Description and molecular analysis of an Italian population of *Centrorhynchus globo caudatus* (Zeder, 1800) Lühe, 1911 (Acanthocephala: Centrorhynchidae) from *Falco tinnunculus* (Falconidae) and *Buteo buteo* (Accipitridae)

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**Abstract**

*Centrorhynchus globo caudatus* (Zeder, 1800) Lühe, 1911 (Centrorhynchidae) was reported in birds of prey. Our population from *Falco tinnunculus* Linnaeus (Falconidae) and *Buteo buteo* Linnaeus (Accipitridae) in northern Italy was morphologically distinct from others described elsewhere. The worms are elongate and cylindrical. Proboscis long, apically truncated and bare, with wider base and variably faint constriction at point of attachment of receptacle. Large anterior hooks well rooted; posterior spiniform hooks with reduced roots; transitional hooks with scutiform roots in-between. Four tubular cement glands extend into prominent ducts overlapping a large Saéffgen’s pouch. Bursa large, with sensory plates. Vagina with laterally slit orifice in sub-ventral pit of globular terminal extension. Thick-shelled eggs ovoid without polar prolongation of fertilization membrane. In our specimens, proboscis hooks, receptacle, male reproductive system, and lemnisci especially in males varied in size from those from Ukraine, India, Egypt, Kyrgyzstan, Russia, Georgia, Armenia and Asian Soviet Republics. Our description of the Italian specimens includes new morphological information supported by scanning electron microscopy and microscope images, molecular analysis and energy dispersive X-ray analysis (EDXA) of hooks. Additional new details of proboscis hook roots, micropores and micropore distribution are described. Metal composition of hooks (EDXA) demonstrated high levels of calcium and phosphorous, and high levels of sulphur in core and cortical layers of eggs. The molecular profile based on sequences of 18S and cytochrome c oxidase 1 genes is also provided, as well as phylogenetic reconstructions including all available sequences of the family Centrorhynchidae, although further sequences are needed in order to clarify their phylogenetic relationships.

**Introduction**

Golvan 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. *Centrorhynchus*, on the other hand, has long and cylindrical trunk with anterior dilation, transverse anastomoses of secondary lacunar vessels, 3–4 very long cement glands and truncated cylindrical anterior proboscis with slight posterior dilation. There are over 98 valid species of *Centrorhynchus* Lühe, 1911 known from birds throughout the world (Amin, 2013). Much of the early descriptions of *Centrorhynchus globo caudatus* (Zeder, 1800) Lühe, 111 (Centrorhynchidae) by Zeder (1800) and Rudolphi (1802) were repeated by subsequent observers. The distribution of *C. globo caudatus* appears to extend throughout Europe, Asia and Africa in many species of birds of prey of the genera *Anthus* Becstein ( Pipits), *Aquila* Brisson (true eagles), *Asio* (Linnaeus) (typical owls); *Athene* Boie (owls), *Buteo* Lacépède (buzzards), *Circus* Lacépède (harrier-hawks), *Falco* Linnaeus (falcons and kestrel), *Glaucidium* Boie (pigey owls), *Milvus* Lacépède (kites), *Ottos* Pennant (Eurasian, Old World, scops owls), *Strix* Linnaeus (wood owls) and *Tyto* Millberg ( barn owls) (see Petrochenko, 1950, 1958; Florescu & Ienistea, 1984; Hoklova, 1986; Lisitsyna, 2019). Insects serve as intermediate hosts for species of *Centrorhynchus*, while amphibians, reptiles and occasionally insectivorous mammals serve as second intermediate or paratenic hosts. Nelson & Ward (1966) collected juvenile specimens of *C. globo caudatus* from the long-eared hedgehog, *Hemichasmus auritus* Gmelin, in the Egyptian El Tahreer Province, and Torres & Puga (1966) collected cystacanthas of specimens of *Centrorhynchus*...
sp. from two species of frogs in Chile – *Eupsophus calcaratus* Günther and *Eupsophus roseus* Dumeril.

Reasonably adequate versions of the description of *C. globocaudatus* by these authors from Ukraine, Kyrgyzstan and former Soviet states, respectively, among others listed in table 1, were used for comparison with our Italian specimens. Other brief descriptions usually based on one or two male or female specimens were also reported from other localities in Bulgaria (Dimitrova *et al.*, 1997), Hungary (Dimitrova *et al.*, 1995), India (Gupta & Gupta, 1972), Slovakia (Komorová *et al.*, 2015) and West Africa (Dimitrova & Gibson, 2005). The brief descriptions in Meyer (1932) and Yamaguti (1963) are worth noting. Two reports on synanthropic birds and their parasites in southern Italy by Dipineto *et al.* (2013) and Santoro *et al.* (2010) made reference to *C. globocaudatus* from the common kestrel, *Falco tinnunculus* Linnaeus, but no descriptive accounts are known for this acanthocephalan from Italy. Our descriptive account is based on collections from northern Italy at Ferrara. Our findings on *C. globocaudatus* recognize a new morphological variant and add new descriptive information that expands our understanding of this interesting acanthocephalan. For instance, the anterior five sub-apical hook roots were each found to be unique in shape and size rather than appearing identical, as has been noted in previous reports using only line drawings.

As usual in most acanthocephalan families, few sequences are available for centrhexnychids. For *C. globocaudatus*, only two sequences, corresponding to 18S and 28S ribosomal DNA (rDNA) genes, have been published to date (García-Varela *et al.*, 2020a). If we consider published sequences of the genus, the number can be only expanded to six for the 18S gene, and even fewer sequences (4) are available for cytochrome c oxidase 1 (cox1).

In the present study, new scanning electron microscopy (SEM) and microscopic images, energy dispersive X-ray analysis (EDXA), micropore and related studies, and DNA analysis expand the body of knowledge about *C. globocaudatus* in particular and the genus *Centrorhynchus* in general.

### Materials and methods

#### Collections

The birds examined for this study were collected between June 2018 and August 2019 from the countryside or villages around Ferrara (44°50′N, 11°37′E). Moribund birds injured in car accidents or those unable to fly are first brought to Centro Recupero Fauna Selvatica (Center for Recovery of Wild Fauna) (CRAS), often with police (Section of Wild Fauna) intervention, for first aid, and are hospitalized with a unique CRAS number. If the birds do not recover or die, they are sent to the Experimental Zoonoprophylactic Institute of Ferrara at St Modena for necropsy, recovery of parasites and identification of possible infectious agencies such as West Nile Virus, then incinerated.

A total of 110 clearly identifiable specimens of *C. globocaudatus* were collected from 13 infected common kestrels (falcons), *F. tinnunculus*, and 32 from four infected common buzzard, *Buteo buteo* (table 1). Specimens from both host species were examined as follows: 16 specimens for SEM and EDXA, six for molecular analysis (three from each host species) and 12 males and 13 females (nine and ten from *F. tinnunculus*, and three and three from *B. buteo*, respectively) for microscopical study. Other specimens remain in O.M.A.’s personal collection. Freshly collected specimens were extended in water until proboscides everted then fixed in 70% ethanol for transport to our Arizona, USA laboratory for processing and further studies.

#### Methods for microscopical studies

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 micrometres, unless otherwise noted; the range is followed by the mean values between parentheses. Width measurements represent

<table>
<thead>
<tr>
<th>Month</th>
<th>Juveniles</th>
<th>Adults</th>
<th>Juveniles</th>
<th>Adults</th>
<th>Location in Ferrara</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2018</td>
<td>0/0</td>
<td>3/3 (11)*</td>
<td>0/0</td>
<td>0/0</td>
<td>Masi Torello, Cona</td>
</tr>
<tr>
<td>July 2018</td>
<td>1/1 (4)</td>
<td>1/2 (10)</td>
<td>0/0</td>
<td>0/0</td>
<td>Copparo, Contrapò, Ferrara</td>
</tr>
<tr>
<td>August 2018</td>
<td>0/2 (14)</td>
<td>1/1 (26)</td>
<td>0/0</td>
<td>0/0</td>
<td>Copparo, Ferrara, Fiscaglia</td>
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<tr>
<td>September 2018</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>—</td>
</tr>
<tr>
<td>October 2018</td>
<td>0/0</td>
<td>2/2 (4)</td>
<td>1/1 (11)b</td>
<td>1/1 (12)</td>
<td>Portomaggiore Formignana, Quartiere, Gualdo, Voghera Vigarano</td>
</tr>
<tr>
<td>November 2018</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>—</td>
</tr>
<tr>
<td>December 2018</td>
<td>0/0</td>
<td>0/0</td>
<td>1/1 (2)</td>
<td>0/0</td>
<td>Rovigo</td>
</tr>
<tr>
<td>January 2019</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>1/1 (7)</td>
<td>Cordea</td>
</tr>
<tr>
<td>August 2019</td>
<td>0/0</td>
<td>2/2 (41)</td>
<td>0/0</td>
<td>0/0</td>
<td>Bondeno, Portomaggiore</td>
</tr>
<tr>
<td>Total</td>
<td>1/3 (18)</td>
<td>9/10 (92)</td>
<td>2/2 (13)</td>
<td>2/2 (19)</td>
<td>Ferrara Province</td>
</tr>
</tbody>
</table>

*Number of birds infected/number examined (acanthocephalans collected).

bThese may be specimens of *C. globocaudatus*, but their identity could not be verified because of extreme contraction.
maximum width. Trunk length does not include proboscis, neck or bursa.

Microscope images were created using 10× and 40× objective lenses of a BH2 light Olympus microscope (Olympus Optical Co., Osachi-shibamiya, Okaya, Nagano, Japan) attached to an AmScope 1000 video camera (United Scope LLC, dba AmScope, Irvine, California, USA), linked to an ASUS laptop equipped with a high-definition multimedia interface system (Fremont, California, USA). Images from the microscope are transferred from the laptop to a USB and stored for subsequent processing on a computer.

Specimens were deposited in the University of Nebraska State Museum’s Harold W. Manter Laboratory (HWML) under collection number 139,404 (voucher specimens on one slide), Lincoln, Nebraska, USA.

SEM
Specimens that had been fixed and stored in 70% ethanol were processed for SEM following standard methods (Lee, 1992). These included critical-point drying in sample baskets and mounting on SEM sample mounts (stubs) using conductive double-sided carbon tape. Samples were coated with gold and palladium for 3 min using a Polaron #3500 sputter coater (Q150 TES, Quorum: www.quorumtech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in an FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon, USA) scanning electron microscope, with digital images obtained in the Nanolab software system (FEI, Hillsboro, Oregon, USA) and then transferred to a USB for future reference. Samples were received under low vacuum conditions using 10 KV, spot size 2, 0.7 torr using a gaseous secondary electron detector.

EDXA
Standard methods were used for preparation similar to the SEM procedure. Specimens were examined and positioned with the aforementioned SEM instrument, which was equipped with a Phoenix energy-dispersive X-ray analyser (FEI, Hillsboro, Oregon, USA). X-ray spot analysis and live scan analysis were performed at 16 Kv with a spot size of 5, and results were recorded on charts and stored with digital imaging software attached to a computer. The TEAM (Texture and Elemental Analytical Microscopy) software system (FEI, Hillsboro, Oregon, USA) was used. Data were stored in a USB for future analysis. The data included weight percent and atom percent of the detected elements following correction factors.

Ion sectioning of hooks
A dual-beam SEM with a gallium (Ga) ion source (GIS) was used for the liquid metal ion source (LMIS) part of the process. The hooks of the acanthocephalans were centred on the SEM stage and cross-sectioned using a probe current of between 0.2 nA and 2.1 nA according to the rate at which the area was cut. The time of cutting was based on the nature and sensitivity of the tissue. Following the initial cut, the sample also underwent a milling process to obtain a smooth surface. The cut was then analysed with X-ray at the tip, middle and base of hooks for chemical ions with an electron beam (Tungsten) to obtain an X-ray spectrum. Results were stored with the attached imaging software.

The intensity of the GIS was variable according to the nature of the material being cut.

Molecular methods
Total genomic DNA (gDNA) was extracted from four specimens of C. globocaudatus (two ex. B. buteo and two ex. F. tinunculus) preserved in ethanol 70% using a Qiagen™ (Valencia, California, USA) DNeasy® Tissue Kit and following the manufacturer’s instructions. Partial nuclear small subunit (SSU) rDNA (18S rDNA) and partial fragments of mitochondrial cox1 gene were amplified (50 μl total volume) using ExcelTaq™ SMOBIO® PCR Master Mix (Taiwan) containing 5x concentrated master mix – that is, a mixture of recombinant Taq DNA polymerase, reaction buffer, magnesium chloride (2 mM), deoxynucleotide triphosphates (dNTPs) (0.2 mM) and enzyme stabilizer; 0.25 μM of each polymerase chain reaction (PCR) primer and 2 μl of extracted gDNA. Primer pairs and amplification conditions used for both genes were as described in Amin et al. (2019a). In every PCR run, one negative and one positive control were included. PCR amplicons were sequenced directly for both strands using the same PCR primers.

Sequences were assembled and edited using ContigExpress implemented in the software Vector NTI Advance® version 10.3.0 (Thermo Fisher Scientific, Massachusetts, U.S.) and submitted to GenBank under accession numbers MT993836–MT993837 (18S rDNA) and MT992255–MT992256 (COI). Sequences were aligned using Muscle implemented in MEGA version 6 (Tamura et al., 2013), together with all published 18S and cox1 sequences of species within the family Centrorhynchidae available in GenBank. Detailed data on the sequences used on both alignments and phylogenetic reconstructions can be found in table 2. Echinorhynchus truttae Schrank, 1788 (Echinorhynchidae) was used as outgroup in both datasets (AY830165 and FR856883 in 18S and cox1 datasets, respectively). Both alignments (18S: 764 nt positions, of which four were excluded prior to analysis; cox1: 668 nt positions, of which 29 were excluded prior to analysis) were used for comparative sequence analysis.

Gblocks 0.91b implemented on the Phylogeny.fr site (Dereeper et al., 2008) was used to select blocks of evolutionarily conserved sites. Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used for phylogenetic tree reconstruction after determination of the best-fit model of nucleotide substitution with jModelTest version 2.1.4 (Darriba et al., 2012) using the Akaike Information Criterion and the Bayesian Information Criterion, respectively. For the 185 dataset, the best-fitting model selected for the ML algorithm was the GTR + G model (nst = 6, rates = gamma, ngammacat = 4), while for BI it was K80 + G (nst = 2, rates = gamma, ngammacat = 4). For the cox1 dataset, the best-fitting model selected for ML algorithm was the GTR + G model (nst = 6, rates = gamma, ngammacat = 4), while for BI it was HKY + G (nst = 2, rates = gamma, ngammacat = 4). ML analyses were performed in PhyML version 3.0 (Guindon et al., 2010) with a non-parametric bootstrap of 100 replicates. BI analyses were carried out with MrBayes version 3.2.6 (Ronquist et al., 2012) on the CIPRES Science Gateway version 3.3 (Miller et al., 2010). Log likelihoods were estimated over 10,000,000 generations using Markov chain Monte Carlo searches on two simultaneous runs of four chains, sampling trees every 1000 generations. The first 25% of the sampled trees were discarded as ‘burn-in’, and a consensus topology and nodal support estimated as posterior probability values (Huelsenbeck et al., 2003).
were calculated from the remaining trees. Pairwise genetic distance matrices were calculated using the ‘uncorrected p-distance’ model implemented in MEGA version 6.

Results

The distribution of *C. globocaudatus* is known from at least ten genera of birds of prey in Europe, Asia and Africa (table 3). Our Ferrara, Italy, population of *C. globocaudatus* became available as a result of the regional monitoring of birds of prey to assess the health of wild fauna and to identify the risk of parasitic and infectious agents, especially West Nile virus, for domestic animals, farm animals and humans. Our collection (table 1) shows a markedly higher intensity of infection in *F. tinnunculus* than in *B. buteo*, but rather similar prevalence rates. The comparable feeding modality of both bird species, mostly on young mammals like voles, less frequently on game and passerine birds, lizards, snakes, frogs, toads and invertebrates like arthropods, may be at play (Bergman, 1961; Cramp & Brooks, 1992; Viitala et al., 1995).

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank ID</th>
<th>Location</th>
<th>Host species (Order: Family)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><em>Centrorhynchus aluconis</em> (Müller, 1780)</td>
<td>MN057695</td>
<td>Unknown</td>
<td><em>Strix aluco</em> (Strigiformes: Strigidae)</td>
<td>García-Varela et al. (2020a)</td>
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<td><em>Centrorhynchus clitorideus</em> (Meyer, 1931)</td>
<td>MT113355</td>
<td>Swabi, Khyber Pakhtunkhwa Province (Pakistan)</td>
<td><em>Athena noctua</em> (Strigiformes: Strigidae)</td>
<td>Muhammad et al. (2020)</td>
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<tr>
<td><em>Centrorhynchus conspectus</em> Van Cleave &amp; Pratt, 1940</td>
<td>U41399</td>
<td>Unknown</td>
<td>Unknown (bird of prey)</td>
<td>Near &amp; Nadler (1995)*</td>
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<tr>
<td><em>Centrorhynchus globocaudatus</em> (Zeder, 1800) Lühe, 1911</td>
<td>MN057696</td>
<td>Unknown</td>
<td>Unknown (bird of prey)</td>
<td>García-Varela et al. (2020a)</td>
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<tr>
<td><em>Centrorhynchus nickoli</em></td>
<td>MT161621</td>
<td>Veracruz (Mexico)</td>
<td><em>Didelphis virginiana</em> (Didelphimorphia: Didelphidae)</td>
<td>García-Varela et al. (2020b)</td>
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<tr>
<td><em>Centrorhynchus sp.</em></td>
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<td>DQ089716</td>
<td>Unknown</td>
<td><em>Falco peregrinus</em> (Falconiformes: Falconidae)</td>
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<tr>
<td><em>Sphaerirostris lanceoides</em> (Petrochenko, 1949)</td>
<td>MT475588</td>
<td>Swabi, Khyber Pakhtunkhwa Province (Pakistan)</td>
<td><em>ardeola grayii</em> (Ciconiiformes: Ardeidae)</td>
<td>Muhammad et al. (2020)</td>
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<tr>
<td><em>Sphaerirostris picae</em> (Rudolphi, 1819)</td>
<td>MG931939</td>
<td>Yuyao, Zhejiang Province (China)</td>
<td><em>Bufo gargarizans</em> (Bufonidae)b</td>
<td>Kang &amp; Li (2018)</td>
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<td><em>Sphaerirostris picae</em> (Rudolphi, 1819)</td>
<td>MK471355</td>
<td>Swabi, Khyber Pakhtunkhwa Province (Pakistan)</td>
<td><em>Dendrocitta vagabunda</em> (Passeriformes: Corvida)</td>
<td>Muhammad et al. (2019)</td>
</tr>
</tbody>
</table>

*a*Direct submission in GenBank.

*b*Acanthocephalans retrieved from their amphibian intermediate/paratenic host.
Table 3. Morphometric comparisons of key taxonomic characters between populations of *Centrorhynchus globocaudatus* from various bird hosts in different geographical locations in Asia, Europe and Africa.

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td></td>
<td>Ferrara (Italy)</td>
<td>Ukraine</td>
<td>Kyrgyzstan</td>
<td>Borg El Arab (Egypt)</td>
<td>Russia, Georgia, Armenia, other Asian Soviet republics</td>
</tr>
<tr>
<td>Sample size</td>
<td>12 MM, 13 FF</td>
<td>7 MM, 14 FF, —, —</td>
<td>11 MM, 12 FF</td>
<td>11 MM, 12 FF</td>
<td>&gt;11 MM, 12 FF, four juveniles</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Trunk L × W (mm)</td>
<td>9.00–17.50 (13.05) × 0.50–0.82 (0.65)</td>
<td>11.95–18.8 (15.56) × 0.76–1.08 (0.93)</td>
<td>15.00–20.00 × 0.91–1.00</td>
<td>15.00–20.00 × 0.90–1.31</td>
<td>15.00–18.00 × 0.8</td>
<td>15.00–20.00 × 0.80–1.00</td>
</tr>
<tr>
<td>Proboscis L × W</td>
<td>946–998 (977) × 364–426 (384)</td>
<td>920–1,130 (1,050) × 300–354 (320)</td>
<td>890–1,000 × 440 (posterior)</td>
<td>540–1,000 × 440 (posterior)</td>
<td>700–1,000 × 400 (posterior)</td>
<td>0.70–1.10 × 0.40–0.52 (posterior)</td>
</tr>
<tr>
<td>Longest hook L</td>
<td>50–55 (51), 57–65 (60)</td>
<td>50–68 (57.6)</td>
<td>44</td>
<td>50</td>
<td>30–40, 50–60</td>
<td>30–40, 50–60</td>
</tr>
<tr>
<td>Proboscis receptacle L × W</td>
<td>1.10–1.55 (1.26) × 0.23–0.37 (0.29)</td>
<td>1.27 × 0.31</td>
<td>1.27–1.40 —</td>
<td>1.10–1.30 × 0.30–0.45</td>
<td>0.95–1.40 × 0.30–0.45</td>
<td></td>
</tr>
<tr>
<td>Lemnisci L × W (mm)</td>
<td>1.20–1.46 (1.31) × 0.08–0.15 (0.12)</td>
<td>0.92–1.90 (1.50) × to anterior testis</td>
<td>1.36 × —</td>
<td>—</td>
<td>1.80–2.20 × 0.12</td>
<td>1.36–2.20 × 0.12</td>
</tr>
<tr>
<td>Cement gland L × W (mm)</td>
<td>3.50–6.45 (5.18) × 0.17–0.27 (0.23)</td>
<td>7.83–11.08 (8.82) × —</td>
<td>8.25 × —</td>
<td>8.25 × 0.22–0.36</td>
<td>9.00–12.00 × 0.10</td>
<td>8.25–12.00 × 0.10</td>
</tr>
<tr>
<td>Cement gland duct L (mm)</td>
<td>2.00–3.00 (2.50) × 0.12–0.30 (0.18)</td>
<td>—</td>
<td>2.45 × —</td>
<td>2.45 × —</td>
<td>2.45 × —</td>
<td>—</td>
</tr>
<tr>
<td>Saeftigen’s pouch, L × W (mm)</td>
<td>1.62–2.50 (2.29) × 0.20–0.37 (0.26)</td>
<td>1.40–2.65 (2.21) × —</td>
<td>2.52 × 0.29</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bursa L × W (mm)</td>
<td>1.00 × 0.87 (n = 1)</td>
<td>2.00 × 1.30</td>
<td>—</td>
<td>0.74 × 1.10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk L × W (mm)</td>
<td>23.12–38.75 (29.94) × 0.70–0.90 (0.80) (anterior)</td>
<td>15.01–40.00 (20.18) × 0.80–1.40 (1.06)</td>
<td>37.00–55.00 × —</td>
<td>21.00–55.00 × —</td>
<td>20.00–35.00 × 1.00</td>
<td>20.00 × 55.00 × 1.00–1.50</td>
</tr>
<tr>
<td>Sample size</td>
<td>—</td>
<td>—</td>
<td>0.72–1.39 (0.96) (posterior)</td>
<td>1.10–1.50 (posterior)</td>
<td>—</td>
<td>1.10–1.50</td>
</tr>
</tbody>
</table>

(Continued)
We describe our northern Italian population morphologically as a geographical variant because of its distinct variability from other descriptive accounts elsewhere where information is available. Descriptive reports variably document its morphology using only line drawings. Among these, we consider the line drawings in Petrochenko (1950) and Lisitsyna & Greben (2015) to be the most complete and adequately representative. Minor exceptions are noted. We, therefore, opted not to duplicate these line drawings of the species and, instead, produce SEM and microscope images for the first time for documentation and introduce new information on the EDXA to discern hook metal composition, Ga hook cuts, micropores and molecular analysis for the first time. Additional details of such structures as proboscis hook roots are described and inaccuracies and errors are being corrected.

The following morphological description is based on the microscopical examination of 25 specimens (12 males, 13 females) and others used in the SEM studies. These study specimens were collected from the common kestrel, *F. tinnunculus* (19 specimens), and the common buzzard, *B. buteo* (six specimens), and a complete set of measurements of each were made before later collections in 2019 were made.

### Morphological description of our population from Ferrara Province, Italy

**Centrorhynchus globocaudatus** (Zeder, 1800) Lühe, 1911 (figs 1–3, 4a–d)

**General.** With characters of the genus *Centrorhynchus* and the family Centrorhynchidae, as defined by Amin et al. (2015). Shared structures markedly larger in females than in males (table 3). Trunk long, cylindrical, slightly wider anteriorly in males and with prominent posterior swelling and terminal knob in females. Cuticle with cross-striations, especially anteriorly, and lacunar system with prominent transverse secondary lacunar canal. Body wall with many fratured nuclei, and micro pores with diverse diameter and distribution in different trunk regions (fig. 2d–f). Proboscis elongate cylindrical, gradually widening posteriorly (fig. 1a), apically truncated and bare (fig. 1b), but apical organ occasionally evident by its anterior invaginaton (fig. 1c, d). Proboscis widening posteriorly past faint constriction at point of attachment of receptacle. Proboscis with 29–33 longitudinal rows of 19–22 hooks each. Anterior 5–7 hooks strongest (fig. 1e), with prominent core and thin cortical layer (fig. 2b, c) and prominent posteriorly directed roots variable in shape and size (fig. 5b); third or fourth hooks longest. Ventral surface of hooks with many pebble-like protrusions, especially near base (fig. 1f). Next 5–7 hooks transitional, spiniform, with prominent scutiform X-shaped roots at area of receptacle attachment near proboscis constriction. Subsequent posterior spiniform hooks (fig. 2a) with smaller scutiform roots becoming simpler and directed anteriorly in posteriormost hooks. Anterior hooks appear in alternating longitudinal rows, but they and all spiniform hooks also appear in definite spiral rows (fig. 1a). Neck relatively short with clear cuticular separation from basal proboscis region (fig. 2a). Proboscis receptacle (PR) double-walled, about 0.5 × (posterior) — 1.62 (1.3) × 0.25 — 1.40 × 0.25.039 × 0.38, L: length, W: width, H: hook.

**Table 3.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Proboscis L × W (mm)</th>
<th>Hook rows × H/row</th>
<th>Proboscis receptacle L × W (mm)</th>
<th>Longest hook L (mm)</th>
<th>Longest hook W (mm)</th>
<th>Eggs L × W (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present paper</td>
<td>1.50–1.90 (1.10) × 0.41–0.47 (0.44) (posterior)</td>
<td>—</td>
<td>5.0–5.7 (5.4–5.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
<td>45–46 (45–60)</td>
</tr>
<tr>
<td>Lisitsyna &amp; Greben (2015)</td>
<td>1.72–2.24 (2.05) × 0.22–0.25 (0.23)</td>
<td>—</td>
<td>5.0–57 (54.3–60.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
<td>45–46 (45–60)</td>
</tr>
<tr>
<td>Petrochenko (1950)</td>
<td>2.4–2.9 (2.5) × 0.43 (0.44)</td>
<td>—</td>
<td>5.0–57 (54.3–60.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
<td>45–46 (45–60)</td>
</tr>
<tr>
<td>Ward (1964)</td>
<td>2.4–2.9 (2.5) × 0.43 (0.44)</td>
<td>—</td>
<td>5.0–57 (54.3–60.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
<td>45–46 (45–60)</td>
</tr>
<tr>
<td>Lisitsyna (2019)</td>
<td>2.4–2.9 (2.5) × 0.43 (0.44)</td>
<td>—</td>
<td>5.0–57 (54.3–60.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
<td>45–46 (45–60)</td>
</tr>
<tr>
<td>Blatchley (1906)</td>
<td>2.4–2.9 (2.5) × 0.43 (0.44)</td>
<td>—</td>
<td>5.0–57 (54.3–60.7) × 0.5–0.65 (0.6–0.7)</td>
<td>50–57 (54.3–60.7)</td>
<td>45.5–51 (45.6–60.7)</td>
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<td>45.5–51 (45.6–60.7)</td>
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</tr>
</tbody>
</table>

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**Table 3.** (Continued)
transitional spiniform hooks at faint constriction. Lemnisci sac-ciform, equal, slightly shorter than receptacle but may reach level of anterior testis.

**Males** (based on 12 mature specimens with sperm). See table 3 for measurements and counts. Testes in anterior fifth of trunk, in tandem, not large, elliptical, nearly equal, contiguous or slightly overlapping. Four cement glands, tubular, tightly contiguous, often appearing as long cord, staggering anteriorly at posterior testis and extending posteriorly into prominent cement gland ducts (CGDs) (fig. 5b) discharging into bursa. Robust Saefftigen’s pouch prominent, elongate-drop-shaped, widest anteriorly, contiguous with posterior end of cement glands (fig. 5c) and overlapping CGDs. Bursa large, longer than wide, with oblong sensory pits in round elevated rims. Common sperm duct, cement glands duct and Saefftigen’s pouch jointly end in bursa. Gonopore terminal.

**Females** (based on 13 gravid adults). Few females had ovarian balls. See table 3 for measurements and counts. Posterior end of trunk inflating abruptly then constricting into terminal prominent papilla (fig. 3a, b). Female reproductive system largely masked by dark eggs, usually in two lateral fields along worm length. Vagina complex, subterminal, with three sphincters and slit orifice opening at base of terminal globular papilla. Wall of papilla thick, continuous with body wall, with prominent muscle band holding dorsal and ventral sides together (fig. 5d, arrow). Uterus comparatively long: uterine bell short with few but prominent cells. Eggs ovoid, with thick concentric shells, and surface varying between smooth (fig. 3c) and tuberculated (fig. 3d), with occasional sperms evident (fig. 3e). Three eggshells evident (arrows) and embryonic acanthor and its components seen in Ga-cut sections (fig. 3f).

**Taxonomic summary**

**Italian hosts examined.** The common kestrel, *F. tinnunculus* Linnaeus (Falconidae), and the common buzzard, *B. buteo* Linnaeus (Accipitridae).

**Locality.** Around Ferrara (44°50’N, 11°37’E), Province of Ferrara, Italy.

**Localization.** Intestine.

**Specimens deposited.** HWML collection number 216359.
EDXA
The unique metal composition of hooks (EDXA) (tables 4–6 and figs 6–10) demonstrated a considerably high level of calcium and phosphorus in hook tip, a middle but low level of sulphur and negligible levels of other metals (table 4 and figs 6, 7). The eggs had the highest levels of sulphur in the cortical and core areas, but high levels of phosphorus only in the core and not in the cortical layer (table 6 and fig. 10).

Micropores
The electron-dense micropores present throughout the epidermal surface of the trunk of *C. globocaudatus* are described. They have been found in various regions of the trunk in different diameters and distributions (fig. 4a, b).

Molecular results
Two identical partial 18S rDNA sequences (760 and 833 nt length) and two *cox1* mitochondrial DNA (mtDNA) sequences (651 and 668 nt length) differing by 1 nt (0.16% divergence) were generated, one from each host (i.e. *B. buteo* and *F. tinnunculus*). Figures 11 and 12 display consensus trees constructed from 18S rDNA and *cox1* alignments, respectively.

According to phylogenetic analyses based on the 18S rDNA gene (fig. 11), present newly generated sequences were identical to that of *C. globocaudatus* published by García-Varela et al. (2020a) (MN057696). Therefore, these three sequences formed a strongly supported clade. The sequences that differed the most from this clade corresponded to *Centrorhynchus nickoli* (MT161621) and *Centrorhynchus microcephalus* (AF064813) (by 0.051 and 0.045% (39 and 34 nt), respectively), although the rest of the *Centrorhynchus* 18S sequences differed from newly generated sequences by 0.033–0.041% (25–31 nt) (table 7).

In phylograms based on the *cox1* gene, present sequences formed a well-supported clade apart from all other centrorhynchid sequences included in the analyses (fig. 12). The only other clade displaying high support included *Sphaerostris* sequences, with 11–30 nt (0.017–0.047%) difference among them, and was embedded among *Centrorhynchus* sequences forming a sub-clade. However, nucleotide difference highly increased if *Sphaerostris* sequences are compared with newly generated ones (0.248–
0.255%, 158–163 nt difference) and other species of the genus *Centrorhynchus* (0.194–0.264%, 124–169 nt; table 7). Other *Centrorhynchus* cox1 sequences differed with present newly generated sequences by 0.250–0.277% (160–177 nt; table 7), and showed very weakly supported phylogenetic relationships.

**Remarks**

A morphometric comparison between our Italian specimens and those from other geographical locations where comparative measurements and counts were available is shown in table 3. *Centrorhynchus globocaudatus* has been reported from many other locations in Asia, Africa and Europe without taxonomic descriptions, leaving their morphologic variability unaccounted for. Most measured specimens were collected from *F. tinmunculus*, thus eliminating host species as a factor in observed differences in sizes (table 3). Our specimens had markedly longer proboscis hooks and larger receptacle, and smaller male reproductive system (testes and cement glands) and lemnisci, especially in males compared to specimens from Ukraine, India, Egypt, Kyrgyzstan, Russia, Georgia, Armenia and Asian Soviet Republics. The Indian specimens probably had an erroneous testis size of 468 × 360. Additionally, females from Ukraine and Kyrgyzstan had more proboscis hook rows, and those from Ukraine had relatively larger eggs (table 3). Such quantitative variations distinguishing the Italian population as a geographical variant have been previously demonstrated in the comparable case of *Mediorhynchus papillosus* Van Cleave, 1916 (Gigantorhynchidae) by Amin & Dailey (1998). Amin & Dailey (1998) studied the key taxonomic characteristics in various geographical populations of *M. papillosus*, which has a wide range of distribution in at least 73 species of birds outside of North and South America in Asia from Taiwan to the east into China, many of the former Soviet Republics and to Eastern Europe to the west. Amin & Dailey (1998) compared measurements of specimens from birds in Maryland, Colorado (their study material), Taiwan, Yakutia, Trans-Baikal, Lower Yansi River basin, the Volga basin and Oren Byreg, Ukraine, Bulgaria, China and Brazil, and demonstrated a distinct geographically based variability, especially in the size of proboscis and its armature, neck,
receptacle and testes, that appeared related to geographical restrictions, intermediate and definitive host specificity and distribution, and host feeding behaviour. “The U.S. population from Colorado and the Taiwanese population [were shown to be] at the opposite ends of the spectrum” by Amin & Dailey (1998), who dismissed the possibility of elevating them to a specific status. The population variant of *C. globocaudatus* from Italy is, nevertheless, comparable to the east–west-intraspecific clinal variants of *M. papillosus* and could have been considered as a distinct species, but this notion is dismissed here also for the same reasons.

**Discussion**

A qualitative comparison between the Italian specimens and those from other geographical locations in table 1 and elsewhere shows discrepancies that may be related to artifacts or inaccuracy of recording observations. These discrepancies are to be expected considering the large number of descriptive accounts of this common acanthocephalan that have been reported since 1800. These discrepancies are rectified below to avoid misinterpretations of the correct descriptive account of *C. globocaudatus*. Intraspecific variabilities, such as differences in the number of anterior, transitional and posterior spiniform hooks/row, will not be included.

**Setting the record straight**

- Lisitsyna & Greben (2015) stated that the proboscis has a ‘maximum width at the anterior part’ of males and females and their Fig. 3a shows a posterior proboscis constriction. The proboscis is actually widest posteriorly and Floreanu & Ienistea (1984, Fig. 21) and our observations (fig. 1a) demonstrate this character best.
- Lisitsyna & Greben (2015) stated that ‘Gonopore subterminal in both sexes.’ Actually, the male gonopore is terminal (Fig. 20). The bend at the posterior end of the male trunk makes the gonopore appear subterminal.
- Nelson & Ward (1966) examined four juvenile specimens from the long-eared hedgehog, *H. auritus* Gmelin, from Egypt, showing a ‘nearly cylindrical’ proboscis also widest anteriorly (their
Table 4. Chemical composition of a Ga (LMIS)-cut hook (cross and longitudinal cuts) for Centrohyynchus globocaudatus using X-ray scans (EDXA).

<table>
<thead>
<tr>
<th>Elementsa</th>
<th>Cross-tip cut</th>
<th>Cross-mid cut</th>
<th>Long cut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Edge</td>
<td>Middle</td>
<td>Arch</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0.06b</td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0.43</td>
<td>1.62</td>
<td>0.14</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>12.38</td>
<td>20.96</td>
<td>9.01</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>4.38</td>
<td>0.30</td>
<td>1.81</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>0.09</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>29.99</td>
<td>46.59</td>
<td>25.26</td>
</tr>
</tbody>
</table>

*aCommon protoplasmic elements (C, N, O) and processing elements (Au, Pd, Ga) omitted. Given in WT%.

*bBolded figures are used to generate spectra in figs 6 and 7.

Table 5. Chemical composition of tip cuts of hooks at three levels of proboscis of Centrohyynchus globocaudatus from Falco tinnunculus.

<table>
<thead>
<tr>
<th>Elementsa</th>
<th>Apical hook</th>
<th>Middle hook</th>
<th>Basal hook</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Edge</td>
<td>Centre</td>
<td>Edge</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.16</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0.70</td>
<td>1.02</td>
<td>0.37</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>11.37</td>
<td>18.96</td>
<td>8.65</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>5.13</td>
<td>0.47</td>
<td>10.01</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>0.06</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>20.03</td>
<td>41.69</td>
<td>16.23</td>
</tr>
</tbody>
</table>

*aCommon protoplasmic elements (C, N, O) and processing elements (Au, Pd, Ga) omitted. Given in WT%.

*bBolded figures are used to generate spectra in figs 8 and 9.
Table 6. Chemical composition of a Ga (LMIS)-cut egg of *Centrorhynchus globocaudatus* using X-ray scans (EDXA).

<table>
<thead>
<tr>
<th>Elements*</th>
<th>Egg Edge</th>
<th>Egg middle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>0.45b</td>
<td>0.01</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>1.24</td>
<td>0.01</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>12.94</td>
<td>8.04</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>0.11</td>
<td>0.34</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>0.47</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*Common protoplasmic elements (C, N, O) and processing elements (Au, Pd, Ga) omitted. Given in WT%.

*Bolded figures are used to generate spectra in fig. 10.

Fig. 6, with unusually few (26–28) longitudinal rows of 20–22 hooks each.

- Only Ward (1964) and the present paper document longer ventral and shorter dorsal proboscis hooks (table 3). All other accounts include only one set of measurements of hooks overlooking their dorso–ventro differentiation.
- Dimitrova et al. (1997), however, noted slightly longer latero-ventral hooks (60–65 long) than dorso-lateral hooks in one male worm from *F. tinnunculus*, but their other male worm from *Falco vespertinus* Linnaeus did not show such differentiation.
- Ward (1964) referred to spiniform hooks on the posterior proboscis as being ‘without roots.’ Actually, these hooks have small scutiform roots, becoming more simple and directed anteriorly in posteriormost hooks, as recognized in our Italian specimens and many other reports.
Ward (1964) also stated that 'hard-shelled embryos of mature females elliptical, with middle membrane slightly evaginated at poles.' Such 'evagination' was not evident in our Italian specimens, nor has it been reported elsewhere.

Gupta & Gupta (1972) studied one male worm from Milvus migrans in Chandigarh, India, and also reported four cement glands, which has been confirmed by Dezfuli et al. (2020) on histological grounds. It is easy to confuse the number of cement glands as they often appear to coalesce in one thick cord.

Bhattacharya (2000) examined one male worm from Ottus sp. in Tripura, India, and reported '2 or 3' cement glands. Bhattacharya (2007) later reported four cement glands (see above).

Bhattacharya (2000) also reported that second, third and fifth hooks from anterior are 'strongest.' This may be true for the fifth hook, but not likely the second or third.

Gupta & Gupta (1972) created a very confused presentation of their work marred by erroneous interpretation of ordinary anatomical structures. They noted that the trunk is aspinose, but indicate that the neck is 'short, rectangular and measures 0.180 × 0.378 mm. It is armed with 30 longitudinal rows of spines and 5–6 spines in each row.' Their Fig. 14 shows the PR inserting at the anterior end of their 'neck.' The authors clearly confused the posterior proboscis with a neck; not a common mistake.

Gupta & Gupta (1972) further indicated that the posterior testis could not be measured because it was 'distorted.' However, their Fig. 13 clearly delineated a posterior testis as distinctly outlined as the anterior testis.

Dimitrova et al. (1995) examined one male from Falco cherrug Gray in Hungary and reported 8–9 anterior hooks, with anteriormost hooks unusually long, 2–3 transitional hooks and 12–13 posterior spiniform hooks. Their disproportional and inaccurate Fig. 2, however, shows 9–10 hooks/row on an atypically spheroid anterior proboscis and only 2–5 spiniform hooks/row on the posterior proboscis.

Dimitrova & Gibson (2005) examined only two female specimens. They indicated that 'posterior trunk terminates in

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**Fig. 7.** Energy dispersive X-ray spectrum of a longitudinal GA-cut hook arch of a Centrorhynchus globocaudatus specimen showing high levels of calcium and phosphorus (see bolded figures in table 4). Insert: SEM of proboscis hooks and a longitudinal GA-cut section of a hook.
rounded conical papilla,’ but their Figs 6b and 2c of two female posterior ends show no such papillae. They also report ‘vagina with 2 sphincters.’ We find three vaginal sphincters.

**Micropores**

The micropores of *C. globocaudatus*, like those reported from other species of the Acanthocephala, are associated with internal crypts and vary in diameter and distribution in different trunk regions corresponding to differential absorption of nutrients. We have reported micropores in a large number of acanthocephalan species (Heckmann et al., 2013) and in a few more since, and demonstrated the tunnelling from the cuticular surface into the internal crypts by transmission electron microscopy. Amin et al. (2009) gave a summary of the structural–functional relationship of the micropores in various acanthocephalan species including *Rhadinorhynchus ornatus* Van Cleave, 1918, *Polymorphus minutus* (Goeze, 1782) Lühe, 1911, *Moniliformis moniliformis* (Bremser, 1811) Travassos (1915), *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos (1916, 1917) and *Sclerocollum rubrimaris* Schmidt & Paperna, 1978. Wright & Lumsden (1969) and Byram & Fisher (1973) reported that the peripheral canals of the micropores are continuous with canalicular crypts. These crypts appear to ‘constitute a huge increase in external surface area . . . implicated in nutrient up take.’ Whitfield (1979) estimated a 44-fold increase at a surface density of 15 invaginations per 1 μm² of *M. moniliformis* (Bremser, 1811) Travassos, 1915 tegumental surface. The micropores and the peripheral canal connections to the canaliculi of the inner layer of the tegument of *Corynosoma strumosum* (Rudolphi, 1802) Lühe, 1904 from the Caspian seal *Pusa caspica* (Gmelin) in the Caspian Sea were demonstrated by transmission electron micrographs in Amin et al. (2011).

**EDXA**

Results of the X-ray scans of the Ga-cut hooks (dual-beam SEM) of *C. globocaudatus* show differential composition and distribution of metals in different hook parts, with calcium and phosphorus levels being highest at the middle of hook tip and mid
cuts, as well as at the basal arch of hooks where tension and strength are paramount for hook function. The sulphur levels were very low throughout but relatively higher at the edge of hook tip cuts (table 4 and fig. 6). The chemical elements present in the hooks are typical for acanthocephalans (Heckmann et al., 2007, 2012). Note the moderate outer layer (fig. 6) of the hook that relates to the sulphur content in the hook of C. globocaudatus, which is different than in other acanthocephalans. The high sulphur content shows up in the outer edge of X-ray scans of hooks (tables 4 and 5; Amin et al., 2018). The hook centre in mid cuts has a different chemical profile than the cortical layer (table 4). The distribution of metals varied along the longitudinal axis of hooks on the proboscis, with apical and middle hooks having the highest levels of phosphorous and calcium but the lowest levels of sulphur compared to basal hooks (table 5 and figs 8, 9). The increased level of phosphorous (9.01% weight) in the developing embryo at the centre of eggs but not in its cortical layer (1.24) is noteworthy (table 6). Amin & Larsen (1989) found high levels of phospholipids in the core and nucleus of ripe eggs but none in the unripe eggs or ovarian balls of Neoechinorhynchus cylindrus (Van Cleave, 1913) Van Cleave, 1919. Sheema (2018) reported intense acid phosphate activity in the eggs of Echinorhynchus veli George & Nadakal, 1978 in agreement with the histochemical observations of Amin & Larsen (1989), Crompton (1963) in P. minutus (Goeze, 1782) Lühe, 1911 and Cain (1970) in M. hirudinaceus (Pallas, 1781) Travassos, 1917. Sheema (2018) noted intense phosphatase activity in eggs associated with accelerated cellular metabolism. Anantaraman & Ravindranath (1976) observed that the protein in the wall of the acanthor of Acanthosentis sp. was rich in aromatic and sulphur-containing amino acids, which agrees with our observations in table 5.

X-ray scans (EDXA) provide insight into the hardened components – for example, calcium, sulphur and phosphorus – of acanthocephalan hooks. The EDXA appears to be species specific, as in fingerprints. For example, EDXA is shown to have
significant diagnostic value in acanthocephalan systematics – for example, *Moniliformis cryptosaudi* Amin, Heckmann, Sharifdini & Albayati, 2019 was erected based primarily on its EDXA pattern (Amin et al., 2019b). Our methodology for the detection of the chemical profile of hooks in the Acanthocephala has also been used in other parasitic groups including the Monogenea (Rubtsova et al., 2018; Rubtsova & Heckmann, 2019) and Cestoda (Rubtsova & Heckmann, 2020). We also provide chemical and molecular data to explain and clarify our findings.

**Molecular discussion**

The results of phylogenetic analyses further support the description of the material described herein as *C. globocaudatus*. Indeed, present sequences unequivocally group with members of the same species published by García-Varela et al. (2020a) in the 18S phylogram. Unfortunately, no other sequences of this species are available to perform comparisons among different populations by *cox1* gene.

Although low bootstrap values are observed in most nodes of the SSU gene-based tree, due to low genetic signal, DNA sequence identity, determined according the alignment average distance under the $p$-distance model, yielded a value of 0.05 (95% identity). Therefore, alignment accuracy is, by far, high enough to build a reliable phylogenetic tree (Kumar & Filipski, 2006).

Actually, the SSU gene would be more adequate to address inter-generic or inter-familiar phylogenetic relationships rather than interspecific ones, as is the case. Unfortunately, as already noted by Steinauer et al. (2019), the study of the relationships among centrorhynchid taxa based on molecular data is difficult due to the low number of sequences available – in particular, no SSU gene sequences are available for other genera of Centrorhynchidae. Thus, there is a great lack of information on the genus under study and any additional phylogeny that could be provided for *Centrorhynchus* can be valuable; this is especially true in the present case, in which a new geographical variant characterized by novel morphological aspects is described.

**Fig. 10.** Energy dispersive X-ray spectrum of the centre of the edge of a Ga-cut egg of a *Centrorhynchus globocaudatus* female specimen showing a markedly high levels of sulphur (see bolded figures in table 6) and low levels of other metals tested. Insert: SEM of a part of Ga-cut egg; see fig. 3f for detail.
Fig. 11. BI phylogram reconstructed using two newly generated 18S rDNA sequences of *Centrorhynchus globocaudatus* and retrieved sequences from GenBank for *Centrorhynchidae*. Outgroup: *Echinorhynchus truttae*. Newly generated sequences are highlighted in bold. Nodal support from ML and BI analyses are indicated as BI/ML. Bootstrap values lower than 70 and posterior probability values lower than 0.9 are omitted. Scale bar indicates expected number of substitutions per site.

Fig. 12. ML phylogram reconstructed using two newly generated cox1 sequences of *Centrorhynchus globocaudatus* and retrieved sequences from GenBank for *Centrorhynchidae*. Outgroup: *Echinorhynchus truttae*. Newly generated sequences are highlighted in bold. Nodal support from ML and BI analyses are indicated as BI/ML. Bootstrap values lower than 70 and posterior probability values lower than 0.9 are omitted. Scale bar indicates expected number of substitutions per site.
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Table 7. Matrix of the pairwise 18S (left) and cox1 (right) nucleotide genetic distances among *Centrorhynchus* and *Sphaerirostris* species.
Outcomes of phylogenetic analyses based on mtDNA data (Muhammad et al., 2020) suggest that *Sphaerirostris* is a sister group of *Centrorhynchus aluconis*, conforming a sub-clade within *Centrorhynchus*, which would, therefore, be a paraphyletic taxon. Our results seem to agree with this suggestion; however, the weak bootstrap support and the nucleotide differences associated to these relationships in phylogenetic analyses hamper its confirmation. In addition, the genera *Centrorhynchus* and *Sphaerirostris* have been well differentiated based on morphology (see Table 1 in Amin & Canaris, 1997). Specifically, one of the clearest diagnostic features allowing discrimination between both genera, the lacunar system pattern, is recognized as one of the most important taxonomic criteria in the classification of the Acanthocephala at the generic and higher levels (Amin & Canaris, 1997). The short spindle-shaped trunk and short globular anterior proboscis in *Sphaerirostris* clearly distinguish it from *Centrorhynchus*, with a long cylindrical trunk and long cylindrical anterior proboscis, among other features.

The two *cox1* available sequences of *Sphaerirostris lanceolata* were obtained from cystacanths present in amphibians and ardeid birds (i.e. *Ardeola grayi*), respectively (Kang & Li, 2018; Muhammad et al., 2020), and the only *cox1* available sequence of *Sphaerirostris picae* was obtained from adults infecting the corvid *Dendrocitta vagabunda* (see Muhammad et al., 2019). Indeed, while *Sphaerirostris* mostly infects passerine birds, *Centrorhynchus* mainly infects birds of prey instead. Therefore, ecological associations between parasites and their hosts further support morphological studies in marking a differentiation between these two centrorhynchid genera. Interestingly, a study based on 18S DNA gene analysis revealed the relatively close relationship between these two centrorhynchid genera. Moreover, a study based on 18S DNA gene analysis revealed the relatively close relationship between these two centrorhynchid genera. Specifically, one of the clearest diagnostic features allowing discrimination between both genera, the lacunar system pattern, is recognized as one of the most important taxonomic criteria in the classification of the Acanthocephala at the higher levels (Amin & Canaris, 1997). The short spindle-shaped trunk and short globular anterior proboscis in *Sphaerirostris* clearly distinguish it from *Centrorhynchus*, with a long cylindrical trunk and long cylindrical anterior proboscis, among other features.

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Conflicts of interest. None.

Ethical standards. The authors declare that they have observed all applicable ethical standards.

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