal apodeme appears in line with aedeagus at lateral view (Morrone 2000).

For Curculioninae, the weevils of this subfamily can be identified through its antennal club which is usually ovoid, pilose and showed clear sutures, with exposed prementum in ventral view (Anderson 2002; Marvaldi & Lanteri 2005; Marvaldi & Morrone 2000; Morrone 2000). The snout is usually longer than head, with cylindrical and elongated shaped that projected forward. These weevils have no dermal lobes that separate the tarsus claws, and the aedeagal pedon lacks the lateral line or groove (Morrone 2000). In addition, the aedeagal apodeme in Curculioninae appears to bend down from the aedeagus at lateral view (Morrone 2000).

Apart from that, the species of the Entiminae can be easily recognized by its wide and shortening snout, obviously different from the other curculionids, and having its prementum covering the maxilla (Anderson 2002; Morrone 2000). Almost all weevils of this subfamily have a mandible that bears a deciduous process which immediately breaks off after emergence of the adults that leaves a visible scar on its mandible. In addition to that, the Entiminae also possess only one spine on the inner angle at the apex of hind tibia, as well as showing less sexual dimorphism as compared to the other species of Curculionidae (Anderson 2002).

For Lixinae and Molytinae, the snout shapes are diverse, either short and wide, or long and slendered. Lixinae are mostly grey or having dull-coloured body and scale pattern (Anderson 2002). The weevils can also be identified by the large tooth at the apex of the hind tibia, along with a short, globular and telescoping labial palpi (Anderson 2002; Morrone 2000). For Molytinae, the weevils have a large, hook-like apical tooth on the hind tibia, or have a many different types of modification to the apex of the hind tibia associated with the tooth development (Anderson 2002; Morrone 2000).

Using the multivariate analyses, the elytron, pronotum and femur length were the diagnostic characters in differentiating the 10 weevils in cluster 1 (Dryophthorinae + Cryptodermatinae). The overlapping of several species, as shown by *C. sordidus* with *O. longicollis* and *O. haemorrhoidalis* with *S. cinereus* indicating the size similarities. Moreover, the clustering of *C. sordidus* and *O. longicollis* were also supported in both phylogenies. Meanwhile, *O. haemorrhoidalis* and *S. cinereus* were separated from the other larger-sized weevils (total length > 30mm), in which both possessed slendered and elongated body shape.

In cluster 2 (Curculioninae + Entiminae + Lixinae + Molytinae), both multivariate analyses suggested that the elytron, pronotum, total length (TL) of body, and mesofemur length (F2L) were the significant characters for distinguishing the examined weevils from four subfamilies. The overlapping between two *Episomus* species with *H. squamosus* and *L. confusus* were expected as they shared similar body size. *Episomus* can be differentiated through coloration and patterns on its pronotum; *H. squamosus* can be recognised by its metallic green or yellow coloured body whereas *L. confusus* can be identified by its cylindrical body shaped with brownish coloration.

CONCLUSION

In conclusion, the relationships among 43 examined weevils from seven subfamilies of Dryophthorinae, Cryptodermatinae, Curculioninae, Entiminae, Lixinae and Molytinae including Brentinae were successfully assessed using a combination of qualitative and quantitative data. Additionally, the monophyletic status of Dryophthorinae was also strongly supported in both phylogenies. However, the addition of more informative morphological characters especially at the tribes or genus level is highly recommended, in order to fully resolve the complexities and reconstruct a more robust phylogeny within the subfamily of Dryophthorinae in future. Moreover, the statistical analyses conducted on 17 species of Curculionidae were able to describe the morphological variations in size. The canonical variate analysis (CVA) was the most effective method to discriminate the examined species which were not fully resolved by the phylogenies. Thus, these morphometrics findings showed that the weevils can be identified mostly from the elytron, pronotum, total length and femur length.

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REFERENCES

Ahmad, A., Ramani, R., Sharma, K.K., Vidyarthi, A.S. & Ramamurthy, V.V. 2014. Distinction of Indian commercial lac insect lines of *Kerria* spp. (Homoptera: Coccoidea) based on their morphometrics. *Journal of Insect Science* 14(263).

Anderson, R.S. 2002. Curculionidae. In. Arnett, R.H. Jr., Thomas, M.C. & Skelley, P. (eds.). *American Beetles. Polyphaga: Scarabaeoidea through Curculionoidea,* pp. 722-815. Boca Raton: CRC Press LLC.

Alonso-Zarazaga, M.A. & Lyal, C.H.C. 1999. *A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera) (Excepting Scolytidae and Platypodidae)*. Barcelona: Entomopraxis.

Daly, H.V. 1992. A statistical and empirical evaluation of some morphometric variables of honey bee classification. In. Sorensen, J.T. & Foottit, R.G. (ed.). *Ordination in the Study of Morphology, Evolution and Systematic of Insects. Applications and Quantitative Genetic Rationales*, pp. 127-155. Amsterdam: Elsevier Science Publishing Inc.

Davis, S.R. 2014. Morphology, phylogeny and evolutionary development in the weevils (Insecta: Coleoptera: Curculionoidea). PhD Thesis, University of Kansas.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution 7*: 1193-1204.

Foottit, R.G. 1992. The use of ordination methods to resolve problems of species discrimination in the Genus *Cinara* Curtis (Homoptera: Aphidoidea: Lachnidae). In. Sorensen, J.T. & Foottit, R.G. (ed.). *Ordination in the Study of Morphology, Evolution and Systematic of Insects. Applications and Quantitative Genetic Rationales*, pp. 193-221. Amsterdam: Elsevier Science Publishing Inc.

Foottit, R.G. & Sorensen, J.T. 1992. Ordination methods: their contrast to clustering and cladistic techniques. In. Sorensen, J.T. & Foottit, R.G. (eds.). *Ordination in the Study of Morphology, Evolution and Systematic of Insects. Applications and Quantitative Genetic Rationales*, pp. 1-10. Amsterdam: Elsevier Science Publishing Inc.

Hair, J.F., Rolph, E.A., Roland, L.T. & William, C.B. 1995. *Multivariate Data Analysis*. New Jersey: Prentice Hall.

Hazmi, I.R. & Sharifah, A.S.S. 2017. Fireflies population and the aquaculture industry (Coleoptera: Lampyridae) of the Sungai Sepetang, Kampung Dew, Perak, Malaysia. *Serangga* 22(2): 217-237.

Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308-309.

Jaramillo-O, N., Dujardin, J.P., Calle-Londono, D. & Fonseca-Gonzales, I. 2015. Geometric morphometrics for the taxonomy of 11 species of *Anopheles* (*Nyssorhynchus*) mosquitoes. *Medical and Veterinary Entomology* 29: 26-36.

Kaiser, H.F. 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement* 20: 141-151.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotides sequence. *Journal of Molecular Evolution* 16: 111-120.

Lawrence, J.F., Slipinski, A., Seago, A.E., Thayer, M.K., Newton, A.F. & Marvaldi, A.E. 2011. Phylogeny of the coleopteran based on morphological characters of adults and larvae. *Annales Zoologici* 61(1): 1-217.

Lopardo, L., Giribet, G. & Hormiga, G. 2011. Morphology to the rescue: Molecular data and the signal of morphological characters in combined phylogenetic analyses-a case study from mysmenid spiders (Araneae, Mysmenidae), with comments on the evolution of web architecture. *Cladistics* 27(3): 278-330.

Luqman, H.A., Noor-Nasuha, A.A., Dzulhelmi, M.N., Nurul-Fatihah, A.L., Muhamad-Fahmi, M.H., Teo, T.M., Idris, A.B. & Hazmi, I.R. 2018. Diversity and composition of beetles (Order: Coleoptera) in three different ages of oil palms in Lekir oil palm plantation, Perak, Malaysia. *Serangga* 23(1): 58-71.

Maddison, W.P. & Maddison, D.R. 2017. Mesquite: A modular system for evolutionary analysis. Version 3.40. http://mesquiteproject.org

Marcellinus, I.S.D., Sazali, S.N., Hazali, R., Razak, A.I.A. & Fitri, F.N.A.H.A. 2020. Species composition and ecological distribution of the subfamily Cicindelinae Latreille, 1801 (Coleoptera: Carabidae) based on voucher specimens in Sarawak. *Borneo Journal of Resource Science and Technology* 10(2): 190-195.

Marvaldi, A.E. & Lanteri, A.A. 2005. Key to higher taxa of South American weevils based on adult characters (Coleoptera, Curculionoidea). *Revista Chilena de Historia Natural* 78: 65-87.

Marvaldi, A.E. & Morrone, J.J. 2000. Phylogenetic systematics of weevils (Coleoptera: Curculionoidea): A reappraisal based on larval and adult morphology. *Insect Systematics and Evolution* 31: 43-58.

Minitab. 2013. Minitab 16 statistical software. Minitab Inc., State College, Pennsylvania, USA.

Morrone, J.J. 2000. Mexican weevils (Coleoptera: Curculionoidea): A preliminary key to families and subfamilies. *Acta Zoologica Mexicana (nueva serie)* 80: 131-141.

Muhaimin, A.M.D., Norela, S., Halim, M. & Yaakop, S. 2017. Altitudinal accumulation and diversity of leaf beetle (Coleoptera: Chrysomelidae) at Fraser’s Hill, Pahang. *Serangga* 22(2): 171-184.

Musthafa, M.M., Rajandram, D. & Abdullah, F. 2019. Diversity and abundance of beetle at Kuala Kelapor, National Park, Malaysia. *Serangga* 24(2): 198-215.

Nor-Izyani, A.Z., Dzulhelmi, M.N., Azuan, A., Luqman-Hakim, A., Andi, S., Madihah, H., Siti-Asmah, M., Nur-Athirah, A., Lailatul-Nadhirah, A. & Faszly, R. 2019. Kepelbagaian kumbang tanah (Coleoptera: Carabidae) di ladang sawit Endau-Rompin, Pahang, Malaysia. *Serangga* 24(1): 91-102.

Nurul-Ain, S. & Arumugam, N. 2019. Beetles diversity (Order: Coleoptera) of R.E.A.C.H Biodiversity Centre, Cameron Highlands, Pahang, Malaysia. *Serangga* 24(2): 58-67.

Sanmartin, I. & Martin-Piera, F. 1999. A morphometric approach to the taxonomy of the genus *Ceramidan* (Coleoptera: Scarabaeoidea: Melolonthidae). *Canadian Entomologist* 131: 573-592.

Sazali, S.N., Hazmi, I.R., Abang, F., Rahim, F. & Jemain, A.A. 2018. Morphometric study of palm weevils, *Rynchophorus vulneratus* and *R. ferrugineus* (Coleoptera: Curculionidae) in view of insular and mainland population of Malaysia. *Pertanika Journal of Tropical Agriculture Science* 41: 1329-1340.

Seago, A.E., Giorgi, J.A., Li, J. & Slipinski, A. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution* 60: 137-151.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.

Van Rensburg, L.J., Chimimba, C.T., Bastos, A.D. and Chown, S.L. 2003. Morphometric measurement selection: an invertebrate case study based on weevils from sub-Antartic Marion Island. *Polar Biology* 27: 38-49.

Wiens, J.J. 2004. The role of morphological data in phylogeny reconstruction. *Systematic Biology* 53: 653-661.

Zimmerman, E.C. 1993. *Australian weevils. Volume III. Nanophyidae, Rhynchophoridae, Erirhinidae, Curculionidae: Amycterinae.* Melbourne: CSIRO.