A multivariate analysis of some Digenean species collected from several Red Sea fishes in Saudi Arabia

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Abstract: A group of ten Digenean species needs a considerable interest to analyze and discuss their phylogeny and classification. Phylogenies and classification of these trematodes are poorly discussed in Saudi Arabia. The present study aimed to better understand the phenetic relationship between the genera and species of ten Digenean parasites obtained from some commercial fishes of the Red Sea at Jeddah city, Saudi Arabia, within their families. Three types of analysis were performed with statistical V7 software, Cluster, Principal Component Analysis (PCA) and non parametric Multi-Dimensional Analysis (MDA). These analyses were based on the ten species of Digenea (Operational Taxonomic units, henceforth OTUs) described by Thirty-two morphological characters.The result showed that the position of the present species in the phenogram is identical to their taxonomic relationships, and the different Digenea studied are distinguished into four subgroups recording families, Lepocreadiidae, Hemiuridae, Cryptogonimidae, Angiodictyidae, Acanthocolpidae and Fellodistomidae. PCA explains 62.4% of the total observed variations. The percentages of the morphological variations within the components recorded were 24.9%, 19.4% and 18.3% for the first, second and third axes, respectively. The main characters utilized for the separation (characters with high loading factor >0.7) are those pertaining to the following, location of oral sucker, shape of pharynx, location of ovary, distance between anterior testis and ovary, uterus, shape of ventral sucker, ventral sucker location, testes location, egg size and shape of ovary. MDA confirms the separation of ten Digenean species into four subgroups representing six families which is conforming with that of Cluster and PCA analyses. These results stressed the importance of some morphological features (Highest loading factor > 0.7) as an indicator of the relationships.
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Key words: Digenean species, Operational Taxonomic Units, Cluster analysis, Phenogram, Red Sea fishes, Principal Component Analysis, Multi-Dimensional Analysis, Morphological analysis.

1. Introduction

The class Digenea contains nearly 18,000 nominal species, it represents the largest group of intestinal parasites (Olsen *et al.,* 2001 and Nolan and Cribb, 2005). The great diversity of Digenea required a considerable interest to analyze and discuss largely their phylogeny and classification (Olsen *et al.,* 2003). Fish parasites, especially the abundant trematodes, introduce information about habits and ecology of their individual hosts (McCaulely, 1966). Digenean systematics still not stable at both higher and lower taxonomic levels (Olsen and Tkach, 2005). Information derived from morphology is used in a series of mathematical algorithms and form the basis of numerical systematic (Mattison *et al.,* 1994). Two leading schools of taxonomic philosophy namely, phenetics and phylogenetics, are derived from numerical taxonomy. Phenetics depends mainly on similarity between taxa. Whereas, phylogenetics stress the importance of genealogical descent. Both schools have been applied recently to helminthes (Mattison *et al.,* 1994). La Rue (1957) has joined the external and internal morphology as well as life cycle information

into classification which constitutes the base of the systems used today. A lot of work based on morphology and life cycle such as Gibson (1966), Yamaguti (1971), Gibson *et al.* (2002), Jones *et al.* (2005) and Bray *et al.* (2008). Brooks *et al.* (1985), Pearson (1992) and Brooks and Mclennan (1993) used a cladistic process to estimate the relationships among the Digenea. The first phylogenetic analysis providing specific character matrices was not attempted until Cribb *et al.* (2001). They combined a newly coded morphological matrix with new molecular data from the small subunit ribosomal RNA gene (ssrDNA) from 75 Digenean species representing 55 families, combined with 56 adult and larval morphological characters for these families resulted in a well-resolved tree (Olsen *et al.*, 2003, p734). Olsen *et al.* (2003) developed this work to include 77 families. They discussed the monophyly of some Digenean groups at the lower taxonomic level.

The present study aims to apply Cluster, Principal Component Analysis (PCA) and non parametric Multi-Dimensional Analysis (MDA) to better understand the phenetic relationships of 10 Digenean

species within their families and important phylogenetic characters between these genera and species.

2. Materials and Methods
2.1. Data Collection
Ten Digenean species were used in the present study. Fish hosts, *Holocentrus sammara*, *H. spiniferus*, *Siganus rivulatus*, *Lethrinus nebulosus*, *Pherapon jarbua* and *Lutjanus johni* were collected from the Red Sea, Jeddah city, Saudi Arabia (Table 1, Figure 1).

Digenean parasites were collected from the alimentary tract of the dissected fishes, relaxed, fixed in formalin 10% and stained in borax carmine or in alum carmine, dehydrated in an ascending series of ethanol, cleared in clove oil and mounted in Canada balsam. Camera lucida was used to draw the specimens; the morphological description was based on fully mature species. Type specimens of all the species described in this study are deposited in the Biological Science Department, Girls section, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia.

**Table 1** List of Digenean species collected from different Red Sea fish hosts in Saudi Arabia (Figure1)

OUT No.	Digenean species	Family	Host
1	*Lipedapedon sammari*	Lepocreadiidae	*Holocentrus sammara*
2	*L. spiniferi*	Lepocreadiidae	*H. spiniferus*
3	*Aponurus sigani*	Hemiuridae	*Siganus rivulatus*
4	*Sterrhurina lethrini*	Hemiuridae	*Lethrinus nebulosus*
5	*Eurycreadium rivulatum*	Fellodistomidae	*Siganus rivulatus*
6	*Stephanostomum pheraponi*	Acanthocolpidae	*Pherapon jarbua*
7	*Hexangium loossi*	Angiodictyidae	*Siganus rivulatus*
8	*H.ecsomi*	Angiodictyidae	*Siganus rivulatus*
9	*Paracryptogonimus lutjani*	Cryptogonimidae	*Lutjanus johni*
10	*Pseudosiphoderoides sargasi*	Cryptogonimidae	*Lutjanus johni*

### 2.2. Observations on morphological characters
More than 20 specimens were used for the recorded characters of the present species. Table 2 shows the characters and character states scored for studying the morphology of Digenean species. A total

of 32 characters were recorded for each specimen. All characters are qualitative, eight of these characters were scored as binary while the rest were scored as multistate characters. The characters were then averaged into one OTU score for each character.
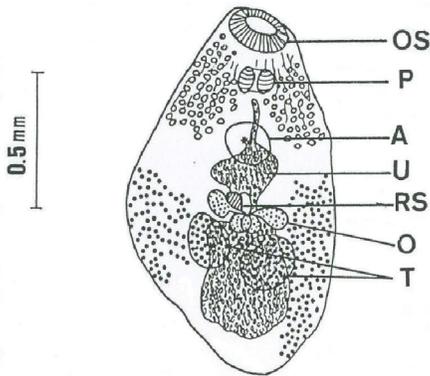

Lepidapedon sammari (1)   *L. spiniferi*   (2)
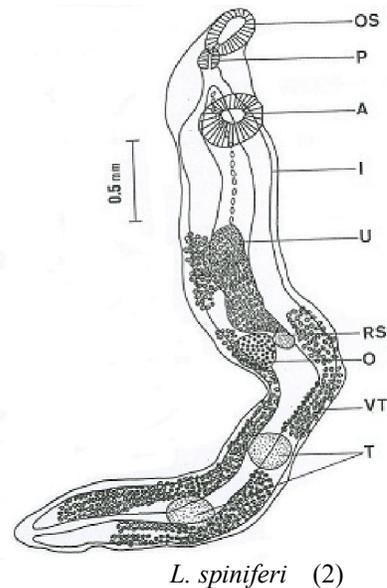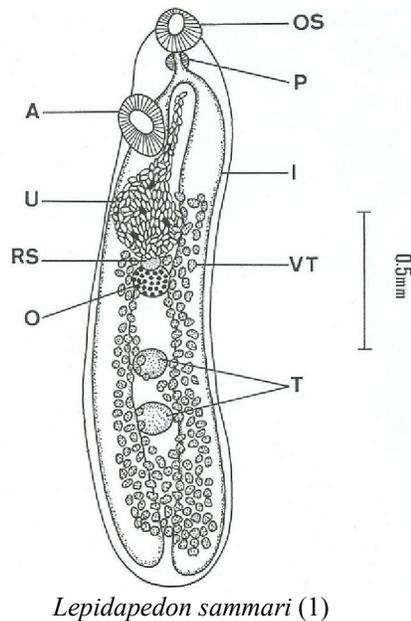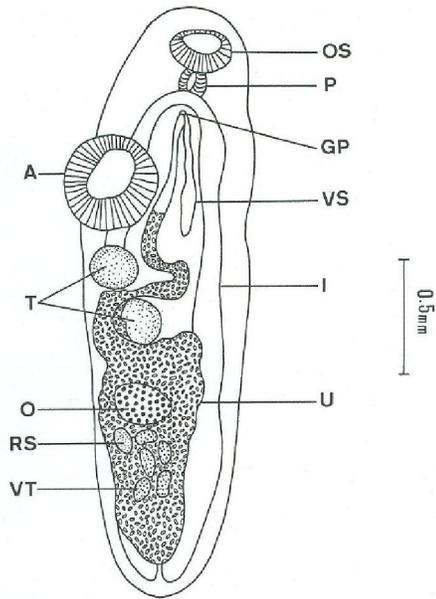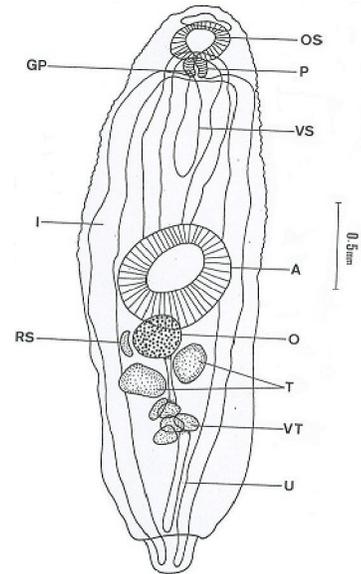
Aponurus sigani (3*)*　　　　　　　　*sterrhurina lethrini* (4)
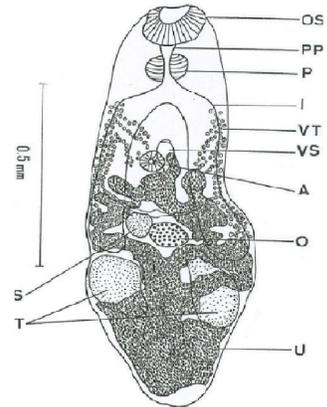
*Paracryptogonimus lutjani*　　(9)　　　　　*Pseudosiphoderoides sargasi* (10)

Figure 1: General appearance of the 10 Digenean OTUs studied (After Alzanbagi 1984)
Abbreviations: A= acetabulum, CS= cirrus sac, GP= genital pore, I= intestinal caecum, O= ovary, OE= oesophagus, OS= oral sucker, P= pharynx, PP= prepharynx , RS= receptaculum seminis, T= testes, U= uterus, VS= vesicula seminalis, VT= vitellaria.
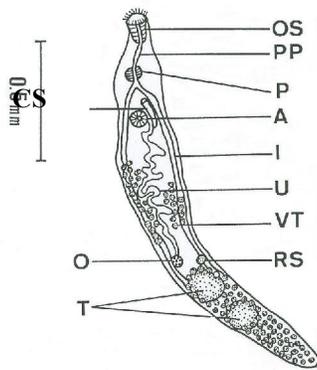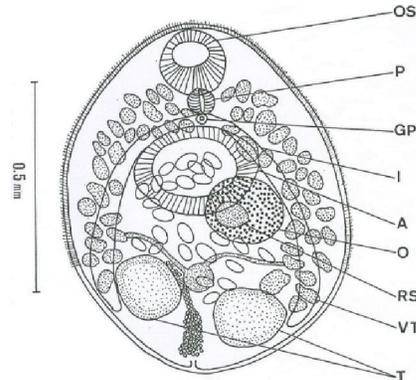
Fig 1 continued
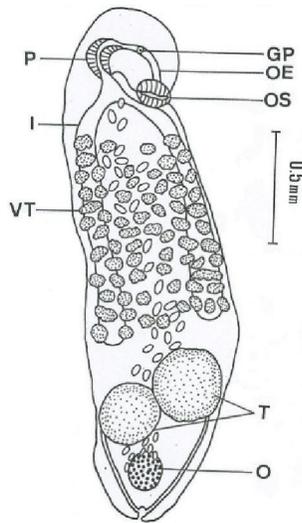
Hexangium loossi (7*)*
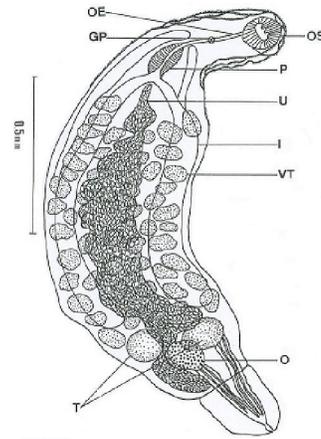
H. ecsomi (8)

Stephanostomum pheraponi (6)

Eurycreadium rivulatum (5)

2.3. Data analysis

Three types of analyses were performed with statistical V7 software: first, Cluster analysis using linkage distance (Norusis, 1999), second, Principal Component Analysis (PCA), using Pearson correlation as a coefficient. This analysis was used to calculate the distance matrix based on standardized data (Rohlf, 1993) and third, non parametric Multi-Dimensional scaling Analysis (MDA) (Sneath and Sokal, 1973) used to distribute the distortions between large and small distances resulting from PCA. These analyses were based on 10 species of Digenea (Operational Taxonomic units, henceforth OTUs) described by 32 morphological characters, coded 1-32 (Table 2). The morphological characters

stated of the Digenea were determined by direct observations or from published data (Table1).

3. Results

3.1. Cluster analysis

Figure 2 shows the phenogram comprising all OTUs in the present study. Two main clusters at values of 16, 20 linkage distance (LD), including two groups, can be distinguished. The first cluster, with two subgroups (LD 2, 3), includes two families, three genera and four species. The first subgroup (LD 2) consists of OTUs of *Lipedapedon sammari* (1) and *L. spiniferi* (2) are put together in one clade. In the second subgroup are located OTUs of *Aponurus sigani* (3) and *Sterrhurina lethrini* (4). They are nested together in a clade at 3linkage distance.

Table 2: Characters and character states used in morphometric analysis of Digenean species

Character	Character state	code	Character	Character state	code
1- Preoral lobe	Absent	0	11-Oesophagus	Absent	0
	Present	1		Very short	1
				Long	2
2- Body	Slender	1	12- intestinal caeca	-Could not seen	1
	Elongate	2		- Extending to posterior third	2
	Flattened	3		-Terminating near posterior extremity	3
	Flattened oval	4			
	Fusiform	5		-Enter ecsoma	4
	Elliptical	6	13-Testes shape	Rounded	1
3- Tegument	Smooth	1		spherical	2
	Serrate	2		Oval	3
	Small spiner	3			
	Spinous	4	14- T. position	Tandem	1
	Glandular	5		Diagonal	2
4- Oral sucker	Elongate	1		Oblique	3
	Rounded	2			
	Spherical	3	15-Testes Location*	-At the middle third of body	1
	Circular	4		- At the mid of the middle third	2
	Oval	5		-At the mid of the post. third	3
	Cone shape	6		-At the junction of middle with post. third	4
5-O.S. Location*	Terminal	1		- At the posterior third	5
	Sub terminal	2		- At the posterior end	6
			16-Genital pore	-At level of pharynx	1
6-Ventral sucker*	Absent	0		-Near mid. oesophageal level	2
	Rounded	1		-In front of acetabulum	3
	Circular	2		-Median preacetabular	4
	Oval	3		-Behind intestinal bifurcation	5
				-Midway between suckers	6
7-V.S. Location*	- Absent	0	17- Ovary*	Rounded	1
	-At the anterior third	1		Globular	2
	-At the junction of anterior with middle third	2		Oval	3
		3		Lobed	4
	-At the anterior part of middle third	4	18-Ovary location*	-In the ant. half of middle third -In the mid of middle third	1
	-At the mid of middle third			-At the ant. part of post. third - At posterior part of middle third	2 3 4
8-Circumoral spines	Absent	0		- At the posterior third	5
	present	1			
9- Prepharynx	Absent	0			
	Short	1	19- Distance between ant. testis & ovary*	shorter than testis length longer than testis length	1 2
	Long	2			
10- Pharynx*	Elongate	1			
	Rounded	2			
	Circular	3			
	Oval	4			

*Characters showing highest factor loading (>0.7)

Table 2: Continued

Character	Character state	code	Character	Character state	code
20-Uterus*	-Between ovary and acetabulum	1	27- Seminal vesicle	Could not seen	1
	-pass anteriorly between the two testes	2		Elongated	2
				Saccular	3
	- From the post. edge of the ovary	3	28- S. V. location		
	-Extend to intracaecal field	4		Could not seen	1
	- Extend to the post. end of body	5		Preacetabulum	2
				Overlapping acetabulum	3 4
	-Fill the body behind acetabulum	6		Nearest acetabulum end	
21- Eggs	Nonoperculated	1	29- Cirrus pouch	Could not seen	1
	Operculated	2		Slender	2
22-Egg Size*	less than 25μm	1	30- Excretory vesicle	Could not seen	1
	more than 25μm	2		Two arms	2
				Glandular	3 4
23-Vitellaria	Follicular	1		V-shape	
	Lobes	2			
	Separate	3	31- Exc. V. Location	Could not seen	1
				Dorsal to pharynx	2
				At the level of pharynx	3
24-Vit. location	-Behind acetabulum	1	32- Ecsoma	Absent	0 1
	-Behind posterior testis	2		Present	
	-Post ovarian	3			
	- Overlapping lobes from pharynx to testes -At the middle third	4 5			
	-Roughly arranged in rows	6			
	-In the posterior region	7			
	-Between pharynx and ovary	8			
25-Seminal receptacle shape	Rounded	1			
	Pyriform	2			
	Oval	3			
	Elongate oval (ellipsoidal)	4			
26- S. R. Location	In front of ovary	1			
	Overlapping ovary	2			
	Right to ovary	3			
	Posterolateral to ovary	4			
	Postovarian	5			

*Characters showing highest factor loading (>0.7)

The second cluster consists of two subgroups, they contain, 5 genera with 6 species belonging to four families. In the first subgroup is the family Cryptogonimidae with two genera and two species. It consists of OTUs of *Paracryptogonimus lutjani* (9) and *Pseudosiphoderoides sargasi* (10). The second subgroup contains 4 species in 3 genera and 3 families.

It consists of OTUs of *Hexangium loossi* (7), *H. ecsomi* (8) (family: Angiodictyidae) in a single clade whereas, *Stephanostomum pheraponi* (6) (Family: Acanthocolpidae) and *Eurycreadium rivulatum* (5) (Family: Fellodistomidae) are located in a separate lineages.

Linkage Distance
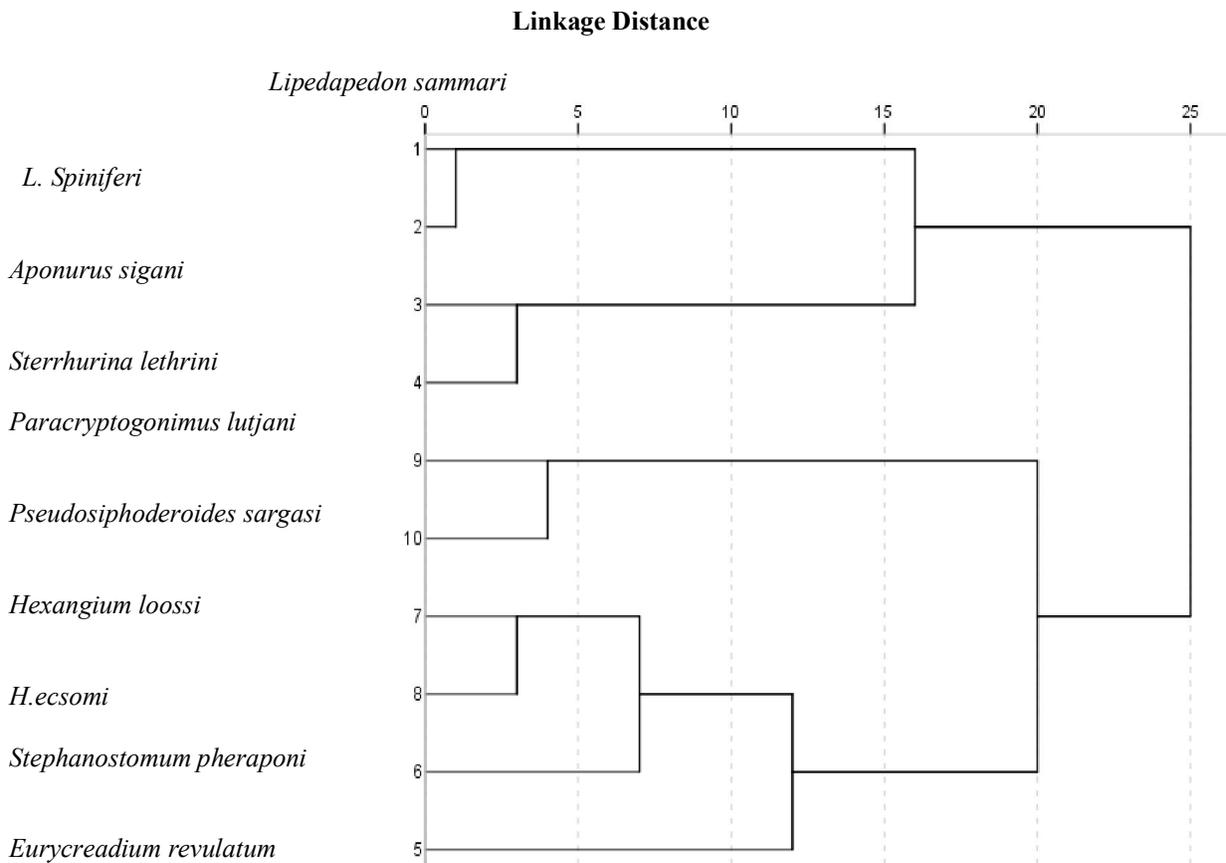
Figure 2: Phenogram of the 10 Digenean species belonging to 6 families: Lepocreadiidae, Hemiuridae, Cryptogonimidae, Angiodictyidae, Acanthocolpidae and Fellodistomidae.

3.2. Principal Components Analysis (PCA)

The principal component axes explain 62.4% of the total observed variations. On the first axis (24.9% of the total variation), segregations are demonstrated between four groups. Group (1): *Lepidapedon sammari* (1) and *L. spiniferi* (2); Group (2): *Aponuru sigani* (3) and *Sterrhinura lethrini* (4); Group (3): *Paracryptogonimus lutjani* (9) and *Pseudosiphoderoides sargasi* (10) and group (4): *Hexangium loosi* (7), *H. ecsomi* (8), *Stephanostomum pheraponi* (6) and *Eurycreadium rivulatum* (5) (Figure 3). The main characters utilized for the separation (characters with high loading factor >0.7) are those pertaining to the location of oral sucker, shape of pharynx, location of ovary, distance between anterior testis and ovary and uterus. The second axis (19.4% of the total variation) reveals a split between four groups. This separation is based mainly on the shape of acetabulum and its location, testes location and egg size. The third axis (18.3% of the total variation) shows a separation between species based on shape of ovary (Table 3). Some of these characters are similar while others are distinguishing characters. Details of PCA characters with highest loading factor (>0.7) for families Lepocreadiidae, Hemiuridae, Cryptogonimidae and Angiodictyidae are shown in (Table 4). Acanthocolpidae is characterized by terminal acetabulum, elongate pharynx, spherical ovary at the posterior part of the middle third, distance between anterior testis and ovary less than one testis, testes in the posterior end. Whereas, PCA characters of Fellodistomids are; sub terminal oral sucker, oval pharynx, rounded ovary at the middle third, distance between anterior testis and ovary more than one testis, testes in the posterior end.
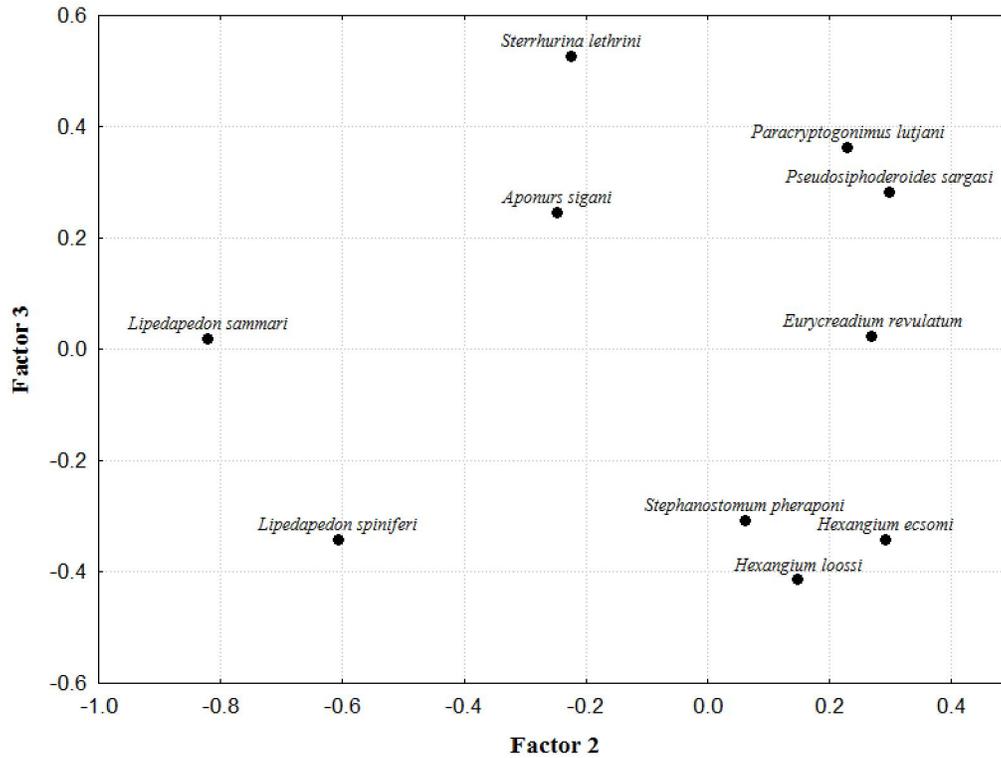
Figure 3: Scatter plot of the 10 OTUs plotted against the second principal component by the third principal component
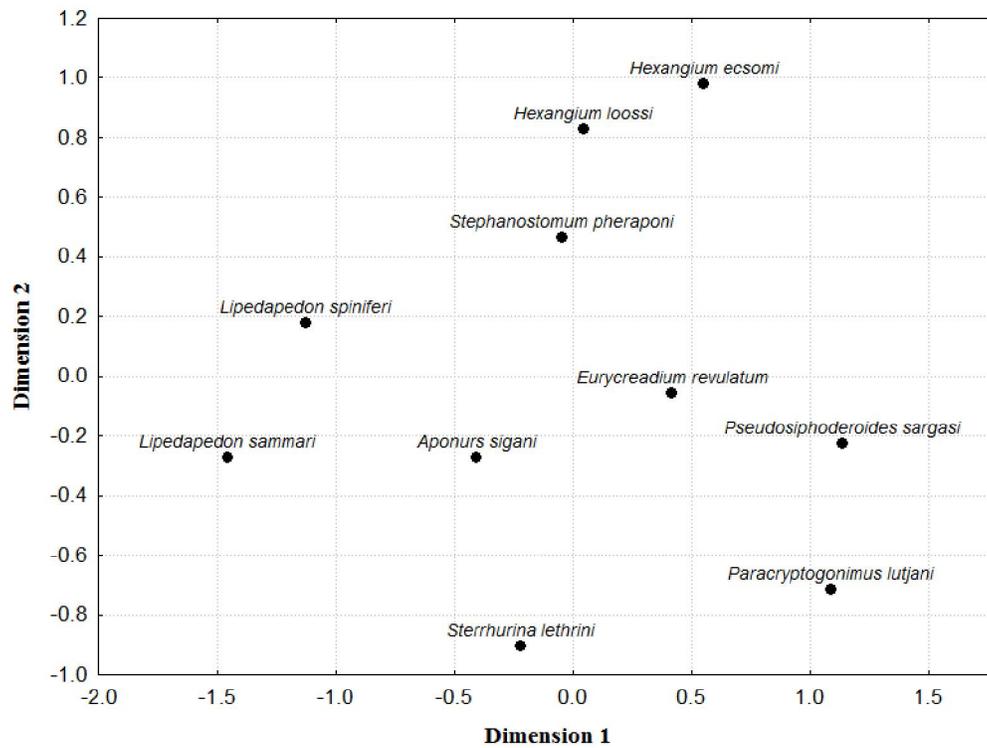
Figure 4: Two dimensional ordering plot with multidimensional scaling of morphological distances between the 10 OTUs of the Digenean species.

Table 3: Morphological characters showing highest factor loading (Marked loadings >0.7)

Characters	Factor1	Factor 2	Factor 3
1- Preoral lobe	-0.627413	0.044406	-0.519321
2- Body	-0.547130	0.256452	0.556520
3- Tegument	-0.549822	-0.020981	0.215495
4- Oral sucker	-0.086333	0.100095	0.434855
5- O. S. Location*	-0.718878*	-0.029234	-0.305819
6- Acetabulum*	0.448374	0.856886*	-0.115508
7Acetabulum Location*	-0.348988	0.709556*	0.173849
8- Circumoral spines	0.248267	-0.341682	0.372164
9- Prepharynx	0.060542	-0.300990	0.532606
10- Pharynx*	0.760903*	0.227376	-0.046761
11-Oesophagus	-0.183695	-0.188555	0.062673
12- intestinal caeca	0.228134	0.277233	-0.644130
13- Testes	-0.386680	0.601045	0.532703
14- T. position	-0.693813	-0.433514	-0.372531
15- T. location*	-0.338980	-0.816946*	0.318571
16- Genital pore	-0.533109	-0.479190	-0.614149
17- Ovary*	-0.162848	0.359824	0.750822*
18- Ovary location*	-0.735853*	-0.330174	0.475958
19- Distance between ant. testis & ovary*	0.804345*	-0.360398	-0.091747
20-Uterus*	-0.700584*	0.550540	0.311652
21- Eggs	0.310611	-0.099272	-0.194586
22- Egg Size*	0.460977	-0.702056*	-0.401285
23-Vitellaria	-0.367437	0.406338	-0.564802
24- Vit. Location	-0.657195	-0.215275	0.684229
25-Seminal receptacle	0.364506	0.501529	-0.438649
26- S. R. Location	-0.371709	-0.531515	-0.215512
27- Seminal vesicle	-0.357963	0.622869	-0.519055
28- S. V. location	-0.445441	0.681529	-0.161705
29- Cirrus pouch	0.248267	-0.341682	0.372164
30- Excretory vesicle	-0.595438	-0.370772	-0.363716
31- Exc. V. Location	-0.608547	-0.214745	-0.434089
32- Ecsoma	-0.627413	0.044406	-0.519321
Total variability (%)	24.880864	19.402794	18.255230

Table 4: Distinguishing and similar characters with highest loading factor (> 0.7) obtained from PCA (Table 3).

OTUs (Fig. 1)	Distinguishing characters	Similar characters
Lepidapedon sammari (1)	Pharynx circular, acetabulum oval, uterus between ovary &acetabulum, testes at the middle third.	Terminal oral sucker, acetabulum lateral at the anterior third of the body, ovary rounded in the mid half of middle third, distance between anterior testis and ovary more than one testis, egg size more than 25μm.
L. spiniferi (2)	Pharynx oval, acetabulum circular, uterus extend to post. end. testes at the mid of post. third.	
Aponurus sigani (3)	Pharynx elongate, ovary oval at ant. part of post. third, acetabulum circular located at the junction of ant. with middle third, testes in the middle third, distance between anterior testis & ovary more than one testis.	Subterminal oral sucker, uterus extend to posterior end, egg size more than 25μm
Sterrhurina lethrini (4)	Pharynx circular, ovary rounded at middle third, acetabulum oval located at the middle third, testes in the anterior part of post. third, distance between anterior testis & ovary less than one testis.	
Paracryptogonimus lutjani (9)	Oral sucker subterminal, uterus extend from ant end of acetabulum to posterior extremity, ovary four lobes	Rounded pharynx, ovary in the middle third, distance between anterior testis & ovary less than one testis, circular acetabulum at the posterior part of middle third, testes at the junction of middle with posterior third, eggs less than 25 μm.
Pseudosiphoderoides sargasi (10)	Oral sucker terminal, uterus fill the body behind acetabulum, ovary oval.	
Hexangium loossi (7)	Ovary is rounded	Subterminal oral sucker, elongate pharynx, ovary at the posterior third, distance between anterior testis & ovary less than one testis, uterus extend to post. end, acetabulum absent, testes are in the mid of post. third, egg size more than 25μm.
H. ecsomi (8)	Ovary is oval	

3.3. Multidimentsional scaling analysis (MDA)

The plot of 10 OTUs on axes of multidimensional scaling analysis is shown in (Figure 4). The analysis confirms the separation of the 10 Digenean species into four subgroups representing six families which is conforming with that of cluster and PCA analyses.

4. Discussion and conclusion

Phylogenetic classifications are based on the discovery of appropriate levels of generality for homologous traits and the recognition of groupings supported by synapomorphic traits. A number of protocols have been advanced for determining the plesiomorphy and apomorphy of traits in a study group (Brooks *et al.,* 1985, p2). PCA characters encountered in this study are compared with those found in other phylogenetic studies of digeneans.

Lepocreadioidea are of considerable interest biologically as they comprise important groups of worms in range of marine habitats (Bray *et al.,* 2009). Family Lepocreadiidae is represented in the present data set by one genus and two species, *Lipedapedon sammari* and *L. spiniferi*, are assembled together in one clade. They are similar in the location of acetabulum which is considered a synapomorphic character for the present two species distinguishing them from the rest of the genus *Lepidapedon* (Al-Zanbagi, 1984 and Abdel Aal *et al.,* 1985). Terminal oral sucker, location and shape of ovary, distance between anterior testis and ovary and egg size are similar in the two species. Recent studies of the lepocreadiidae have stressed the importance of terminal genitalia as an indicator of relationships. The position of the ovary and distance from the anterior testis are clearly phylogenetic characters (Bray and Gibson, 1997 and Bray, 2005). The non-homology of other PCA characters in the present species is indicated by the shape of pharynx, shape of acetabulum, uterus and testes location which is contrary to the non homologic characters of Manter (1966), Watson (1984) and Bray and Cribb (2001).

Family Hemiuridae is nested in the second clade containing two genera, *Aponurus sigani* and *Sterrhurina lethrini*. Brooks *et al.* (1985) provided the first phylogenetic systemic analysis of the group using the taxa and characters listed by Gibson and Bray (1979). Principal component analysis shows a similarity in the oral sucker location, uterus and egg size. The reduction of vitellarium is a synapomorphy for the higher Hemiurata and its occurrence elsewhere can be argued as homoplasious (Olsen *et al.,* 2003). The absence of ecsoma is plesiomorphic for all digeneans and is present in all members of Hemiuridae (Abdel Aal *et al.,* 1984 and Leon-Regagnon *et al.,* 1998). In order to include *Aponurus sigani* in the Hemiuridae, some authors have described vestigial ecsomas. Phylogenetic analysis of the hemiuriforms indicates that the absence is apomorphic within the Hemiuridae (Leon-Regagnon *et al.,* 1996; 1998).

In the second group, family Cryptogonimidae is located in a separate clade containing two genera, *Paracryptogonimus lutjani* and *Pseudosiphoderoides sargasi*. Shape of pharynx, location of ovary, distance between anterior testis and ovary, location of acetabulum, testes location and egg size are seems to be similar characters within the two genera studied. They are differing in oral sucker location, uterus and shape of ovary. The Cryptogonimidae is a large widespread digenean family comprising at least 24 genera with more than 200 species. Cryptogonimids are no longer separated into subfamilies due to insufficient knowledge of the phylogenetic relationships between lineages within the family (Miller and Cribb, 2008). They have disused all subfamilies previously recognized in this family due to obvious difficulties in their differentiation and the uncertain relative 'weight' of various morphological characters used in the systematic of this group. Tkach and Bush (2010, p210) suggested that Cryptogonimidae and its genera need a combined molecular and morphological investigations to aproximate their systematic problems and illuminate many questions of their evolution and host associations.

The last sub group of the present phenogram is made of three families: Angiodyctyidae, Acanthocolpidae and Fellodistomidae. The family Angiodictyidae contains one genus (*Hexangium*) with two species, *H. loosi* and *H. ecsomi*. They exhibit a great limit of similarity in nearly all PCA characters except the shape of ovary. This suggests that the clade exhibits an unusual amount of homoplasy at the generic level. The presence of ecsoma and preoral lobe are not characters of the genus *Hexangium* (Al-Zanbagi, 1984). *Hexangium ecsomi* has all the diagnostic characters of the genus *Hexangium* except the presence of ecsoma and preoral lobe (Al-Zanbagi, 1984). This supports the point of view that these characters are considered as apomorphic characters for *H. ecsomi*.

The family Acanthocolpidae has to some extent generalized plagiorchiidan morphology with a spinous tegument, without external seminal vesicle and a uterine seminal receptacle (Olsen *et al.,* 2003). In the present phenogram, family Acanthocolpidae is in a separate lineage represented by the species *Stephanostomum pheraponi*. Bray and Cribb (2003) summarized some major characters of *Stephanostomum* species, initially dividing the genus by vitellarium type, <10% of hind body devoid of follicles and >10 % of hindbody devoid of follicles. None of the clades recovered shared the same vitelline distribution. Circum-oral spine number, ventral hiatus and uroproct are important diagnostic characters of *Stephanostomum*

species (Bray *et al.,* 2005, p289). These characters are contrary to the present PCA characters of *Stephanostomum pheraponi.* They include

Fillodistomes are distinguished by their smooth tegument, Y- or V-shaped excretory vesicle, post-gonadal uterus, fairly restricted vitelline field, uterine seminal receptacle and an anterior opening of Laurer's canal (Bray, 1988, p101). Family Fillodistomidae is represented in the present set of data by the species, *Eurycreadium rivulatum* which is located in a separate lineage in the present phenogram. From the description of Al-Zanbagi (1984), the generic characters based on Yamaguti (1971) of the present species are: sub terminal oral sucker, acetabulum located at the middle third of the body and testes at the posterior end. These characters are in coinciding with that of PCA characters with highest loading factor (>0.7).

To summarize the findings of this study it can be said that PCA characters of the studied digenean species may be in accordance or contrary to their diagnostic characters. In *Lipedapedon sammari* and *L. spiniferi* (Lepocreadiidae), terminal acetabulum is a synapomorphic character distinguishing these species from the rest of the genus. The presence of ecsoma is an apomorphic character in *Aponurus sigani* (Hemiuridae) while, its presence is an apomorphic one in *Hexangium ecsomi* (Angiodictyidae). In *Stephanostomum pheraponi* (Acanthocolpidae), PCA characters are contrary to those described by Bray and Crib (2003) and Bray *et al.* (2005). Cryptogonimidae and its genera need combined molecular and morphological investigations to approximate their systematic problems. PCA characters of *Eurycreadium rivulatum* (Fillodistomidae) are in accordance with its diagnostic characters. The outcome of the present results demonstrated that numerical analysis of the digenean characters is conforming to their morphology. This will further help in the understanding of host specificity and geographical distribution of digenean taxa. Biodiversity and evolution of these taxa need more attention and discussion in Saudi Arabia.

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