PHYLOGENETIC ANALYSIS OF Sphaerirostris picae (ACANTHOCEPHALA: CENTRORHYNCHIDAE) BASED ON LARGE AND SMALL SUBUNIT RIBOSOMAL DNA GENE

RADWAN N.A.
Department of Zoology, Faculty of Science, University of Tanta, Tanta, Egypt.
*Corresponding Author: Email- nahla_ahmad_mohamed@yahoo.com

Received: December 10, 2012; Accepted: December 18, 2012

Abstract- The purpose of the present study was to add new 18S and 28S DNA gene sequences data to Sphaerirostris picae (Rudolphi, 1819) Golvan, 1960 and analyze the generated sequences to define the taxonomic placement of genus Sphaerirostris and providing a better resolution inside the Palaeacanthocephala. Two regions: 18S and 28S of nuclear ribosomal DNA of S. picae were amplified using polymerase chain reaction and sequenced following the instructions of GATC German company facility. Mealign module in the DNASTar Lasergene V7 was used to design a forward and reverse primer of 28S DNA gene. 18S and 28S DNA gene sequences of S. picae were aligned with sequences for both genes of Palacanthocephalans retrieved from GenBank. Results were analyzed using distance matrix methods UPGMA. The resulting phylogenetic trees suggest a paraphyletic arrangement of the two Palaeacanthocephala orders; Echinorhynchida and Polymorpha depending on the placement of the three echinorhynchids, Transvera, Rhadinorhynchus and Gorgorynchoides in the polymorphid clade. The present study is the first to generate gene sequences of genus Sphaerirostris and discuss its relationships within Palaeacanthocephala. Further comprehensive studies should be done for other species of genus Sphaerirostris and family Centrorhynchidae as all based on molecular phylogenetic analysis to solve their taxonomic overlapping.

Keywords- Sphaerirostris picae, Acanthocephala, Ribosomal DNA, Degenerate primer, Phylogenetic analysis

Citation: Radwan N.A. (2012) Phylogenetic Analysis of Sphaerirostris picae (Acanthocephala: Centrorhynchididae) Based on Large and Small Subunit Ribosomal DNA Gene. International Journal of Parasitology Research, ISSN: 0975-3702 & E-ISSN: 0975-9182, Volume 4, Issue 2, pp.106-110.

Copyright: Copyright©2012 Radwan N.A. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Introduction

Acanthocephalans have been related to Rotifera, Nematoda, Nematomorpha and Gastrotricha [1]. Most phylogenetic analyses of phylum Acanthocephala, based on structural and molecular data, have focused on its relationships to other invertebrate phyla, however studies deal with the inter-relations between different Acanthocephala families and species with uncertain taxonomy are still out of focus.

Verweyen, et al. [2] reported that the first molecular phylogenetic analyses of Acanthocephala made by Garey, et al. [3] confirmed the major taxonomic grouping of the traditional classifications. In this grouping, Palaeacanthocephala is placed close to the Eoacanthocephala, with the Archiacanthocephala being the most basal taxon. According to Herlyn, et al. [4], the monophyly of the Palaeacanthocephala is still a matter of debate. Consequently, the assumed phylogeny of taxa within the Acanthocephala varies, placing either archiacanthocephalans [1,5] or palaeacanthocephalans [6] at the base of the taxon.

Molecular data using 18S and/or 28S DNA gene sequences of family Centrorhynchidae are very limited and has indicated that the classification based on the structure of the proboscis and shape and number of its hooks is not consistent with phylogenetic relationships [3].

Amin, et al. [7] reported that, since the erection of Sphaerirostris (family Centrorhynchidae) as a subgenus of Centrorhynchus Lühne, 1911 [8], its taxonomy has been in a state of confusion and largely dependent on the use of proboscis armature, especially the number of proboscis hooks rows. This character has proven to be extremely variable through the genus, so a good number of synonymies were made. The authors accelerated that this genus is in need to a serious taxonomic revision that will be enhanced by different types of analyses based on the molecular criteria.

The purpose of the present study was to add new 18S and 28S DNA gene sequences data to S. picae as a member of the family Centrorhynchidae (Palaeacanthocephala). Such data may help in understanding and defining the taxonomic placement of genus Sphaerirostris and providing a better resolution inside the Palaeacanthocephala. The phylogenetic relationships of S. picae were examined with 21 acanthocephalan species belonging to 9 related families that all are belongings to the class Palaeacanthocephala.
Phylogenetic Analysis of Sphaerirostris picae (Acanthocephala: Centrorhynchidae) Based on Large and Small Subunit Ribosomal DNA Gene.

as inferred from partial 18S and 28S DNA sequences. The study used an algorithm ClustalW2 for designing degenerate primers which are of particular value in amplifying homologous genes from different organisms.

Materials and Methods

Specimen and DNA Isolation

S. picae was collected from the small intestine of the Hooded crow Corvus corone cornix, Linnaeus 1758, which is a common resident inhabiting cultivated land and wooded terrain in Nile Delta and valley in Egypt. For identification, worms were preserved in 70% ethanol, stained with Mayer’s carmine, mounted in Canada balsam [9] and identified according to Dimitrova, et al. [10,11]. Specimens were stored at -20°C until nucleic acid was extracted. DNA extraction followed Chloroform-Isoamyl alcohol method [12]. Three replicates of one worm were homogenized on ice in TE buffer (10 mM Tris–HCl, pH 7.5, 10 mM NaCl, 1 mM EDTA) and digested by adding 400µl of TE buffer, 40µl 10% sodium dodecyl sulphate (SDS) and 10µl of 20mg/ml proteinase K, then incubated in 55°C overnight. The supernatant was extracted twice with buffered phenol (pH 8.0) and once with chloroform/isomyl alcohol (24:1). The aqueous layer (~ 400µl) was collected and 16µl of 5 M NaCl and 420µl ice cold isopropanol were added. The sample was cooled in the refrigerator at -20°C for 10 min and centrifuged for 5 min at 15,000 rpm. The supernatant was poured off and after complete drying of the pellet, 50µl of the TE buffer was added and left for 30 min to complete dissolving. The sample was stored at -20°C till use.

Designing the Degenerate Primers of 28S DNA Gene

The DNA sequences of 28S DNA gene of 23 species accession numbers from AB500157.1 to AB500179.1 were retrieved from the sequence database of NCBI. FASTA file format of the previous sequences was used in the algorithm type of the Blast (Basic local alignment search tool). Expected values (E-values) was calculated to infer homology and distance of similarity between the sequences [13].

The mealign module impleknted in the DNASTar Lasergene V7 used the Clustal W2 algorithm for alignment collected sequences and produced the conserved blocks, which were used to design the degeneracy primers. All primer characteristics were adjusted. Primer Blast tool [14] was used to detect the homology between the designed primers and targeted templates in the GenBank database (http://www.ncbi.nlm.nih.gov/tools/prime-blast), the position of degeneracy nucleotides, and the uniqueness for the forward and reverse primers.

The PCR insilico module (Ruslan- Kalender version 4.0.8 university of Helsinki, Finland) was used to produce all the details of the PCR program such as the optimum temperature for the forward and reverse primer, the predict PCR product, and the positions of primers on the template sequences.

DNA Amplification and Sequencing

Two regions of nuclear ribosomal DNA were amplified using polymerase chain reaction (PCR). The first is the 18S DNA using the primers:

forward.5´GGGCGGTAAGACGCAAGTGT TT-3´ and reverse. 5´GGAAGGGGCTCGCAAGTTATT 3´ [2].

The second is 28S DNA gene using degenerate designed primers:

forward 5´GTAAGGCGCAGTAAGTGAAGT -3´ and reserve 5´TACACACTCTGAGTAGA A AC C AIC CA3´.

PCR mixture (50µl) contained 25µl Maxima Hot Start PCR Master Mix (Fermentas), 1µl final concentration of each primer, 17µl nuclease-free and 6µl of template DNA solution. PCR cycling parameter for 18S DNA gene included initial denaturation period at 95°C for 10 min, annealing at 58°C for 1 min and extension at 72°C for 45 seconds followed by another 35 cycles at the same denaturation, annealing and extension temperatures and finished with a 10 min final extension at 72°C (after the modification of Garey, et al. [2]). A negative control (no template) was also performed for every PCR run to determine any potential contamination of the PCR products.

PCR products were evaluated by electrophoresis of 4µl PCR mixture through 1% agarose gel against 1 kb ladder (Fermentas), and cleaned up using Gene JET™ PCR purification Kit (fermentas) and the purified DNA was stored at -20°C. 2µl of PCR product was sequenced using an ABI big dye terminator kit following the instructions of GATC German company facility and an ABI 3730xl DNA sequencer.

Phylogenetic Analysis

In addition to the 18S and 28S DNA gene sequences generated in this study, additional 21 sequences for 18S DNA and 16 for 28S DNA genes of other Paleacanthocephalans and one out group were obtained from the GenBank [Table-1]. The ClustalW2 algorithm was used to visualize the consensus sequences and the dendogram. The dendograms were built depending on the molecular based evolution set by Pearson and Lipman [15]. The matrix methods UPGMA (Unweighted-Pair-Group Methods with Arithmetic Mean) was used to present the dendograms and the relation between homologous sequences based on the produced multiple sequence alignment [16].

Results

DNA Sequencing and Data Set

The sequence obtained for 18S DNA is a 385 nucleotides long including primers with 59% GC content [Fig-1], while 28SDNA sequence is a 282 nucleotide long, including primers with 58% GC content [Fig-2]. The 18S and 28S DNA sequences for S. picae are in the process of publication in the nucleotide data set in GenBank (NCBI). The homologous sequences for the two generated genes were retrieved from the GenBank and processed all together by mealign tool of DNASTar Lasergene V7 to produce the multiple sequence alignment. Highly conserved blocks were detected, the best position of consensus sequence was assigned.

Phylogenetic Analysis

Molecular analyses of both 18S and 28S DNA using matrix unweighted pair-group method with arithmetic mean (UPGMA) revealed the degree of genetic similarity between S. picae and all retrieved species [Fig-2], [Fig-3] using full optimization and 1000 bootstrap replicates.
Table 1- Species related to class Palaeacanthocephala and represented in the phylogenetic analyses with Gene bank accession numbers.

| Order               | Family         | Species                              | 18rDNA Accession | 28rDNA Accession |
|---------------------|----------------|--------------------------------------|------------------|------------------|
| Polymorphida        | Centrorhynchida| Centrorhynchus sp.                    | AY830155         | AY829104         |
| Polymorphida        | Sphaerirostris picae | Present study | Present study |
| Polymorphida        | Polymorphida  | Polymorphus sp                        | AF01938          |
|                    |                | Profilicollis botulus                 | EU267805         | EU267818         |
|                    |                | Pseudocorynosoma constrictum         | EU267800         | EU267812         |
|                    |                | Corynosoma strumosum                 | EU267804         | EU267816         |
|                    |                | Andracantha gravida                  | EU267802         | EU267814         |
|                    |                | Southwellina hispida                 | EU267807         |                  |
|                    |                | Artyrhynochynchus brevis             | AF064812         |
|                    |                | Ibiryynchus dimophia                | GQ981436         | GQ981437         |
| Polymorphida        | Plagiorhynchida| Plagiorhynchus cylindraceus          | AF001839         | AY829102         |
| Echinorhynchida     | Rhabdorhynchida| Gorgorhynchoïdes bulloclipi          | AY830154         | AY829103         |
|                    |                | Rhabdorhynchus sp                    | AY062433         | AY829099         |
| Echinorhynchida     | Transvenida    | Pararadiorhynchus sp                 | HM545903         |
|                    |                | Transvena annulospinosa              | AY830153         | AY829098         |
| Echinorhynchida     | Cavisomida     | Filisoma bucentum                    | AF064814         | AY829110         |
| Echinorhynchida     | Artyrhynochiida| Acanthocephaloides propinquus        | AY830149         | AY829100         |
| Echinorhynchida     | Pomphorhynchida| Pomphorhynchus laevis               | AY216124         |
| Echinorhynchida     | Echinorhynchida| Echinorhynchus gadi                   | AY216123         | AY216146         |
| Echinorhynchida     | Polymorphida   | Polymorphus sp                       | AF001838         |
| Echinorhynchida     | Plagiorhynchida| Plagiorhynchus cylindraceus          | EU267808         | EU267817         |

Fig. 1- Nucleotide sequence of 361 bp fragments of 18S DNA gene of Sphaerirostris picae.

1  TTATGTTTGT CTCCTGTAT GGCTCTACTT GTGGTCTCG
41 GTGAGAGCC ACTGTCCTCT TGGAAAAACT CTGTTGCTCT
81 AATGCCAGCT AACTGCTGA ATATACGCTG ATGGTAGAT
121 GAAATGGGCT CCTGGCCTGG TTTGGTTGAT TACNAAAGC
161 AAAGACATG ATTAATGGCG ACAGACGGCG CATGTCTAT
201 TGGCTGTGCTA AATGGAAT TCTGTAACCA TGGCAAAACA
241 AACACATGCA AAAACATTTG CAAAAATGT TTTCTTAT
281 CACAAACCAA AGTTAAAGGA TCAAAAAAAT TAAATACCC
321 TCTATTTTCT AAGCGTAACCC TGGCGCGGCC AGGAGCTCCC
361 CAAGGCAAATA ACTTGCCAN CCCTT

Fig. 2- Nucleotide sequence of 281 bp fragments of 28S DNA gene of Sphaerirostris picae.

For 18S DNA gene, the analysis yielded a single tree [Fig-3], [Fig-4], where Palaeacanthocephalans show high diversity. The tree represents order Echinorhynchida and order Polymorphida in a paraphyletic arrangement. The three major clades of order Echinorhynchida lack genus Gorgorhynchoïdes, which is allocated it in the polymorphid cluster. The first clade of Echinorhynchida carries both Pomphorhynchus (family Pomphorhynchidae) and Echinorhynchos (family Echinorhynchidae) with a genetic similarity 95.7%. Acanthocephalus (family Echinorhynchidae) and Filisoma (family Cavisomidae) have high genetic similarity 96.8%, the clade that carries both genera is sister to that of Echinorhynchida (family Echinorhynchidae) and Acanthocephaloides (family Artyrhynochinae). The clade carries Transvena and Pararadiorhynchus (family Transvenidae) and Rhabdorhynchus (family Rhabdorhynchidae) appears separate from the other 2 major clades.

Table 1 continues...
Phylogenetic Analysis of Sphaerirostris picae (Acanthocephala: Centrorhynchidae) Based on Large and Small Subunit Ribosomal DNA Gene.

Hexaglandula (97.7%) and the second includes Andracantha with Corynosoma (98.4%).

Analysis based on 28S DNA gene [Fig-5][Fig-6] represents two major clades of Palaeacanthocephala. The first is of Echinorhynchida, which lacks the two closely related genera; Transvena and Rhadinorhynchus (30.3 %genetic similarity), in addition to Gorgorhynchoides which are all allocated in the polymorphid clade. The clade of Transvena and Rhadinorhynchus formed a sister group to Sphaerirostris (family Centrorhynchidae). Echinorhynchus is the most basal genus in the echinorhynchids. It has 98.7% genetic similarity with Acanthocephalus which is related to the same family (Echinorhynchidae), being relatively more close to Acanthocephaloïds (Arhythmicacanthidae) (90% genetic similarity).

Depending on 18S DNA gene analysis, S. picae has high genetic similarity ratio (92.7%) with Centrorhynchus sp (Centrorhynchidae) [Fig-3][Fig-4], while in that of 28S DNA gene [Fig-5][Fig-6], both species are separated by a Echinorhynichids clade encloses Transvena, Rhadinorhynchus and Gorgorhynchoides.

Discussion

The relationship of the Acanthocephala to other invertebrate phyla has been estimated recently by analysis of structural and molecular data. The first molecular phylogenetic analyses within Acanthocephala [2] confirmed the major taxonomic grouping of the traditional classifications. In that analysis, Palaeacanthocephala was placed close to the Eoacanthocephala, while Polyacanthocephalans appeared sister to the Eoacanthocephalans with the Archiacanthocephala being the most basal taxon [3]. Previous studies of acanthocephalans combined data sets of both, 18S and 28S DNA [2,17]. The present study data set adds to the most recent analyses of Palaeacanthocephala relationships by García-Varela and Nadler [17], Garey, et al. [2] and Verweyen, et al. [3].

Palaeacanthocephala include two orders; Echinorhynchida and Polymorphida. According to Verweyen, et al. [3], these species rich taxa include 83 genera and 594 species of Acanthocephalans. Both orders demonstrate high morphological diversity, which may explain why traditional identification keys have distinguished taxa according to their final hosts. Based on traditional classification, Echinorhynchida is divided into 10 families and 339 valid species, while Polymorphida includes only three families and a total of 255 valid species [3].

The present study analyzed the relationships between the three polymorphid families and six of the echinorhynchids families using both 18S and 28S DNA gene analytical methods which revealed the paraphyletic assembly of Palaeacanthocephala. This finding is incorporated by the previous analysis of Herlyn, et al. [4] and Verweyen, et al. [3], however both studies depended only on the analysis of 18S DNA gene. Otherwise, phylogenetic analysis based on morphological and ecological characters of Palaeacanthocephala [5,18] revealed the monophyletic form of this class.

The present 18S DNA gene analysis indicates that the family Rhadinorhynchidae (Echinorhynchida) is paraphyletic, where the analysis allocates Gorgorhynchoides bullocki in the Polymorphid clust.

The replacement is confirmed by the present analysis based on 28S DNA gene, where Rhadinorhynchus sp which belongs to the same family (Rhadinorhynchidae), presents together with Gorgorhynchoides bullocki in the same position. This finding was in agreement with the previous reports of Verweyen, et al. [3].
et al. [6] and Garcia-Varela and Nadler [17]. In addition, Shih, et al. [19] traditionally classified Rhadinorhynchus pristis as a member of order Polymorphida. It is worth mentioning here that Gorgorhynchoids has trunk hooks on its praesoma (only some echinorhynchid acanthocephalans have irregularly arranged hooks on the trunk) while the regular distribution of hooks on the trunk is one of the most common features within the polymorphids.

Based on 18S and 28S DNA gene analysis, the polymorphid; Plagiorhynchus cylindraceus is placed between Polymorphid and Echinochorychid clades. Morphologically, this species has some Echinochorychid characters, where the cylindrical trunk has anterior hooks and the males have six cement glands [3].

An interesting finding in the present study, revealed by 18S DNA gene analysis, is the relatively close relation of Sphaerirostris picae (family Centrorhynchidae) with Plagiorhynchus cylindraceus (Plagiorhynchidae) when compared with the relation of S. picae with the other species. Morphologically, the later species is characterized by cylindrical fusiform aspinose trunk, slender lemnisci, and 6 elongate tubular cement glands in the males. Sphaerirostris has the same characters except only 4 cement glands. Otherwise, Amin, et al. [7] reported the presence receptacle process at the anterior end of the proboscis receptacle of S. picae, this trait distinguishes this species from all other species of its genus. A similar structure was reported previously in the proboscis of Plagiorhynchus digiticephalus (Plagiorhynchidae) [20]. These facts might introduce a justification of the close relation of both genera.

The present analysis of 18S DNA gene shows high similarity ratio (92.7%) between Sphaerirostris and Centrorhynchus. Dollfus and Golvan [21] listed both Centrorhynchus teres Westrum, 1981 and Centrorhynchus (Sphaerirostris) picae Rudolphi,1819 as valid species. The present analysis of 28S DNA gene separates Sphaerirostris from Centrorhynchus by two clades represents three Echinochorychid species. Morphologically, Golvan [8] erected Sphaerirostris Rudolphi, 1819 as subgenus of Centrorhynchus Lühe,1911, later he [22] listed 26 species of Sphaerirostris by reversing the synonyms, where the differentiation was based on the proboscis armature [7]. Relations between the two main genera of Family Centrorhynchidae need further analysis based on both morphological and molecular data.

To the best of our knowledge, no data set are available for either 18S or 28S DNA genes of Sphaerirostris, the present study is the first to add gene sequences to this genus. As the taxonomy of Sphaerirostris was largely based on the proboscis armature, and this character has proven to be extremely variable [7], further comprehensive studies should be done for other species of this genus based on molecular analysis to solve their taxonomic overlapping.

Acknowledgement

The author would like to thank Dr. Abdallah A. Sharaf lecturer in Genetic Engineering Unit, Faculty of Agriculture, University of Ain - Shams for his critical help the molecular work.

References

[1] García-Varela M., Cummings M.P., Perez-Ponce de León G., Gardner S.L., Lacletteam J.P. (2000) Journal of Molecular Evolution., 50, 532-540.

[2] Garey J.R., Near T.J., Nonnemacher M.R., Nadler S.A. (1996) Molecular Phylogenetics and Evolution, 43, 287-292.

[3] Verweyen L., Klimpel S., Palm H.W. (2011) PLoS One, 6(12), e28285.

[4] Herlyn H., Piskurek O., Schmitz J., Ehlers U., Zischler H. (2003) Molecular Phylogenetics and Evolution, 26, 155-164.

[5] Near T.J., Garey J.R., Nadler S.A. (1998) Molecular Phylogenetics and Evolution, 10, 287-298.

[6] Herlyn H., Martini N. and Ehlers U. (2001) Systematic Parasitology, 50, 105-116.

[7] Amin O.M., Heckmann R.A., Halajan A., Eslemi A. (2010) Journal of Parasitology, 96(3), 561-568.

[8] Golvan Y. (1956) Bullettin de l'istitute dafrique Noire (Ser. A.), 18, 732-785.

[9] Amin O.M. (1998) NOAA Technical Report NMFS, 135.

[10] Dimitrova Z. and Genov T. (1992) Folia-Parasitologica, 39(3), 235-247.

[11] Dimitrova Z., Georgiev B., Genov T. (2000) Acta. Zoologica. Bulgariica., 52(3), 3-32.

[12] Benesh D.P., Hasu T., Soumalainen L.R., Valtonen E.T., Tirola M. (2006) International Journal of Parasitology, 36(2), 247-254.

[13] Ezz M.A., Soliman M.H., Gamal El-Din A.Y., Abdelsalam A.Z.E. (2010) Journal of Life Science, 4(6), 1934-7931.

[14] Rozen S., Skaletsky H. (2000) Methods. Molecular Biology. 132, 365-386.

[15] Pearson W.R., Lipman D.J. (1988) Proceeding of Natl. Academic Science, USA., 85(8), 2444-2448.

[16] Sokal R.R., Michener C.D. (1958) University of Kansas Science Bulletin, 28, 1409-1438.

[17] García-Varela M., Nadler S. (2005) Journal of Parasitology, 91, 1401-1409.

[18] Monks S. (2001) Systematic Parasitology, 48, 81-116.

[19] Shih H.H., Chen H.Y., Lee C.Y. (2010) Taiwania, 55(2), 123-127.

[20] Amin O.M., Heckmann R.A., Ha N.V. (2008) Acta. Parasitologiica, 56(1), 67-77.

[21] Dollfus R.P., Golvan Y. (1957) Note Rectificative Bulletin IFAN, Ser. A., 19, 412-416.

[22] Golvan Y. (1994) Res. Parasitology, 54, 135-205.

International Journal of Parasitology Research
ISSN: 0975-3702 & E-ISSN: 0975-9182, Volume 4, Issue 2, 2012