

**Molecular characterization of *Cotugnia polycantha* (Cestoda, Cyclophyllidea, Davaineidae) infecting doves (*Streptopelia senegalensis*) and pigeons (*Columba livia Domestica*) from Egypt**

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**Abstract:** The genomic DNA was extracted from cestode parasites, *Cotugnia polycantha* from two different hosts, doves (*Streptopelia senegalensis*) and pigeons (*Columba livia domestica*). The random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied to differentiate between *C. polycantha* infecting doves and pigeons. Eight primers of arbitrary sequences were used in the PCR reactions. The eight primers screened gave total amplified fragment markers 133.

The total number of unique bands was 25 and the highest polymorphism percentage (63.63%) was obtained among the two specimens. Molecular analysis of the present data, showed that *C. polycantha* infecting doves (*S. senegalensis*) differs from that infecting pigeons (*C. livia domestica*).

So, *C. polycantha* infecting doves could be named *C. polycantha streptopeli* and *C. polycantha* infecting pigeons, *C. polycantha columbi*.

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**Key Words:** Doves – Pigeons – Cestode- Genomic DNA – DNA fragments.

### 1. Introduction

The phylogeny of Eucestoda has been a matter of controversy for a century (Mariaux, 1996; Hoberg et al., 1997). Initial cladistic studies based on morphological characters (Brooks et al., 1991) were attempted to identify and specify the relationships among the major lineages of Eucestoda. This represents the first modern phylogenetic study of these parasites and constitutes a foundation on which to base more detailed analysis (Justine, 1998). Subsequently, using a revised and greatly augmented morphological data base, and for the first time, including spermatological characters, Hoberg et al. (1997) reported that the addition of any new characters of cestodes might increase the accuracy of phylogenetic inference.

Molecular techniques have become widely accepted all over the world. They provide a more specific method than methods conventionally employed in epidemiological studies (Coote, 1990; Erlich et al., 1991; Barker, 1994; Rognlie et al., 1994; Kramer and Schnieder, 1998; Heckerroth and Tenter, 1999; Mostafa et al., 2003 and Aldemir, 2006). Molecular techniques such as PCR and its variants are used for the diagnosis of

parasitic diseases and identification of parasites (Aldemir, 2006).

Molecular approaches are the most effective and accurate means for the detection of many organisms and for screening of genetic variation among populations (Wongsawad and Wongsawad, 2010).

The RAPD technique is based on amplification of a random DNA segment with a single primer of arbitrary nucleotide sequence and using polymerase chain reaction (Welsh and McClelland, 1990; Williams et al., 1990; Mohammedzadeh et al., 2007 and Nuchprayoon et al., 2007).

Molecular techniques based on genomics are very useful for epidemiological and diagnostic tools as well as for research on genetic variation of parasitic organisms (Mas-Coma et al., 2005 and Meshgi et al., 2008)

DNA Polymorphism assay based on random amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR) has been proved useful for analyzing the inter- and intra-specific genetic variations and phylogenetic relationships (Gasser, 2005; Mas-Coma et al., 2005; Nuchprayoon et al., 2007 and Rokni et al., 2010).

The technique is very rapid, simple and generates reproducible fingerprints of the

PCR products. In addition, it neither depends on previous knowledge or availability of the target DNA sequences nor requires DNA hybridization (Mohammedzadeh *et al.*, 2007; Nuchprayoon *et al.*, 2007 and Sripalwit *et al.*, 2007).

Ahmed and Abdel-Moaty (2011) reported that *C. polycantha* infecting doves, *S. senegalensis*, differs from that infecting pigeons, *C. livia domestica*. Sperm ultrastructure was used as a new tool for identification.

In the present study, *C. polycantha* infecting two different hosts, doves *Streptopelia senegalensis* and pigeons *Columba livia domestica*, were compared using random amplified polymorphic DNA (RAPD) analysis for differentiation between them.

## 2. Materials and Methods

### Parasite preparation

Adult tape-worms were collected alive from the small intestine of naturally infected and recently killed doves, *Streptopelia senegalensis*, and pigeons, *Columba livia domestica*, from Sharkia Province, Egypt. The collected parasites were rinsed several times with 0.65% saline solution. Some were mounted as a whole and prepared as permanent slides for investigation and identification. The remaining flukes were kept in 70% ethyl alcohol at  $-20^{\circ}\text{C}$  for DNA extraction.

### Genomic DNA extraction

Genomic DNAs were isolated on a small scale from 1ml of adult worms using multisource genomic DNA, Mini-Prep Kit, Axgene Biotechnology, U.S.A Cat. No.110420-25, according to manufacture manual.

### RAPD analysis

Primers for RAPD were tested in parasite specimens and eight of them were selected due to successful amplification for two specimens. Names and sequences of primers are as shown in table (1).

Table (1): RAPD primers

Name	Sequence
OPA-10	GTGATCGCAG
OPB-O3	CATCCCCCTG
OPA-14	GCTGGTCTGT
OPB-19	ACCCCCGAAG
OPC-01	TTCGAGCCAG
OPC-13	AAGCCTCGTC
OPD-01	ACCGCGAAGG
OPD-13	GGGGTGACGA

PCR analysis was performed in 25  $\mu\text{l}$  volume containing 2.5 mM MgCl<sub>2</sub>, 0.2 mM of dNTPS, 20  $\mu\text{M}$  primer, 50 ng genomic DNA and 1.0 unit Taq DNA polymerase (Bioren, Germany). All reactions were performed in a Perkin Elmer 2400 Thermal cycler. RAPD program was performed as 1 cycle of  $94^{\circ}\text{C}$  for 4 min (primary denaturation), 40 cycles of  $94^{\circ}\text{C}$  for 1 min (denaturation), then  $35^{\circ}\text{C}$  for 1 min. (annealing),  $72^{\circ}\text{C}$  for 1 min (extension) and a final extension step of  $72^{\circ}\text{C}$  for 10 min.

### Detection of PCR products

The products of both RAPD based PCR analyses were detected using agarose gel electrophoresis (1.2% in 1X TBE buffer), stained with ethidium bromide (0.3  $\mu\text{g}/\text{ml}$ ), visually examined with UV transilluminator and photographed using a CCD camera (UVP, UK).

### Data analysis

Clear, unambiguous and reproducible bands recovered through different techniques were considered for scoring. Each band was considered as a single locus. Data were scored as '1' for the presence and '0' for the absence of a given DNA band. Band size was estimated by comparing it with 1-kb ladder (Invitrogen, USA) using Totallab, TL120 1D v2009 (nonlinear Dynamics Ltd, USA).

## 3. Results

Parasitic genomic DNA extracted from *C. polycantha* recovered from two different hosts, *S. senegalensis* and *C. livia domestica* was amplified using 8 primers provided distinct patterns. The RAPD profiles of the two parasites with every primer are shown in figure 1 and table 2. The analysis of RAPD profiles indicated the presence of genetic variation between the two specimens as there

was considerable variation in the RAPD profiles among them using on the basis of number and intensity of bands. The 8 primers selected produced clearly distinguishable band patterns and yielded a total of 133 scoreable RAPD fragments. The 133 bands ranged between 143 and 1367 bp (base pair) in length by comparison with a 1-kb ladder. The number of bands generated by every primer, number of polymorphic bands and number of bands of the two specimens are shown in table 2. The higher number of bands was generated by primer B19 and A14, amplifying the two specimens. The parasitic specimen preparation from *S. senegalensis* produced a higher number of DNA fragments (69 DNA fragments) than that from specimen 2 from *C. livia domestica* (64 DNA fragments) as shown in table 2.

All primers except primer C13 generated specific DNA fragments markers for the both two specimens or one of them. The total

number of unique markers was 25 [15 for (1) and 10 for (2)] as shown in Fig. 1 and table 2.

All primers produced 54 monomorphic bands for the two specimens (1 and 2), while polymorphic ones without unique bands were negative (0) (table 3). All primers produced unique bands and polymorphic with unique (25). The total number of bands was (79) and the highest percentage of polymorphism was (63.63) as shown in (table 3 and Fig. 1).

Seven primers produced DNA unique bands for two specimens.

Primer A10 produced seven unique bands 352, 362, 536, 628, 653, 717 and 968 bp, primers B03 and A14 produced one unique band for each of 672 and 816 bp respectively. Primer B19 produced six bands, 347, 373, 411, 829, 1168 and 1367 bp. Primers C01 and D01 produced three unique bands for each (272, 383 and 1256 bp) and 299, 364 and 1003 bp, respectively. Primer D13 produced four unique bands 143, 254, 343 and 932 bp.

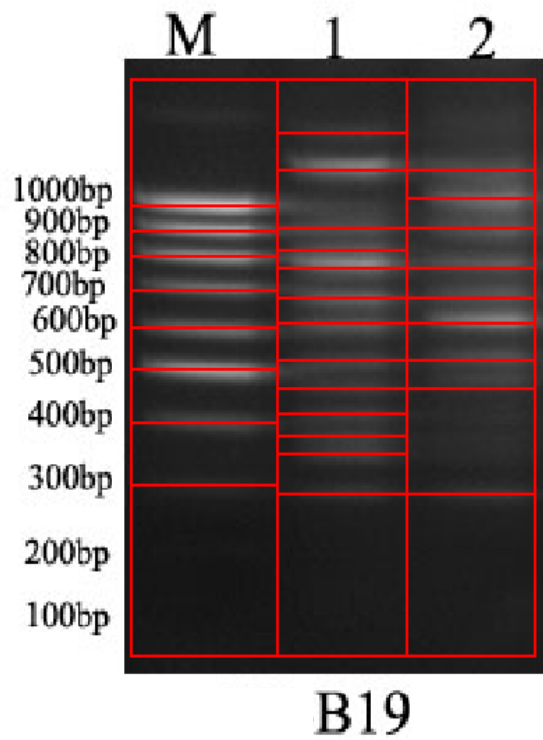
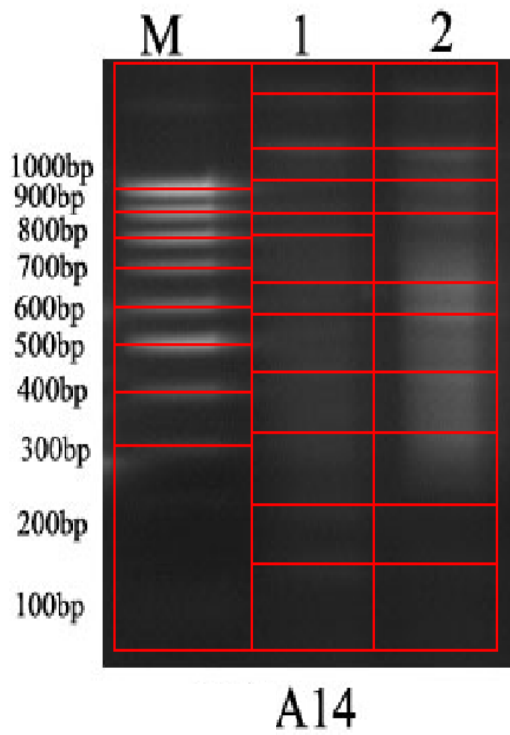
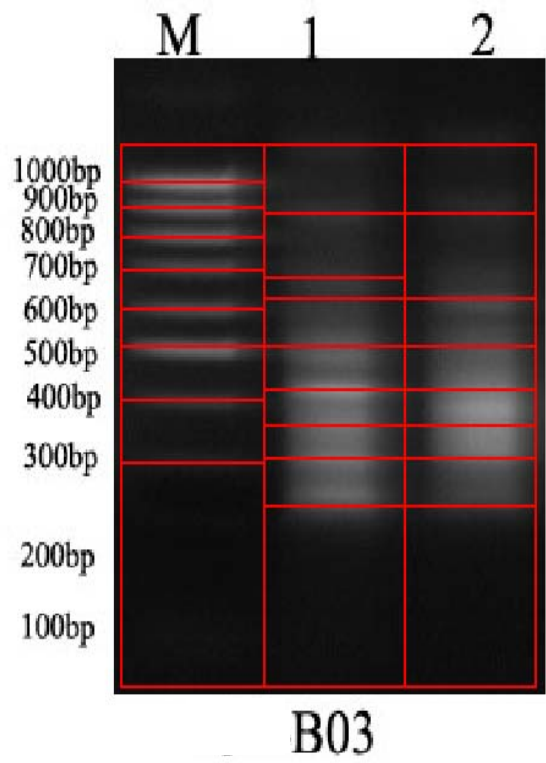
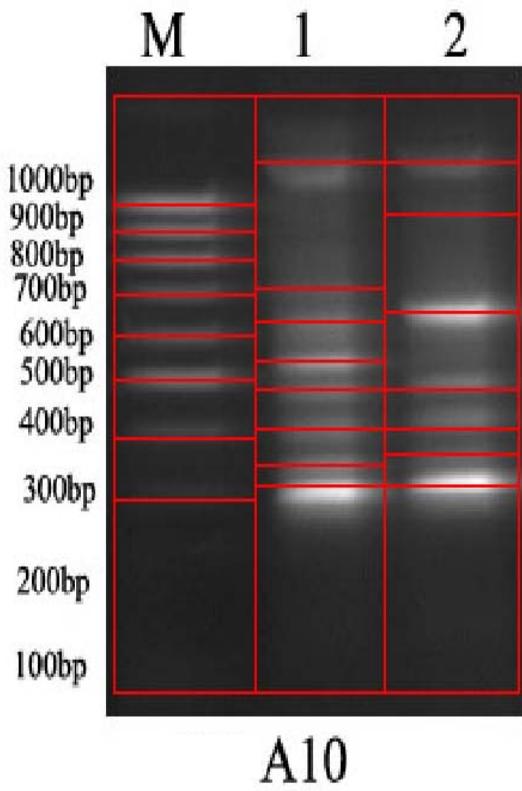
**Table (2):** Number of amplified fragments marker of *C. polycantha* from two specimens based on RAPD-PCR analysis

<i>C. polycantha</i> from		RAPD Primers								Total
		A10	B03	A14	B19	Col	C13	D01	D13	
<i>S. senegalensis</i> (1)	AF	8	8	11	13	7	7	9	6	69
	SM	4	1	1	5	1	0	3	0	15
<i>C. livia domestica</i> (2)	AF	7	7	10	9	8	7	6	10	64
	SM	3	0	0	1	2	0	0	4	10
Total TSM		7	1	1	6	3	0	3	4	25
Total TAF		15	15	21	22	15	14	15	16	133

AF: amplified fragment; SM: marker including either the presence or absence of a band; TAF: total amplified fragment; TSM: total number of specific marker across a each specimen.

**Table (3):** Polymorphism data for 8 primers using Gel Images for the two specimens

Gel image \ Primers	A 10	B 03	A14	B19	Col	C13	D 01	D13	Total
	Monomorphic bands	4	7	10	8	6	7	6	6
Polymorphic (without unique)	0	0	0	0	0	0	0	0	0
Unique bands	7	1	1	6	3	0	3	4	25
Polymorphic (with unique)	7	1	1	6	3	0	3	4	25
Total number of bands	11	8	11	14	9	7	9	10	79
Polymorphism %	63.63	12.5	9.09	42.86	33.33	0%	33.33	40.0	33.53



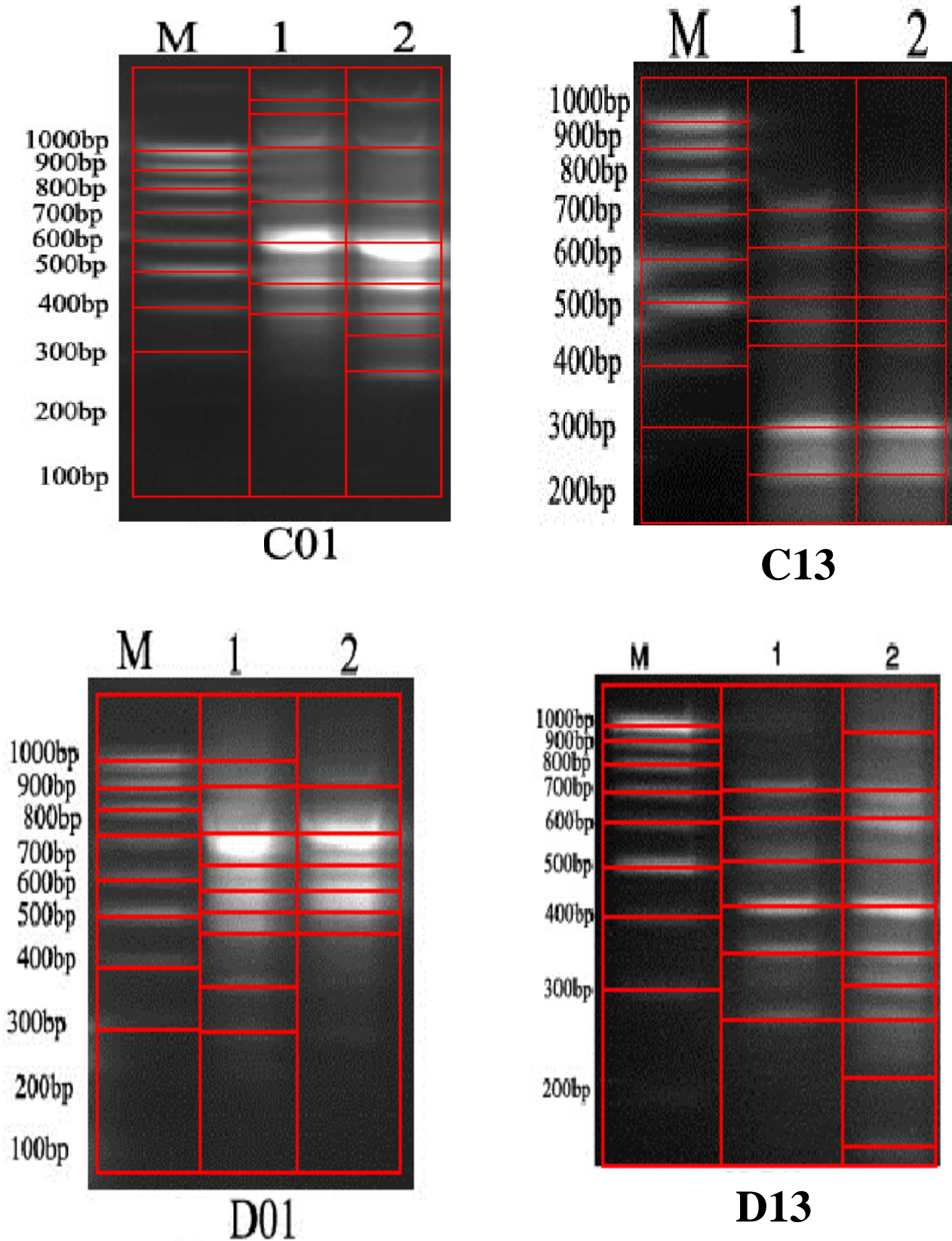


Fig. (1): Agarose gel electrophoresis showing the RAPD profiles of *C. polycantha* infecting doves, *S. senegalensis* (1) and pigeons, *C. livia domestica* (2) generated by 8 primers. 1-kb DNA ladder.



#### 4. Discussion

The taxonomy of different species of cestodes represents insufficiently solved task and the reliable identification of many taxa by classic taxonomic methods is problematic. This is particularly the case for soft bodies animals such as cestodes. However, detailed studies of morphological and genetic variation of different cestode groups have been performed using morphological, biometrical, cytogenetic and isoenzyme approaches (Kralová and Spakulová, 1996; Eom *et al.*, 2002; Maravilla *et al.*, 2003).

However, recent advances in molecular biology, in particular the amplification of specific DNA regions via the polymerase chain reaction (PCR) and the improvement of direct deoxy sequencing techniques may allow to distinguish closely related species by comparing their DNA (Coote, 1990; Erlich *et al.*, 1991; Barker, 1994; McManus and Bowles, 1996 and Mostafa *et al.*, 2003).

Previous studies on molecular phylogenetic analysis using CO1 and 28S rDNA proposed a phylogenetic relationship and the position of 3 taxa, *Taenia solium*, *T. saginata* and *T. asiatica* (Queiroz and Alkire, 1998) suggested that *T. saginata* and *T. asiatica* represent one host-colonization, whereas *T. solium* represents another independent colonization event. This conclusion is further supported by cladistic analysis employing morphological characters in which *T. saginata* and *T. asiatica* were sister species and distantly related to *T. solium* (Hoberg *et al.*, 2001).

Polymerase chain reaction (PCR)-based molecular techniques, such as random amplified polymorphic DNA (RAPD), have been used for differential diagnosis of species and strains and to gain knowledge of the genetic diversity in parasite populations (Bandi *et al.*, 1993; Kaukas *et al.*, 1994; Tighe *et al.*, 1994; Kralová and Spakulová, 1996; Brouwer *et al.*, 2001; Eom *et al.*, 2002).

Simpson *et al.* (1993) proved that RAPD-PCR is undoubtedly a powerful approach for the analysis of genetic variation and identification of genetic markers. So, RAPD is of particular value in the study of the genetic variation of cestodes. The amplified products can be broadly classified into variables (polymorphic) and contents (monomorphic) which can be used as diagnostic markers (Hadrys *et al.*, 1992). The RAPD-PCR was applied to discriminate between three cestode parasites infecting domesticated birds, *Railletina vinagoi*, *Cotugnia polycantha* infecting pigeons and *R. sinensis* infecting chicken (Taha *et al.*, 2006).

Ahmed and Abdel-Moaty (2011) found that *Cotugnia polycantha* infecting doves, *Streptopelia senegalensis* differed from *C. polycantha* infecting pigeons, *Columba livia domestica*, by the ultrastructure of spermatozoa which is considered as a new tool for identification.

In the present study, the (RAPD-PCR) confirm that *C. polycantha* infecting doves, *S. senegalensis*, differ from *C. polycantha* infecting pigeons *C. livia domestica*.

So, *C. polycantha* infecting doves could be named *C. polycantha streptopeli* and *C. polycantha* infecting pigeons, *C. polycantha columbi*.

#### Conclusion

The members of some genera or some species of cestode parasites may exist as a number of phenotypes and genotypes that are closely similar and can not be recognized morphologically, but can be, identified using molecular assays.

The RAPD-PCR technique has proved to be a reliable technique in detecting intra-specific genetic variation between closely similar and closely related parasitic members of the same species.

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