The description of Centrorhynchus globirostris *n. sp. (Acanthocephala: Centrorhynchidae) from the pheasant crow,* Centropus sinensis *(Stephens) in Pakistan, with gene sequence analysis and emendation of the family diagnosis*

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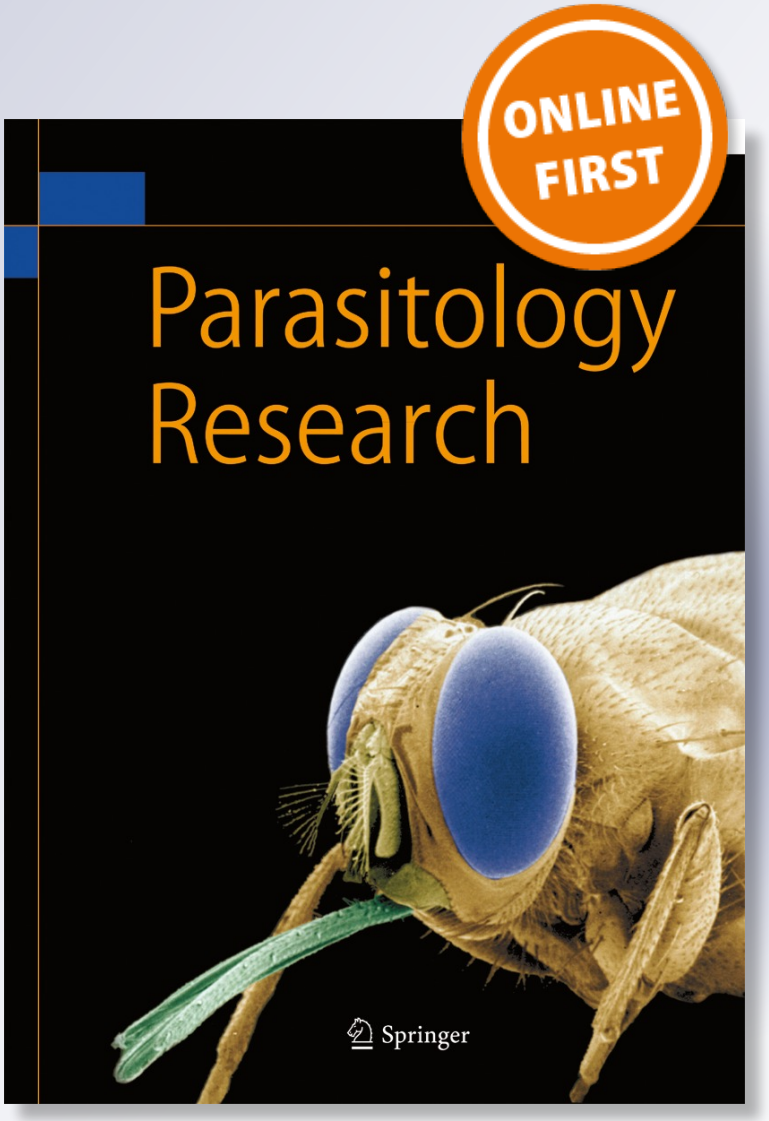

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ORIGINAL PAPER

The description of *Centrorhynchus globirostris* n. sp. (Acanthocephala: Centrorhynchidae) from the pheasant crow, *Centropus sinensis* (Stephens) in Pakistan, with gene sequence analysis and emendation of the family diagnosis

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Abstract A new species of *Centrorhynchus* (Centrorhynchidae) with receptacle insertion at the posterior third of the proboscis is described from the pheasant crow *Centropus sinensis* (Stephens) (Cuculidae) in Pakistan. *Centrorhynchu sglobirostris* n. sp. is similar to the 98 other known species of *Centrorhynchus* Lühe, 1911 in having long cylindrical trunk with anterior dilation and transverse anastomoses of the secondary lacunar vessels. However, specimens of *C. globirostris* differ from all other species of the genus by having a unique globular proboscis not divided into anterior proboscis with rooted hooks and posterior proboscis with rootless spines. Posterior hooks of C. *globirostris* emerge at the level of the receptacle insertion and are uniquely fully rooted. Sequencing and phylogenetic analysis of C. *globirostris* 18S and 28S ribosomal RNA genes reveals the genetic and evolutionary relationships between *C. globirostris* and other members of Centrorhynchidae which have representative orthologs in public databases. Comparison to known acanthocephalans confirms appropriate inclusion in the genus *Centrorhynchus*.

Keywords Acanthocephala · *Centrorhynchus globirostris* n. sp · Centrorhynchidae · Gene sequencing · *Centropus sinensis* · Pakistan

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The pheasant crow, *Centropus sinensis* (Stephens) (Cuculidae) is a large nonparasitic member of the cuckoo order, the Cuculiformes. It is a widespread resident in Asia, from India to China and Indonesia. Pheasant crows are weak fliers that abound in a wide range of habitats from jungle to cultivated lands near major canals and rivers where they forage for insects and small vertebrates such as the saw-scaled vipers. They are also known to eat snails, bird eggs, nestlings, fruits, and seeds (Ali and Ripley 1987; Natarajan 1993; Roberts 1991). The parasitic fauna of the pheasant crow is largely unknown. A small collection of these birds in a Sindh location, Pakistan, yielded a large number of specimens of acanthocephalans. Extensive morphological data suggested that the acanthocephalans collected constitute a new species. This morphological data is supported by analysis of the highly conserved 18S and 28S gene regions of *Centrorhynchus globirostris* which confirms its placement within Centrorhynchidae Van Cleave, 1916 (Golvan 1960).

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Materials and methods

Twelve pheasant crows from Oderolal, Matiari District, Sindh Province, Pakistan (25° 36′ N, 68° 26′ E) were examined for parasites. The intestinal tract of only one bird was found infected with 250 acanthocephalans. The birds were provided under a special collecting permit by the Animal Laboratory, Department of Zoology, University of Karachi.

For microscopical examination, selected specimens of various sizes were placed in water for 2–5 h or until fully extended then fixed in FAA before transferring to 70 % ethanol. Worms were punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4 % hydrochloric acid in 70 % ethanol, dehydrated in ascending concentrations of ethanol (24 h each), and cleared in 100 % xylene then in 50 % Canada balsam and 50 % xylene (24 h each). Whole worms were then mounted in Canada balsam. Measurements are in micrometers, unless otherwise noted; the range is followed by the mean values between parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck, or bursa. Type specimens were deposited in the University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection in Lincoln, NE, USA.

For SEM studies, specimens previously fixed in 70 % ethanol were placed in critical point drying baskets and dehydrated using ethanol series of 95 % and 3 N 100 % for at least 10 min per soak, followed by critical point drying (Lee 1992). Samples were mounted on SEM sample mounts, gold coated, and observed with a scanning electron microscope (XL30 ESEMFEG; FEI, Hillsboro, OR). Digital images of the structures were obtained using digital imaging software attached to a computer.

For genetic analysis, specimens preserved in 70 % ethanol, washed in water, dried thoroughly, and macerated with a razor blade. The macerated tissue sample was then placed in a 1.5-mL microcentrifuge tube and DNA-extracted using the QiagenDNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA) per manufacturer instructions.

PCR primer sequences designed for the amplification of the 18S ribosomal gene (5′-AGATTAAGCCAATGCA TGCGTAAG-3′ and 5′-TGATCCTTCTGCAGGTTCAC CTAC-3′) were previously described for the amplification of *Mediorhynchus africanus* (Amin 2013). Primers for the 28S ribosomal gene were designed based on suspected relatedness to the genus *Centrorhynchus* using GenBank accession number AY830155.1 (forward primer 5′-GAGTTCACAAGTGCGTGAAAC-3′, reverse primer 5′-CTTCGCAATGATAGGAAGAGCC-3′).

PCR amplification was performed using OneTaq DNA polymerase (New England Biolabs, Ipswich, MA, USA). The thermal profile of the PCR amplification consisted of initial denaturation at 95 °C for 2 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 68 °C for 90 s. All PCR products were cloned into *E. coli* using the TOPO TA cloning kit per instructions of the manufacturer (Life Technologies, Grand Island, NY, USA). Plasmids containing the PCR amplified gene sequences were extracted using the QIAprep Miniprep kit per instructions of the manufacturer (Qiagen). Cycle sequencing was performed by the Brigham Young University DNA Sequencing Center on an ABI 3730xl automated sequencer. Sequences generated were compared to those available in GenBank by performing a Blastn search using default parameters (Madden 2002). Top hit results returned were used for sequence comparisons and phylogenetic analyses (Tables 1 and 2). Sequences were uploaded to NCBI using BankIt. GenBank accession number for 18S is KM588206. GenBank accession number for 28S is KM588207. ClustalW multiple sequence alignments were performed using Geneious 7.1.7 (Biomatters, San Francisco, CA). Subsequently, Gblocks (v.91b) was used to remove ambiguously aligned regions, divergent regions, and to correct for multiple substitutions. Aligned sequences were analyzed to find the best-fit model of sequence evolution with PhyML(v3.0) (Dereeper et al. 2008). Using the Akaike information criterion (AIC), the GTR+G model was identified as the most suitable substitution model for phylogenetic analysis. Distance matrices were calculated using the same models, and these data are included for all taxa (Tables. 3 and 4). Data from Tables 1 and 2 were independently used to create phylogenetic analyses. Phylogeny was determined using maximum likelihood (ML) with PhyML. Sequence data were analyzed and used to generate two separate phylogenetic trees by TreeDyn 198.3 (Dereeper et al. 2008). Nodal support was derived by bootstrap analysis (100 replicates).

Table 1 18S GenBank accession numbers of related species used for phylogenetic analyses

Species	18S GenBank accession number
Centrorhynchus globirostris	KM588206
Centrorhynchus conspectus	U41399.1
Centrorhynchus sp.	AY830155.1
Centrorhynchus microcephalus	AF064813.1
Corynosoma validum	JX442170.1
Bolbosoma turbinella	JX442166.1
Arhythmorhynchus frassoni	JX442164.1
Polymorphis brevis	JX442171.1
Gorgorhynchoides bullocki	AY830154.1
Macracanthorhynchus ingens	AF001844.1
Mediorhynchus grandis	AF001843.1

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Table 2 28S GenBank accession numbers of related species used for phylogenetic analyses

Species	28S GenBank accession number
Centrorhynchus globirostris	KM588207
Centrorhynchus sp.	AY829104.1
Corynosoma validum	JX442182.1
Gorgorhynchoides bullocki	AY829103.1
Echinorhynchus truttae	AY829097.1
Bolbosoma turbinella	JX442178.1
Filisoma bucerium	AY829110.1
Arhythmorhynchus frassoni	JX442177.1
Acanthocephaloides propinquus	AY829100.1
Polymorphus trochus	JX442185.1

Results and discussion

A new species of *Centrorhynchus* Lühe, 1911 with elongated cylindrical trunk shape and transverse secondary lacunar vessels is recognized from the pheasant crow *Centropus sinensis* Stephens, 1815 in Pakistan . The new species differs from the other 98 known species of *Centrorhynchus* (Amin, 2013) in having a globular proboscis not divided into anterior rounded part with hooks and posterior cylindrical part with spines, among other features discussed below. The assignment of the new species in Centrorhynchidae is based on the insertion of the anterior proboscis receptacle into the posterior third of the proboscis and DNA studies indicating its affiliation with other species of *Centrorhynchus*. The unique globular proboscis and the rooted posterior spines would suggest a new genus in Centrorhynchidae, but this was not supported by DNA analysis.

Family diagnosis

The diagnosis of Centrorhynchidae is herein emended to allow the inclusion of centrorhynchids with globular proboscis and rooted hooks throughout its length.

Centrorhynchus globirostris n. sp. (Figs. 1–23)

General With characters of the genus *Centrorhynchus*. Shared structures invariably larger in females than in males. Trunk long, cylindrical with prominent anterior ovoid dilation (AOD), prominent anterior-dorsal hump (Figs. 1, 2, 6, and 10), and transverse secondary lacunar canals (TSLC) throughout. TSLC very close in area of AOD but become more widely spaced posteriorly. AOD considerably and proportionally more prominent in younger worms. Body wall with many fractured nuclei and micropores with diverse diameter and distribution in different trunk regions (Figs. 16–19). Proboscis globular, tilted ventrad, not divided into anterior

Table 3 Similarity of *Centrorhynchus globirostris* 18S gene compared to related species

	Ma. ingens	*Me. grandis*	*C.globirostris*	*C.conspectus*	*C.microcephalus*	*Centrorhynchus* sp.	*G.bullocki*	*Ar. frassoni*	*P.brevis*	*B.turbinella*	*Co. validum*
Ma. ingens		98.2 %	*75.9 %*	76.3 %	75.2 %	75.7 %	79.5 %	79.7 %	81 %	80.8 %	81.2 %
Me. grandis	98.2 %		*75.7 %*	76 %	75 %	75.5 %	78.9 %	79.5 %	80.7 %	80.4 %	80.8 %
C. globirostris	*75.9 %*	*75.7 %*		*96.4 %*	*95.7 %*	*96.9 %*	*83.9 %*	*83.8 %*	*85.1 %*	*85.7 %*	*86 %*
C. conspectus	76.3 %	76 %	*96.4 %*		96.8 %	98.1 %	84.5 %	84.2 %	85.4 %	86.5 %	86.7 %
C. microcephalus	75.2 %	75 %	*95.7 %*	96.8 %		98.4 %	83.4 %	82.7 %	84.1 %	85 %	85.3 %
Centrorhynchus sp.	75.7 %	75.4 %	*96.9 %*	98.1 %	98.4 %		84.3 %	83.8 %	85.2 %	86.1 %	86.2 %
G. bullocki	7935 %	78.9 %	*83.9 %*	84.4 %	83.3 %	84.3 %		88.1 %	89.4 %	90.9 %	91.1 %
Ar. frassoni	79.7 %	79.5 %	*83.8 %*	84.2 %	82.7 %	83.8 %	88.1 %		92.4 %	83.2 %	93.4 %
P. brevis	81 %	80.7 %	*85.1 %*	85.4 %	84.1 %	85.2 %	89.4 %	92.4 %		95.2 %	85.6 %
B. turbinella	80.8 %	80.4 %	*85.7 %*	86.5 %	85 %	86.1 %	90.9 %	93.2 %	95.2 %		99.2 %
Co. validum	81.2 %	80.8 %	*86 %*	86.7 %	85.3 %	86.2 %	91.1 %	93.4 %	95.6 %	99.2 %	

Average 18S percent identity among similar taxa to *C. globirostris*. Data retrieved from NCBI BLASTn searches. Percent identity between *C. globirostris* and other species is in italics

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Table 4 Similarity of *Centrorhynchus globirostris* 28S gene compared to related species

	Ar. frassoni	*E. truttae*	*G. bullocki*	*C. globirostris*	*Centrorhynchus* sp.	*P. trochus*	*B. turbinella*	*Co. validum*	*Ac. propinquus*	*F. bucerium*
Ar. frassoni		77.3 %	78.5 %	*90 %*	72.1 %	82.1 %	82.9 %	83.2 %	74.6 %	73.6 %
E. truttae	77.3 %		77.7 %	*90.3 %*	71.2 %	79 %	79.6 %	79.7 %	82.1 %	81.4 %
C. globirostris	*90 %*	*90.3 %*	*90.5 %*		*98.4 %*	*90.4 %*	*90.7 %*	*91.4 %*	*85.1 %*	*85.3 %*
Centrorhhynchus sp.	72.1 %	71.2 %	73.5 %	*98.4 %*		74.1 %	76 %	76.3 %	68.5 %	67.4 %
P. trochus	82.1 %	79 %	81.5 %	*90.4 %*	74.1 %		89.3 %	89.8 %	75.8 %	74.1 %
B. turbinella	82.9 %	79.6 %	82.7 %	*90.7 %*	76 %	89.3 %		95.9 %	76.1 %	75 %
Co. validum	83.2 %	79.7 %	83.5 %	*91.4 %*	76.3 %	89.8 %		95.9 %	76.1 %	75 %
Ac. propinquus	74.6 %	82.1 %	74.2 %	*85.1 %*	68.5 %	75.8 %	76.1 %	76 %		86.1 %
F. bucerium	73.6 %	81.4 %	73.7 %	*85.3 %*	67.4 %	74.1 %	75 %	75 %	86.1 %	

Average 28S percent identity among similar taxa to *C. globirostris*. Data retrieved from NCBI BLASTn searches. Percent identity between *C. globirostris* and other species is in italics

and posterior parts (Figs. 2, 4, and 10–13). Proboscis with 24–25 rows each with 10–11 (usually 10) hooks. Hooks transition from small anteriorly, massive near middle to gradually smaller posteriorly (Figs. 11, 13, and 14). Surface of all hooks ribbed (Fig. 15). Anterior hook roots simple, very long, posteriorly directed and posteriorly forked, becoming gradually smaller posteriorly. Root of hook 5 from anterior with small anterior manubrium. Manubria gradually increase in size in posterior hooks, at level of insertion of receptacle, with the decrease to total absence of posterior root in basal hooks (Figs. 4 and 5). No rootless spines. Neck prominent, widest posteriorly, delimited from trunk with mild anterior trunk girdle (Fig. 13). Proboscis receptacle (PR) double-walled, about twice as long as proboscis with cephalic ganglion at middle. PR not continuous posteriorly where retractor muscles pass and its outer wall with occasional marked nuclei at middle (Fig. 2). PR or its process insert anteriorly near posterior third of proboscis where robust hooks gradually transition into smaller hooks (Figs. 2 and 4). Lemnisci digitiform, equal, about three times as long as proboscis receptacle, with many small but prominent nuclei, attached to body wall with fibrous connectives. Fibrous connectives enveloping lemnisci, with prominent nuclei at posterior end of lemnisci (Fig. 7). Gonopore subterminal in both sexes (Figs. 12 and 21).

Males (based on 17 mature specimens with sperm; 3 juveniles are not included). Trunk 12.50–23.75 mm (18.40) long by 0.47–0.95 mm (0.68) wide just posterior to AOD. AOD 2.92–4.55 mm (3.58) long by 0.75–1.10 mm (0.89) wide. AOD 16–23 (19 %) of trunk length, relatively longer in smaller worms reaching 27–28 % of trunk length in three 7.75–10.0-mm-long juvenile males. Proboscis 603–700 (647) long by 364–468 (399) wide in anterior half. Hook length×width at base, [length of roots] numbered from anterior: (I) 38–55 (45)×12–15 (14), [47–52 (49)], (II) 55–67 (61)×17–20 (19), [65–67 (66)], (III) 60–67 (64)×22–30 (26), [72–82 (77)], (IV) most massive hook 58–70 (64)×30–33 (31), [72–82 (77)], (V) 50–72 (59)×20–22 (21), [40–65 (52)], (VI) 45–50 (48)×17–18 (17), [35–52 (41)], (VII) 42–50 (46)×12–15 (14), [30–50 (38)], (VIII) 37–42 (40)×10–12 (11), [25–27 (26)], (IX) 35–37 (36)×10 (10), [23–25 (24)], (X) 27–37 (31)×7–8 (8), [15–20 (17)]. Neck 187–302 (231) long by 281–343 (285) wide posteriorly. Proboscis receptacle 1.16–1.50 long by 0.17–0.32 wide. Lemnisci 1.51–2.37 mm (1.97) long by 0.12–0.32 mm wide. Testes relatively large, elliptical, nearly equal, not contiguous. Anterior testis 0.60–1.50 mm (0.98) long by 0.27–0.45 mm (0.36) mm wide. Posterior testis 0.57–1.40 mm (1.02) long by 0.19–0.62 mm (0.34) wide. Cement glands 4, tubular, ducted, with thick walls containing many ovoid-elongate nuclei, not contiguous with posterior testis, 6.25–13.12 mm (9.26) long by 0.10–0.32 mm (0.19) wide. Saefftigen's pouch prominent, elongate-drop-shaped, widest anteriorly, contiguous with posterior end of cement glands, 2.00–3.50 mm (2.80) long by 0.17–0.40 mm (0.31) wide anteriorly. Bursa plain, longer than wide (Fig. 20), 1.22–1.75 mm (1.43) long by 0.70–1.50 mm (1.13) in diameter. Ventral common sperm duct, cement glands duct, and Saefftigen's pouch jointly end in bursa. Gonopore subterminal in rounded posterior end with 2 prominent peri-genital glands (Fig. 3, arrow).

Females (based on 14 mostly gravid adults; 1 juvenile not included). Few females had ovarian balls (Fig. 23). Trunk 16.75–43.75 mm (28.31) long by 0.45–1.12 mm (0.79) wide just posterior to AOD. AOD 2.87–5.87 mm (4.20) long by 0.87–1.42 mm (1.12) wide. AOD 12–21 (15 %) of trunk length, relatively longer in smaller worms reaching 35 % of trunk length in one 5.62–mm-long juvenile female. Proboscis 666–759 (732) long by 385–468 (443) wide in anterior half. Hook length×width at base, [length of roots] numbered from anterior: (I) 52–60 (56)×12–17 (15), [50–75 (62)], (II) 65–67 (66)×20–22 (21), [67–85 (77)], (III) 67–72 (69)×22–30 (26), [75–87 (83)], (IV) most massive hook 65–72 (69)×30–42 (35), [90–100 (94)], (V) 57–66 (62)×22–30 (26), [72–80

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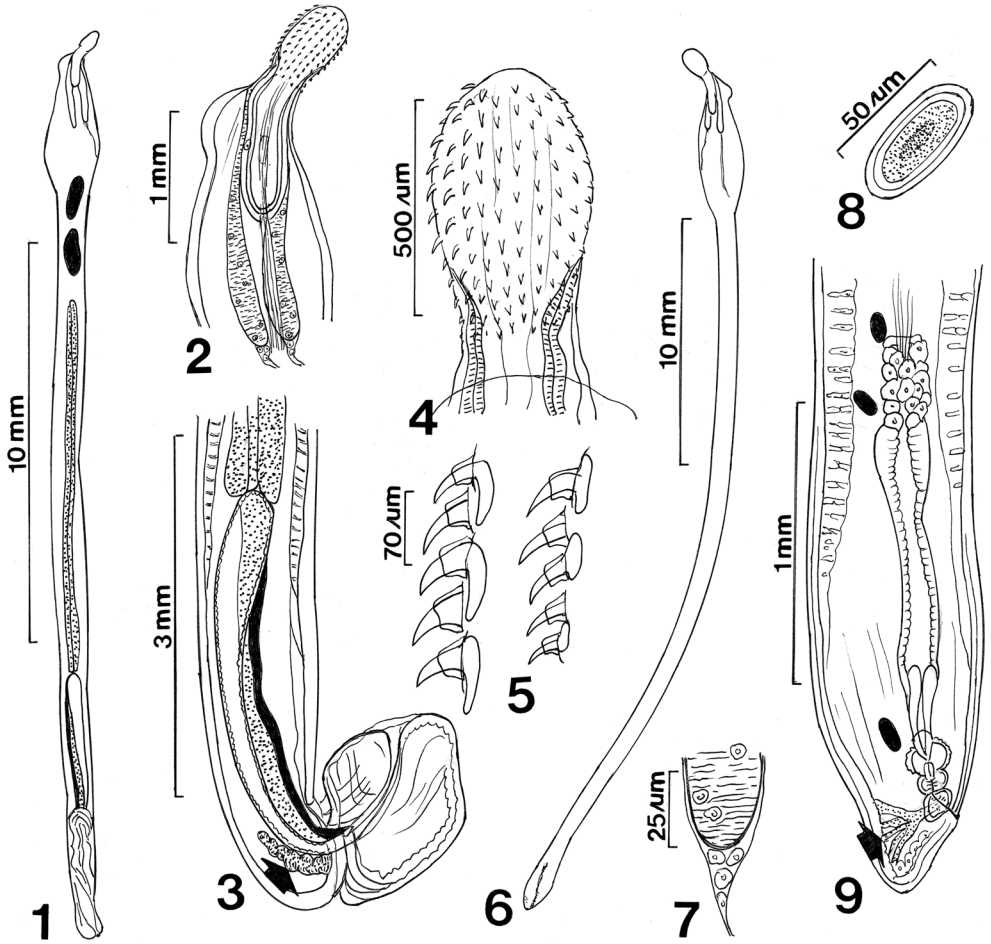

Figs. 1–9 *Centrorhynchus globirostris* from the pheasant crow *Centropussinensis*in Pakistan. *1* Holotype male; note the anterior swelling, anterio-dorsal hump, black common sperm duct, and rounded posterior end; secondary transverse lacunar branching not shown. *2* Anterior part of holotype male; note anterio-dorsal hump and incomplete outer proboscis receptacle wall at posterior end; secondary transverse lacunar branching not shown. *3* Posterior end of a paratype male; note the black ventral common sperm duct and the peri-genital glands (*arrow*). *4* Proboscis and proboscis receptacle insertion of allotype female. *5* A row of proboscis hooks from anterior (upper left) of a paratype male. Every other hook root is deliberately omitted for space considerations. *6* The body shape of a young paratype female. Secondary transverse lacunar branching not shown. *7* The posterior end of a lemniscus showing the anterior nucleated part of the ligament fibers attaching the lemniscus to the body wall. *8* A ripe egg. *9* The posterior end of a paratype female showing the reproductive system; note the complex vagina and long uterus, as well as the thick triangulate ligaments attaching the distal vaginal bulb to the dorsal body wall (*arrow*)

(75)], (VI) 57–62 (59)×17–25 (21), [67–70 (68)], (VII) 50–52(51)×15–25 (20), [42–55 (50)], (VIII) 40–45 (43)×12–15 (14), [30–37 (33)], (IX) 35–42 (39)×10–12 (11), [28–35 (31)], (X) 32–40 (37)×10 (10), [25–27 (26)]. Neck 156–239 (199) long by 312–416 (356) wide posteriorly. Proboscis receptacle 1.22–1.89 mm (1.58) long by 0.27–0.39 (0.33) wide. Lemnisci 1.70–2.87 mm (2.31) long by 0.13–0.37 mm (0.26) wide. Female reproductive system 1.40–2.25 mm (1.74) long (7–9 % length of trunk); percentage markedly higher in younger specimens reaching 14 % in one 5.62-mm-long juvenile. Eggs ovoid, speckled, with concentric shells (Figs. 8 and 22), 45–60 (52) long by 25–28 (26) wide. Vagina complex; distal bulb connected to dorsal body wall with thick triangularly shaped branching fibers (Fig. 9, arrow). Uterus comparatively long; uterine bell short with few but prominent cells. Gonopore subterminal in triangular bluntly pointed posterior end (Figs. 9 and 21).

Molecular description

Amplification of the 18S gene resulted in a nucleotide sequence of 1747 bp, sans plasmid vector sequences. The most similar sequence in GenBank was *Centrorynchus conspectus* (accession number U41399.1) with 96 % identity to the query sequence. PCR amplification of the 28S gene resulted in a product of 707 bp. *Centrorhynchus* sp. (GenBank accession number AY829104.1) was the most similar to the 28S sequence with 98 % identity to the query sequence.

Average percent identity among most similar taxa retrieved from NCBI BLASTn searches were calculated for both targeted

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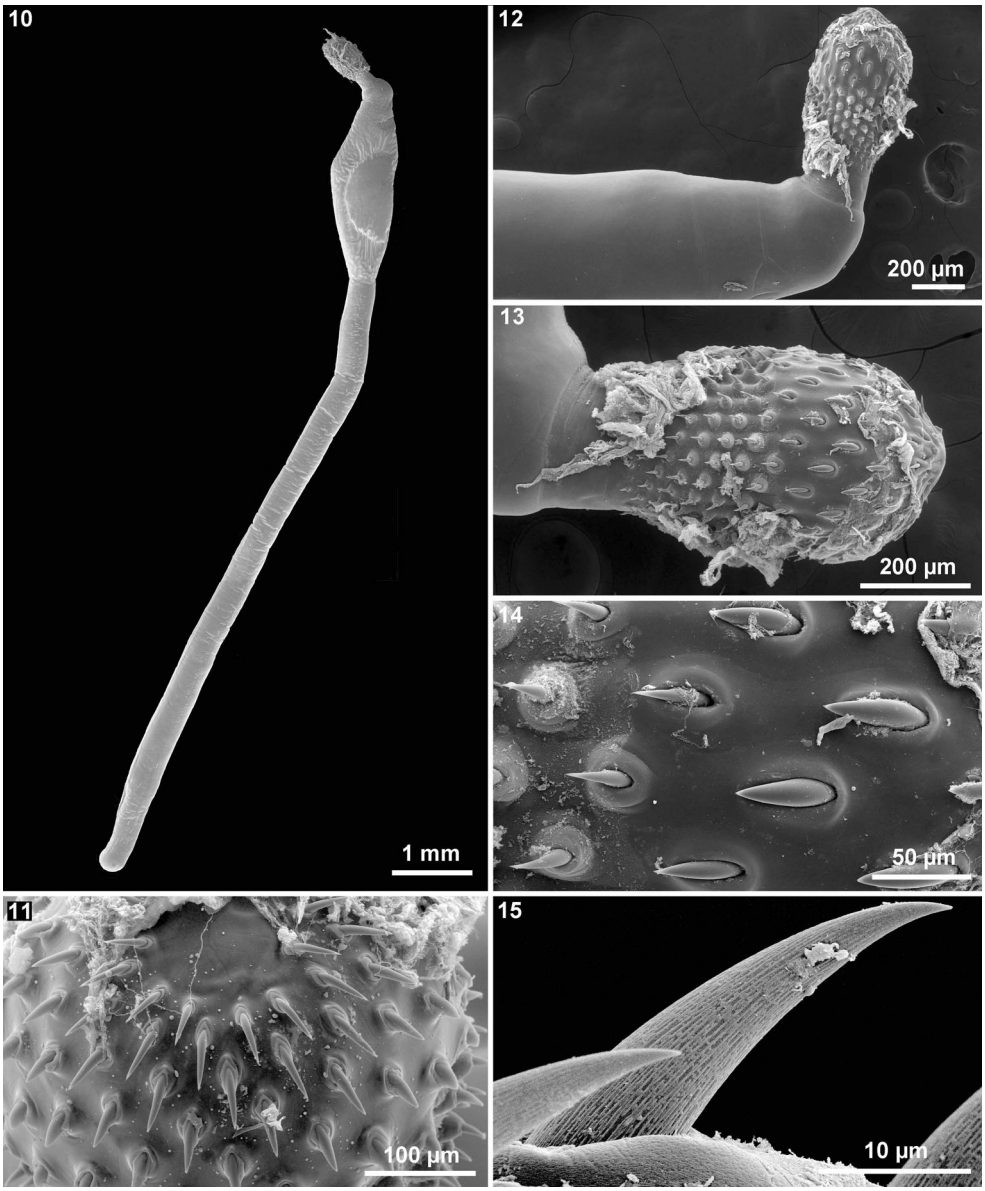

Figs. 10–15 SEM of *Centrorhynchus globirostris* from the pheasant crow *Centropus sinensis* in Pakistan. *10* A paratype male; note the anterior trunk swelling and antero-dorsal trunk hump. *11* Apical view of specimen in Fig. 10 showing a small apical spineless area and the smaller apical hooks. *12* Anterior end of a male specimen with proboscis tilted ventrad. *13* A higher magnification of proboscis in Fig. 12 showing the lack of constriction between larger anterior hooks and smaller posterior hooks. *14* A close view of the transitional zone between the larger anterior hooks (*right*) and smaller posterior hooks (*left*) corresponding to the point of attachment of the proboscis receptacle. *15* A high magnification of an anterior proboscis hook showing the ribbed surface characteristic of all hooks

gene sequences independently. GenBank accession numbers of each taxon are presented in Tables 1 and 2. Multiple alignments of these sequences were used to create independent phylogenetic trees (Figs. 24 and 25). Average 18S genetic divergence within genera *Centrorynchus* and *Corynosoma* was 2.23 and 1.2 %, respectively, indicating a low percentage of nucleotide base changes within defined genera. Average divergence among the genera *Centrorynchus* and *Corynosoma* was 13.9 %.*Centrorhynchus globirostris* 18S sequence was 96.4 % similar *to C. conspectus*, 95.7 % similar to *C. microcephalus*, and 96.9 % similar to *Centrorhynchus* sp. By comparison, based on representative sequences, the genera *Macroacanthorhynchus* and *Mediorhynchus* share 98.2 % similarity.

Concurrently, average 28S genetic divergence among the genera *Centrorynchus* and *Corynosoma* was 23.7 %, as reflected in values given in Table 4. Phylogenetic analysis corresponds with a bootstrap value of 100, nesting *C. globirostris* and *Centrorynchus* on the same branch (Fig. 25). At the 28S locus, *C. globirostris* and *Cntrorhynchus* sp. share 98.4 % sequence similarity. Two other closely related genera, *Corynosoma* and *Bolbosoma*, were found to share 95.9 % sequence similarity.

Phylogenetic relationships as inferred from the 18S gene fragment depict *C. globirostris* forming a strongly supported clade with *C. microcephalus*, *C. conspectus*, and *Centrorhynchus* sp. The monophyly of *C. globirostris* with *Centrorhynchus* is strongly supported (100 % bootstrap support). The 28S gene phylogeny reflects poorer representation of *Centrorhynchus* species found in public databases but supports the relationship of *C. globirostris* within

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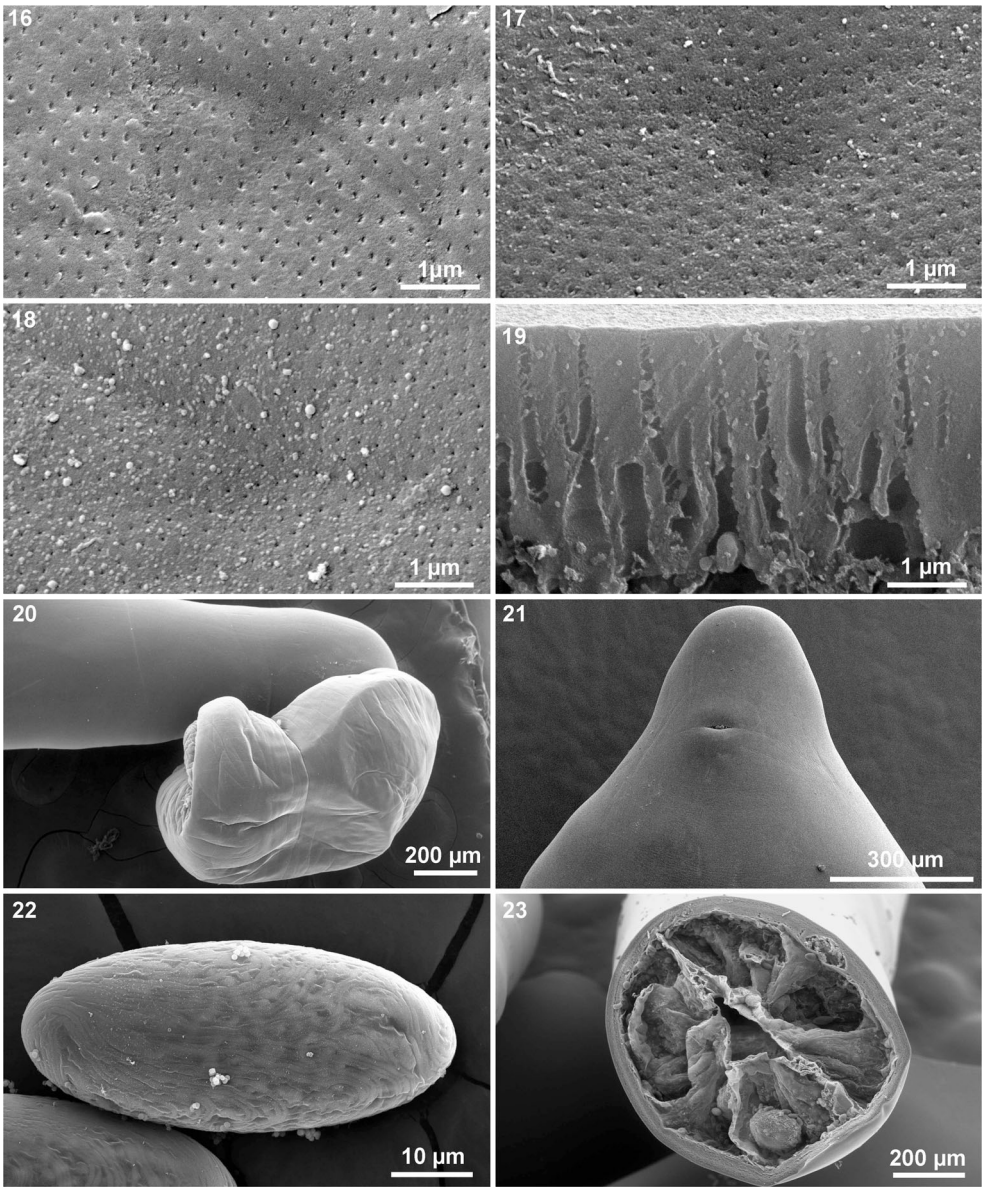

Figs. 16–23 SEM and TEM (Fig. 19) of *Centrorhynchus globirostris* from the pheasant crow *Centropus sinensis* in Pakistan. *16–18* Micropores in the anterior, middle and posterior trunk, respectively. *19* TEM of a cut up section of body wall showing the dermal canaliculi associated with the micropores. *20* Posterior end of a male showing the subterminal position of the bursa and gonopore; when the bursa is retracted, the gonopore appears as a deep subterminal longitudinal slit. *21* The triangular posterior end of a female showing the subterminal position of the gonopore. *22* Egg; note its speckled appearance. *23* A section of the trunk of a female showing some ovarian balls

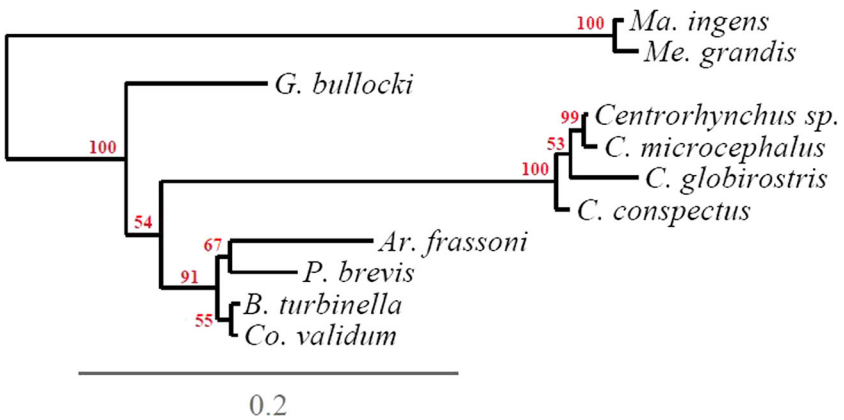

Fig. 24 Phylogenetic analysis of 18S gene sequence. Phylogenetic relationships between *C. globirostris* and similar taxa (Table 1) were estimated using maximum likelihood. Maximum likelihood bootstrap proportions are shown above each branch. Branch lengths are drawn to scale representing the amount of evolutionary change and reported as number of nucleotide substitutions per nucleotide site

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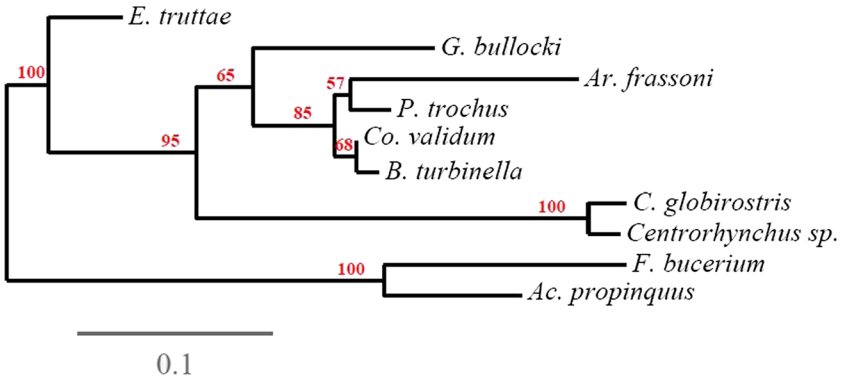

Fig. 25 .Phylogenetic analysis of 28S gene sequence. Phylogenetic relationships between *C. globirostris* and similar taxa (Table 2) were estimated using maximum likelihood. Maximum likelihood bootstrap proportions are shown above each branch. Branch lengths are drawn to scale representing the amount of evolutionary change and reported as number of nucleotide substitutions per nucleotide site

Centrorhynchus (100 % bootstrap support; Fig. 25). Sequence analysis of the ribosomal 18S and 28S genes suggests that *C. globirostris* is highly similar to *Centrorhynchus* and other genera of the family *Polymorphidae*. Extensive morphological and molecular data suggested that the acanthocephalans described here constitute a new species in the genus *Centrorhynchus*.

Taxonomic summary

Type host The pheasant crow *Centropussinensis* (Stephens) (Cuculidae)

Site of infection Intestine

Type locality Oderolal, Matiari District, Sindh Province, Pakistan (25° 36′ N, 68° 26′ E)

Specimens deposited University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection no. 49922 (holotype male), no. 49923 (allotype female), no. 49924 (paratypes). Additional specimens are included with type specimens on same slides.

Etymology The generic name reflects the closeness to the genus *Centrorhynchus*. The specific name describes the shape of the proboscis.

Taxonomic remarks and comparisons

Golvan (1956) erected *Sphaerirostris* Golvan, 1956 as a subgenus of *Centrorhynchus* Lühe, 1911 and included 21 species with short spindle-shaped trunk, polydendritic lacunar system, three or four tubular cement glands, and short globular anterior proboscis with hooks set from cylindrical posterior proboscis with spines at insertion of proboscis receptacle. *Centrorhynchus*, on the other hand, has long and cylindrical trunk with anterior dilation, transverse anastomoses of secondary lacunar vessels, three to four very long cement glands, truncated cylindrical anterior proboscis with slight posterior dilation with hooks constricted at junction with posterior cylindrical proboscis with spines where the proboscis receptacle inserts. *Neolacunisoma* Amin et Canaris, 1997 is similar to *Sphaerirostris* except that the secondary lacunar vessels have transverse anastomoses anteriorly and mostly dendritic posteriorly making *Neolacunisoma* an intermediate taxon between *Centrorhynchus* and *Sphaerirostris*. *Centrorhynchus globirostris* n. sp. is similar to other species of *Centrorhynchus* in trunk shape (Figs. 1, 6, and 10) and arrangement of secondary lacunar vessels but differs by having a globular proboscis not divided into anterior proboscis with hooks and posterior proboscis with spines (Figs. 2, 4, 12, and 13). In *C. globirostris*, the proboscis receptacle or its anterior process insert at the posterior third of proboscis where the anterior large hooks transition to the smaller rooted posterior hooks (Figs. 2, 4, 13, and 14). The posterior hooks are fully rooted with considerable variation in the hook root pattern (Fig. 5). Posterior hooks are basically rootless spines in other members of the three centrorhynchid genera.

All hooks of *C. globirostris* have surface striations. This observation was not reported in any centrorhynchid genera but is known in a few other species including *Dentitruncus truttae* Sinzar, 1955, *Rhadinorhynchus ornatus* Van Cleave, 1918, and *Leptorhynchoides polycristatus* Amin et al., 2013a, by Dezfuli et al. (2008), and Amin et al. (2009), Amin 2013), respectively.

Differences in micropore diameter and distribution were noted in various trunk regions (Figs. 16–19) reflecting differential absorption of nutrients as has been previously described in various genera and species including *L. polycristatus*, *Neoechinorhynchus zabensis* Amin, Abdullah and Mhaisen 2003, *Acanthosentis tilapiae* (Baylis, 1948) in Amin and Heckmann (2012), *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911 in

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Amin et al. (2011), and *Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911 in Heckmann et al. (2011). The mid trunk of *C. globirostris* appears to be the more active region for nutrient uptake (Fig. 17). Wright and Lumsden (1970) and Byram and Fisher (1973) reported that these peripheral pore are continuous with canalicular crypts (see our Fig. 19). These crypts appear to "constitute a huge increase in external surface area…implicated in nutrient uptake." Whitfield (1979) estimated a 44-fold increase at a surface density of 15 invaginations per 1 μm^2 of the tegumental surface of *Moniliformis moniliformis* (see Byram and Fisher 1973).

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