Quadriacanthus species (Monogenea: Dactylogyridae) from catfishes (Teleostei: Siluriformes) in eastern Africa: new species, new records and first insights into interspecific genetic relationships

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Abstract

Background: African catfishes of the families Bagridae and Clariidae are known to be parasitized with monogeneans of Quadriacanthus Paperna, 1961 (Dactylogyridae). The genus remains taxonomically challenging due to its speciose nature and relatively wide host range representing two fish orders, i.e. Siluriformes and Osteoglossiformes, in Africa and Asia. Here, we investigated diversity of Quadriacanthus spp. parasitizing Clarias gariepinus (Burchell), Heterobranchus bidorsalis Geoffroy Saint-Hilaire, and Bagrus docmak (Forsskål) collected in the Lake Turkana (Kenya) and Nile River Basin (Sudan). The interspecific relationships among Quadriacanthus spp. parasitizing catfishes inferred from ribosomal DNA sequences were investigated for the first time.

Methods: A combined morphological and molecular approach was used for description of the new species and for a critical review of the previously described Quadriacanthus spp., by means of phase contrast microscopic examination of sclerotized structures, and assessing the genetic divergence among the species found using rDNA sequences.

Results: Seven species (including four new) of Quadriacanthus were identified. These were as follows: Quadriacanthus aegypticus El-Naggar & Serag, 1986, Quadriacanthus clariadis Paperna, 1961, Quadriacanthus fomicatus n. sp., Quadriacanthus pravus n. sp., and Quadriacanthus zuheiri n. sp. from Clarias gariepinus (Clariidae); Quadriacanthus mandibulatus n. sp. from Heterobranchus bidorsalis (Clariidae); and Quadriacanthus bagrae Paperna, 1979 from Bagrus docmak (Bagridae). For both 18S-ITS1 and 28S rDNA regions, Q. clariadis from a clariid fish was found to be most closely related to Q. bagrae from a bagrid host. Quadriacanthus mandibulatus n. sp. was observed to be the most distant species from the others. The separation of Q. mandibulatus n. sp. from the other species corresponds with the different morphology of its copulatory tube. The copulatory tube is terminally enlarged in Q. mandibulatus n. sp., while the tube in all other congeneres studied is comparatively small and with an oblique tapering termination.

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Background

Monogenea is a diverse group of mostly ectoparasitic flatworms showing great potential as model organisms to study the ecological and evolutionary processes that drive diversification and speciation. The high host specificity shown by most monogeneans enables searches for links between the ecological characteristics of the hosts and the diversity of their parasites [1].

Among monogeneans, Quadriacanthus Paperna, 1961 (Dactylogyridae) represents one of the genera with wider host and geographical distribution. Although this genus comprises mostly gill parasites of African and Asian clariids (Siluriformes, Clariidae), one species has been recorded on bagrids (Siluriformes, Bagridae) and one species on phylogenetically distant notopterids (Osteoglossiformes, Notopteridae) in Africa [2, 3].

The genus was proposed by Paperna [4] for Q. clariadis Paperna, 1961 from the gills of Clarias gariepinus (Burchell) (syn. C. lazerca) collected in Israel and characterized, in part, by having two unequal bars, each with a solid base. Kritsky & Kulo [5] subsequently emended the diagnosis of Quadriacanthus and recognized that the ventral bar is composed of two components articulating medially. Despite the work of these authors, Dubey et al. [6] established Anacornuatus Dubey, Gupta & Agarwal, 1992 for those species of Quadriacanthus that possess a two-piece ventral bar instead of a single-piece ventral bar, as indicated by Paperna [4]. They were evidently unaware of the work of Kritsky & Kulo [5] and hence erred in proposing the new genus. Consequently, Lim et al. [2], who listed 24 species of Quadriacanthus parasitizing Clariidae (Clarias spp. and Heterobranchus spp.) and African Bagrus spp., plus one Quadriacanthus species of doubtful validity, infecting tilapia (see also [5]), synonymized Anacornuatus with Quadriacanthus. However, these authors were not able to ascertain the validity of the two species assigned to Anacornuatus with Quadriacanthus. However, Tripathi et al. [7] added generic characters, redefined the dorsal bar as “T or Y-shaped with mid-posterior process”, and limited the taxon to 25 species (including Anacornuatus postbifidus Dubey, Gupta & Agarwal, 1992 as a new combination within Quadriacanthus). The recent descriptions of three new species from Clarias submarginatus Peters by Bahana et al. [8] brings the number of Quadriacanthus species from siluriform hosts to 28. Nack et al. [3] revealed the presence of a species of Quadriacanthus on a fish host belonging to the Notopteridae (Osteoglossiformes). This finding extends the host range of species of Quadriacanthus to a new family (Notopteridae) and even a new order (Osteoglossiformes).

In a recent survey of monogeneans parasitizing catfishes from Kenya and Sudan, we recovered three described (Q. aegypticus El-Naggar & Serag, 1986, Q. clariadis Paperna, 1961 and Q. bagrae Paperna, 1979) and four new species of Quadriacanthus. We thus aimed to describe these four species and determine their relationships to congeners based on the partial 18S, entire ITS1, and partial 28S rDNA sequences.

Methods

Fish collection

Catfish hosts Bagrus docmak (Forsskål) (Bagridae), Clarias gariepinus (Burchell) and Heterobranchus bidorsalis Geoffroy Saint-Hilaire (Clariidae) (all autochthonous fishes) were collected by hook-and-line or beach seine net, or purchased at local fish markets in five localities in Kenya (Lake Turkana) and Sudan (White and Blue Nile River) during the period 2008–2014 (Table 1; Fig. 1). Fish hosts were identified using the keys given by Bailey [9] and Hopson & Hopson [10]. Scientific and common names of fishes are those provided in FishBase [11] and verified in Eschmeyer et al. [12].

Parasite collection and identification

The gills of freshly killed fishes were extracted and examined in bottled water under a dissecting microscope. Live monogeneans were individually picked from the gills with fine needles and immediately processed. Some specimens were prepared for morphological studies following Musilová et al. [13]. Briefly, they were flattened using coverslip pressure in order to best expose their hard parts, and fixed with a mixture of glycerine and ammonium picrate (GAP). Specimens collected for DNA analyses were bisected using fine needles under a dissecting microscope. Subsequently, one half of the
Table 1: Localities from which siluriform species were collected during 2008-2014

| Locality number | Locality name                                         | Coordinates                           | Year of collection |
|-----------------|-------------------------------------------------------|---------------------------------------|--------------------|
| 1               | Kalokol - Fishing Lodge, Lake Turkana, Kenya          | 3°33′18.26″N, 35°54′56.03″E           | 2008, 2009         |
| 2               | Loyingalani - El Molo Bay, Lake Turkana, Kenya        | 2°49′45.55″N, 36°41′55.32″E           | 2008, 2009         |
| 3               | Todonyang - Omo River Delta, Lake Turkana, Kenya      | 4°27′36.37″N, 35°56′15.44″E           | 2008, 2009         |
| 4               | Fishmarket in Kosti, White Nile, Sudan                | 13°10′18.58″N, 32°40′19.24″E          | 2010, 2014         |
| 5               | Fishmarket in Sennar, Blue Nile, Sudan                | 13°32′31.09″N, 33°37′15.79″E          | 2010, 2014         |

Fig. 1: Map of the localities from which siluriform species were collected during 2008–2014 (see also Table 1)
body (either the posterior part with haptoral sclerites or anterior part containing the male copulatory organ) was fixed in 96% ethanol for later molecular analysis; the other body half was completely flattened under coverslip pressure and fixed with GAP for species identification. The body half in GAP was deposited (one per species) as a hologenophore, i.e. a voucher specimen from which a molecular sample is directly derived (see [14] for terminology). Parasite specimens collected in Kenya were not used for molecular analysis.

The mounted monogenean specimens (or their parts) were studied using an Olympus BX 61 microscope equipped with phase contrast optics, and drawings were made with the aid of a drawing attachment. Measurements, all in micrometres, were taken using digital image analysis (Stream Motion, version 1.9.2) and are presented as the range followed by the mean and the number (n) of specimens measured in parentheses. The dimensions of the body and haptor were obtained from unflannted specimens as the longest measurements in dorsoventral view; measurements of the sclerotized structures (the haptoral and reproductive hard parts) were taken from specimens flattened under coverslip pressure, facilitated by the blotting of excess water with a filter paper. The schemes of measurement for the hard structures are shown in Fig. 2; in essence, the method of measuring the anchors follows the procedures outlined by Řehulková & Gelnar [15]. The numbering of hook pairs (in Roman numerals) follows that recommended by Mizelle [16]. The male copulatory organ is henceforth abbreviated to MCO. For comparative purposes, species of some previously described species were examined: Quadriacanthus agnebiensis N’Doubu, Lambert & Euzet, 1999 (MNHN 572 HF Tk 89); Quadriacanthus clariadis Paperna, 1961 (MRAC 37.160); Quadriacanthus numidus Kritsky & Kulo, 1988 (MNHN 572 HF Tk 91); Quadriacanthus thysi N’Doubu, Lambert & Euzet, 1999 (MNHN 577 HF Tk 94 and 576 HF Tk 93; and MRAC 37416). Note that the authorities of the new taxa were not used for molecular analysis.

DNA extraction, PCR amplification and sequencing
DNA was extracted from 2 to 6 individuals of each collected species using a DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. DNA was stored in AE buffer at -20 °C. Two nuclear ribosomal DNA fragments were used in our analysis: fragment spanning partial 18S rDNA (18S) and entire internal transcribed spacer 1 (ITS1), and fragment of partial nuclear 28S rDNA (28S). Until now, only two 28S sequences for Quadriacanthus kobiensis Ha, 1968 (EF100545, AY841874) and one 18S-ITS1 fragment for Quadriacanthus sp. (HG491496) had been deposited in GenBank. The partial 28S fragment was amplified using primers C1 (forward; 5’-ACC CGC TGA ATT TAA GCA T-3’) and D2 (reverse; 5’-TGG TCC GTG TTT CAA GAC-3’) [18]. The 18S-ITS1 fragment was amplified in one round using primers S1 (forward, 5’- ATT CCG ATA ACG AAC GAG ACT-3’) [19] and IR8 (reverse, 5’-GCT AGC TGC GTT CTT CAT CGA-3’), that anneal to the 18S and 5.8S rDNA genes, respectively [20]. PCRs were performed according to Mendlová et al. [21]. The PCR products were electrophoresed on a Gold View strained agarose gel (2%) and then successful PCRs, in which a single fragment was amplified, were purified using High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). The purified PCR products were sequenced for both strands with the same primers as used in the amplification. Sequencing was carried out using BigDye® Terminator v3.1 Cycle Sequencing Kit and an Applied Biosystems 3130 Genetic Analyzer. Nucleotide sequences of the 18S-ITS1 and partial 28S regions were assembled and edited using Sequencer software (Gene Codes Corporation, Ann Arbor, MI, USA). The final sequences were deposited in the GenBank database under accession numbers KX713993–KX713998 and KX685951–KX685956.

Sequence and phylogenetic analysis
Because no significant differences were found between 18S and ITS1 sequence data (partition homogeneity test, P = 1.00), further analyses were performed based on concatenated 18S-ITS1 sequences. Two datasets (18S-ITS1 and 28S) were used to estimate the interspecific relationships among the Quadriacanthus species. All corrected 18S-ITS1 and 28S sequences were aligned using ClustalW [22] and improved manually using the program BioEdit version 7.1.11 [23]. Alignments were then trimmed automatically using TrimAl v.1.3 [24]. Calculations of genetic distances (Kimura 2-parameter [25]) among sequences of Quadriacanthus species were carried out in MEGA 7 [26].

Maximum likelihood analyses were conducted using MEGA 7 [26] with 1000 rapid bootstrap replicates. Data were modelled according to the K2 + G model. Phylogenetic trees were rooted by including available data from GenBank: Schilbetrema sp. (HG491495) isolated from Schilbe intermedius Rüppell in Africa for the 18S-ITS1 dataset, and Schilbetrema sp. (KP056243) isolated from Pareutropius debauwi (Boulenger) in West Africa for the 28S dataset. Quadriacanthus sp. (HG491496) isolated from Heterobranchus bidorsalis Geoffroy Saint-Hilaire in Senegal (West Africa) was also included in the 18S-ITS1 phylogenetic analysis, and Q. kobiensis Ha, 1968 (AY841874) isolated from Clarias batrachus (Linnaeus) in China was used in the 28S phylogenetic analysis.
Results
Our investigation of the monogeneans from the gills of three catfish species revealed the presence of three previously described and four new species of Quadriacanthus. Bagrus docmak (Bagridae) and Heterobranchus bidorsalis (Clariidae) were each found to harbour one Quadriacanthus species, Q. bagrae and Q. mandibulatus n. sp., respectively. Five Quadriacanthus species were found to infect Clarias gariepinus (Clariidae): Q. aegypticus, Q. clariadis, Q. fornicatus n. sp., Q. pravus n. sp., and Q. zuheiri n. sp. All the species found are morphologically characterized and described below.

Family Dactylogyridae Bychowsky, 1933
Genus Quadriacanthus Paperna, 1961

Quadriacanthus aegypticus El-Naggar & Serag, 1986

Syn. Anacornuatus aegypticus (El-Naggar & Serag, 1986) Dubey, Gupta & Agarwal, 1992

Type-host and locality: Clarias gariepinus (Burchell) (syn. C. lazera) (Clariidae), Lake Manzala and the Demietta branch of the River Nile, Dakahlia Governorate, Egypt [27].

Host: Clarias gariepinus (present study).

Localities: Lake Turkana, Kenya (localities 1–3); Nile River Basin, Sudan (localities 4, 5) (present study).

Site in host: Gill lamellae.

Other records: C. gariepinus (syn. C. lazera), Nile River near Cairo, Egypt [5]; C. gariepinus, Lake Kariba, Zimbabwe [28]; C. gariepinus, River Nile in the Helwan locality, south Cairo, Egypt [29]; C. gariepinus, Nwanedi-Luphephe Dams, Limpopo River System, South Africa [30]; C. gariepinus, Vaal Dam, Gauteng Province, South Africa [31]; C. gariepinus, Lake Tana (Bahir Dar), Ethiopia [32].
Voucher material: MNHN HEL625 (1 specimen; locality 4); MNHN HEL626 (2 specimens; locality 1); MNHN HEL627 (1 specimen; locality 3); IPCAS M-632 (1 specimen from locality 1; 1 specimen from locality 5).

Measurements
[Based on 10 flattened and 3 unflattened specimens in GAP; Fig. 3]. Body length 475–569 (513; n = 3); greatest width 80–125 (108; n = 3). Haptor 95–100 (97; n = 3) long, 110–155 (136; n = 3) wide. Ventral anchor: a = 33–37 (35; n = 10); b = 11–13 (12; n = 10); c = 9–11 (10; n = 10); x/y = 1.2–1.7 (1.4; n = 10). Dorsal anchor: a = 40–44 (42; n = 10); b = 3–5 (4; n = 10); c = 16–21 (19; n = 10); x/y = 1.1–1.2 (1.1; n = 10). Ventral bar: d = 40–46 (43; n = 10). Dorsal bar: e = 39–52 (49; n = 10); f = 21–31 (28; n = 10); g = 10–17 (15; n = 10); h = 14–17 (15; n = 10). Hooks: 7 pairs; i = 11–29 (17; n = 10); hook I 16–19 (17; n = 10); hooks II–V 11–15 (13; n = 10); hook VI 26–29 (29; n = 10); hook VII 18–19 (18; n = 10). Vagina: slightly sclerotized; j = 17–21 (19; n = 10). MCO: k = 39–44 (41; n = 10); l = 36–40 (38; n = 10).

Differential diagnosis
The present specimens closely fit the characters of the original description of Q. aegypticus by El-Naggar & Serag [27] as well as the drawings/measurements provided by Kritsky & Kulo [5] and Douéllou & Chishawa [28] and there is little doubt that they are conspecific. However, it should be mentioned that in the original paper by Douéllou & Chishawa [28] the illustrations of Q. aegypticus were mixed with those of another Quadriacanthus species; their figure 2 (identified as the hap- toral structures, MCO and vagina of Q. aegypticus) clearly represents the sclerotized structures (actually without depiction of a vagina) of another species of Quadriacanthus, most probably those of Q. clariadis, although the morphometrical characteristics of the sclerotized structures correspond to those originally described for Q. aegypticus. Also, Douéllou & Chishawa’s [28] figure 1 (identified as the sclerotized structures of Q. bagrae) and figure 2 are identical (see also remarks to Q. bagrae); indeed, figure 2 was later replaced with the correct version (i.e. illustrating the sclerotized structures of the real Q. aegypticus) as an erratum to Douéllou & Chishawa’s [28] original paper.

Quadriacanthus aegypticus is most similar to Q. clariadis on the basis of the morphology of the haptor sclerites. It differs from the latter species by having (i) noticeably larger ventral anchors in relation to the ventral bar; (ii) a ventral anchor with an elongated shaft (the shaft of the ventral anchor is comparatively shorter in Q. clariadis); (iii) a sclerotized vagina; and (iv) an accessory piece with two claw-like hooks serving as a guide for the distal portion of the copulatory tube and a medial part modified into two protruding diverticula.

Quadriacanthus bagrae Paperna, 1979
Syn. Quadriacanthus clariadis bagrae Paperna, 1979

Type-host and locality: Bagrus docmak (Forsskål) (syn. B. docmak) (Bagridae), Lake Victoria, Uganda [33].
Host: Bagrus docmak (present study).
Locality: Nile River Basin, Sudan (locality 5) (present study).
Site in host: Gill lamellae.
Other records: Bagrus docmak (syn. B. docmak), Albert-Nile near Chobe, Uganda [33]; Bagrus bajad (syn. B. bayad), Lake Albert, Uganda [33]; Bagrus orientalis, River Ruaha, Tanzania [33]; Clarias gariepinus (syn. C. lazera), River Nile near Cairo, Egypt [5]; Clarias gariepinus, Lake Kariba, Zimbabwe [28]; C. gariepinus, River Gomti, Lucknow, State of Uttar Pradesh, India [7].
Voucher material: MNHN HEL628 (1 specimen; locality 5); MNHN HEL629 (1 specimen; locality 5); IPCAS M-633 (2 specimens; locality 5). Hologenophore: MNHN HEL641 (locality 5).
Representative DNA sequences: 18S-ITS1 rDNA (GenBank acc. no. KX713993) and 28S rDNA (GenBank acc. no. KX685951) (see also Table 2).

Measurements
[Based on 10 flattened and 3 unflattened specimens in GAP; Fig. 4]. Body length 781–838 (815; n = 3); greatest width 156–194 (171; n = 3). Haptor 100–124 (109; n = 3) long, 161–194 (179; n = 3) wide. Ventral anchor: a = 27–33 (30; n = 10); b = 9–14 (12; n = 10); c = 8–11 (9; n = 10); x/y = 1.1–1.6 (1.3; n = 10). Dorsal anchor: a = 37–43 (40; n = 10); b = 8–9 (9; n = 10); c = 16–18 (17; n = 10); x/y = 0.9–1.3 (1.2; n = 10). Ventral bar: d = 47–57 (52; n = 10). Dorsal bar: e = 58–73 (64; n = 10); f = 26–33 (29; n = 10); g = 17–23 (20; n = 10); h = 12–15 (13; n = 10). Hooks: 7 pairs; i = 12–25 (15; n = 3); hook I 14–16 (15; n = 3); hooks II–V 12–16 (14; n = 3); hook VI 21–25 (23; n = 10); hook VII 13–15 (15; n = 3). Vagina: not observed. MCO: k = 32–35 (34; n = 10); l = 29–33 (31; n = 10).

Differential diagnosis
This species was elevated from subspecies status under Q. clariadis and adequately redescribed by Kritsky & Kulo [5]. The morphology and measurements of our specimens generally correspond with the redescriptions and later characterization of this species by Tripathi et al. [7]. Small differences were observed in the morphology of the hooks. However, the shapes of the hooks fall within the variation observed in our series. In individual specimens, the shanks of hooks appear to be more or less robust. The report of Q. bagrae by Douéllou &...
Chishawa [28] is erroneous because their drawings and measurements of the haptoral structures and MCO suggest that these authors were dealing with another Quadriacanthus species, most probably with Q. clariadis. Their depiction of the sclerotized structures of “Q. bagrae” (figure 1) shows a ventral bar with elongated arms (each component is more than twice longer than the total length of the ventral anchor, while it is less than twice longer in Q. bagrae) and a dorsal anchor with an elongated bent shaft and short point (in Q. bagrae, the dorsal anchor has a short curved shaft and a moderate point), all of which are characters consistent with specimens identified as Q. clariadis by El-Naggar & Serag [34], Kritsky & Kulo [5], Tripathi et al. [7], and the present study. Moreover, Douëllou & Chishawa [28] themselves supported our opinion by stating that the hooklets (= hooks) of their specimens were similar to those of Q. clariadis rather than to those of Q. bagrae. According to our observations, the morphology of Q. bagrae and Q. clariadis male copulatory organ is very similar; the morphology of haptoral sclerites, however, clearly differs between the two Quadriacanthus species.

Molecular characterization

The sequence of the 18S-ITS1 region of Q. bagrae was 921 bp long, of which 515 bp corresponded to the partial

Table 2 List of Quadriacanthus species used in this study, including their host species, locality (with number in parentheses), total number of isolates and GenBank accession numbers for 18S-ITS1 and 28S sequences

| Parasite species | Host species | Locality of collection* | Isolates | 18S-ITS1          | 28S          |
|-----------------|--------------|-------------------------|----------|------------------|--------------|
| Q. aegypticus   | Clarias gariepinus | LT (1, 2, 3); WN (4); BN (5) | –         | –                | –            |
| Q. bagrae       | Baganag docmak | BN (5)                  | 3         | KX713993         | KX685951     |
| Q. clariadis    | Clarias gariepinus | LT (1, 3); WN (4); BN (5) | 6         | KX713994         | KX685952     |
| Q. fornicatus n. sp. | Clarias gariepinus | WN (4); BN (5)            | 3         | KX713995         | KX685953     |
| Q. mandibulatus n. sp. | Heterobranchus bidorsalis | LT (3); BN (5)         | 5         | KX713996         | KX685954     |
| Q. pravus n. sp. | Clarias gariepinus | WN (4); BN (5)            | 3         | KX713997         | KX685955     |
| Q. zuheiri n. sp. | Clarias gariepinus | WN (4); BN (5)            | 2         | KX713998         | KX685956     |

Abbreviations: LT Lake Turkana, Kenya, WN White Nile (Kosti), Sudan, BN Blue Nile (Sennar), Sudan
*Localities where specimens were collected for molecular analysis are shown in bold
18S rDNA region and 406 bp corresponded to the entire ITS1 region. The sequence of the partial 28S region was 777 bp long. No intraspecific variability was found in 18S-ITS1 or 28S sequences.

Quadriacanthus clariadis Paperna, 1961

Syn. Quadriacanthus clariadis clariadis Paperna, 1979

Type-host and locality: Clarias gariepinus (Burchell) (syn. C. lazera) (Clariidae), Lake Galilee, Israel [4].

Host: Clarias gariepinus (present study).

Localities: Lake Turkana, Kenya (localities 1, 3); Nile River Basin, Sudan (localities 4, 5) (present study).

Site in host: Gill lamellae.

Other records: Clarias gariepinus (syn. C. lazera), Bahr Mouis, River Nile near Zagazig, Egypt [35], Lake Manzala and Demietta Branch, River Nile near Mansoura, Egypt [34], River Nile near Cairo, Egypt [5]; C. gariepinus, River Gomti, Lucknow, State of Uttar Pradesh, India [7]; C. gariepinus, Nwanedi-Luphephe Dams, Limpopo River System, South Africa [30].

Voucher material: MNHN HEL627 (1 specimen; locality 3); MNHN HEL630 (1 specimen; locality 5); MNHN HEL631 (1 specimen; locality 4); MNHN HEL632 (2 specimens; locality 1); IPCAS M-262 (1 specimen from locality 5; 2 specimens from locality 4; 1 specimen from locality 3). Hologenophore: MNHN HEL642 (locality 5).

Comparative material examined: Voucher specimen of Quadriacanthus clariadis Paperna, 1961 (MRAC 37.160).

Representative DNA sequences: 18S-ITS1 rDNA (GenBank acc. no. KX713994) and 28S rDNA (GenBank acc. no. KX685952) (see also Table 2).

Measurements

[Based on 10 flattened and 3 unflattened specimens in GAP; Fig. 5]. Body length 491–564 (518; n = 3); greatest width 89–115 (100; n = 3). Haptor 68–119 (89; n = 3) long, 113–151 (131; n = 3) wide. Ventral anchor: a = 28–31 (29; n = 10); b = 10–13 (11; n = 10); c = 7–10 (8; n = 10); x/y = 1.1–1.9 (1.4; n = 10). Dorsal anchor: a = 45–51 (48; n = 10); b = 5–7 (6; n = 10); c = 15–20 (17; n = 10); x/y = 0.9–1.0 (1.0; n = 10). Ventral bar: d = 53–64 (60; n = 10). Dorsal bar: e = 54–65 (60; n = 10); f = 29–37 (32; n = 10); g = 13–19 (17; n = 10); h = 13–16 (15; n = 10). Hooks: 7 pairs; i = 12–39 (18; n = 10); hook I 18–22 (20; n = 10); hooks II–V 12–15 (14; n = 10); hook VI 33–39 (35; n = 10); hook VII 15–16 (16; n = 10). Vagina: not observed. MCO: k = 26–28 (27; n = 10); l = 24–27 (26; n = 10).

Differential diagnosis

This species was adequately redescribed by Kritsky & Kulo [5]. Examination of the voucher specimen (MRAC 37.160) showed that our specimens are con-specific with this material. The morphology of the
sclerotized structures of our specimens also correspond to that observed by Tripathi et al. [7]. Molnar & Mossalam’s [35] report of Quadriacanthus clariadis contains photographs indicating that the authors found two Quadriacanthus species, i.e. Q. clariadis and Q. aegypticus, but were not able to distinguish between them (see [5]). Quadriacanthus clariadis resembles a number of congeners: Q. aegypticus El-Naggar & Serag, 1986; Q. agnebiensis N’Douba, Lambert & Euzet, 1999; Q. allobychowskiiella Paperena, 1979; and Q. longifilisi N’Douba, Lambert & Euzet, 1999) [27, 33, 36] by its having a ventral anchor with a curved shaft and long point, a dorsal anchor with an elongated bent shaft and short point, and an MCO composed of a straight tapered copulatory tube and an accessory piece with terminal hook(s). The differentiation of Q. clariadis and Q. aegypticus is provided in the remarks for the latter species. Quadriacanthus allobychowskiella is easily separated from Q. clariadis by its dorsal anchor having a large accessory sclerite. In Q. agnebiensis and Q. longifilisi, the hooks of pair VII are markedly longer than those of the corresponding pair in Q. clariadis.

**Molecular characterization**

The combined 18S-ITS1 sequence of Q. clariadis was 920 bp long. This sequence included 514 bp of the partial 18S rDNA region and the complete 406 bp long ITS1 region. The sequence of the partial 28S region was 845 bp long. No intraspecific variability was found in the 18S-ITS1 and 28S sequences.

Quadriacanthus fornicatus Francová & Řehulková n. sp.

**Type-host:** Clarias gariepinus (Burchell) (Clariidae).

**Type-locality:** Nile River Basin, Sudan (locality 5).

**Other locality:** Nile River Basin, Sudan (locality 4).

**Type-material:** Holotype: MNHN HEL633. Paratypes: MNHN HEL634 (1 specimen); IPCAS M-634 (1 specimen).

**Voucher material:** MNHN HEL639 (2 specimens; locality 4); IPCAS M-634 (2 specimens; locality 4). Hologenophore: MNHN HEL643 (locality 5).

**Site in host:** Gill lamellae.

**Representative DNA sequences:** 18S-ITS1 rDNA (GenBank acc. no. KX713995) and 28S rDNA (GenBank acc. no. KX685953) (see also Table 2).

**ZooBank registration:** To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [37], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:ADCB9E56-E8F1-48B6-AD21-BBA5E52D0B39. The LSID for the new name Quadriacanthus fornicatus n. sp. is urn:lsid:zoobank.org:act:6C76112A-B9EA-45E4-9DBB-96B3738A12CF.
Etymology: The specific name is derived from Latin (fornicatus = arched, vaulted) and refers to the shape of the dorsal anchor shaft.

Description
[Based on 7 flattened and 2 unflattened specimens in GAP; Fig. 6]. Body length 384–404 (394; n = 2); greatest width 75–84 (80; n = 2). Haptor 77–84 (80; n = 2) long, 84–95 (89; n = 2) wide. Ventral anchor with moderate base, curved shaft, long point; accessory sclerite small, with subequal wings; a = 26–31 (28; n = 7); b = 11–13 (12; n = 7); c = 7–8 (7; n = 5); x/y = 1.0–1.3 (1.2; n = 7). Dorsal anchor with broad base, curved shaft, long point; accessory sclerite moderate, with poorly differentiated wings; a = 32–39 (35; n = 7); b = 12–13 (12; n = 7); c = 9–14 (12; n = 7); x/y = 0.9–1.2 (1.1; n = 7). Ventral bar composed of two elongated components; d = 44–54 (49; n = 5). Dorsal bar with small anterior shield; mid-posterior process trapeziform, with uneven margins; e = 42–64 (54; n = 6); f = 17–33 (27; n = 6); g = 8–14 (12; n = 7); h = 9–13 (12; n = 7). Hooks: 7 pairs, dissimilar in size; i = 12–29 (15; n = 5); hook I 13–17 (15; n = 7); hooks II–V 12–14 (13; n = 5); hook VI 25–29 (27; n = 7); hook VII 13–14 (14; n = 6). Vagina not observed. MCO comprising copulatory tube and accessory piece; k = 23–29 (26; n = 7). Copulatory tube straight; base simple, without thickened margin or flange; l = 22–26 (24; n = 7). Accessory piece basally articulated to the copulatory tube in the form of a spike-like structure; medial part lightly sclerotized; distal part a hook-like structure with broader base.

Differential diagnosis
Quadriacanthus fornicatus n. sp. could be confused with Q. simplex, a species described on Heterobranchus isopterus in Ivory Coast by N'Douba et al. [36], by having nearly identical haptoral sclerites. However, these species are easily differentiated by the comparative morphology of the MCO. The accessory piece of the MCO in Q. simplex is noticeably simpler than that in the new species.

Molecular characterization
The combined 18S-ITS1 sequence of Q. fornicatus n. sp. was 912 bp long. This sequence included 493 bp of the partial 18S rDNA region and the complete 419 bp-long ITS1 region. The sequence of the partial 28S region was 847 bp long. No intraspecific variability was found in the 18S-ITS1 and 28S sequences.

Quadriacanthus mandibulatus Francová & Řehulková n. sp.

Type-host: Heterobranchus bidorsalis Geoffroy Saint-Hilaire (Clariidae).

Type-locality: Nile River Basin, Sudan (locality 5).

Other locality: Lake Turkana, Kenya (locality 3).

Type-material: Holotype: MNHN HEL635. Paratypes: MNHN HEL635 (1 specimen); MNHN HEL636 (1 specimen); IPCAS M-635 (1 specimen).

Voucher material: MNHN HEL637 (3 specimens; locality 3); IPCAS M-635 (2 specimens; locality 3). Hologenophore: MNHN HEL644 (locality 5).

Fig. 6 Quadriacanthus fornicatus n. sp. ex C. gariepinus (Sudan). Sclerotized structures. Abbreviations: Va, ventral anchor; Vb, ventral bar; Da, dorsal anchor; Db, dorsal bar; HI-HVII, hooks; Mco, male copulatory organ.
Comparative material examined: Type-specimens of Quadriacanthus thysi N’Douba, Lambert & Euzet, 1999 (holotype MNHN 577 HF Tk 94; paratypes 576 HF Tk 93 and MRAC 37416).

Site in host: Gill lamellae.

Representative DNA sequences: 18S-ITS1 rDNA (GenBank acc. no. KX713996) and 28S rDNA (GenBank acc. no. KX685954) (see also Table 2).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [37], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:ADCB9E56-E8F1-48B6-AD21-BBA5E52D0B39. The LSID for the new name Quadriaacanthus mandibulatus n. sp. is urn:lsid:zoobank.org:act:AD61A17B-E49C-49B6-B47F-A5CCA2ED2D21.

Etymology: The specific name is derived from Latin (mandibula = an insect mandible; treated as an adjective) and reflects the insect mandible appearance of the dorsal bar process.

Description
[Based on 10 flattened and 5 unflattened specimens in GAP; Fig. 7]. Body length 569–840 (734; n = 5); greatest width 132–148 (139; n = 5). Haptor 140–184 (158; n = 5) long, 164–192 (178; n = 5) wide. Ventral anchor with narrow base, shaft sharply (at about 90°) bent medially, slightly recurved (poorly differentiated) point; accessory sclerite Y-shaped, with two subequal wing-like processes; a = 28–30 (29; n = 10); b = 12–14 (13; n = 10); c = 9–15 (13; n = 10); x/y = 1.0–1.3 (1.1; n = 10). Dorsal anchor with small base, elongated shaft bent (at about 90°) proximally and waved distally, short recurved point; accessory sclerite triangular; a = 72–86 (79; n = 10); b = 8–9 (8; n = 10); c = 24–27 (26; n = 10); x/y = 0.9–1.4 (1.1; n = 10). Ventral bar composed of two elongated components articulating medially; d = 54–68 (62; n = 10). Dorsal bar broadly V-shaped, with small anterior shield; large mid-posterior process, an insect mandible-like sclerotized structure distally passing into a lightly sclerotized membrane (often with a needle-like distal margin); e = 84–102 (95; n = 10); f = 28–41 (34; n = 10); g = 12–18 (15; n = 10); h = 13–17 (15; n = 6). Hooks: 7 pairs, dissimilar in size; i = 12–74 (28; n = 10); hook I 14–18 (16; n = 10); hooks II–V1 2–17 (14; n = 10); hook VI 67–74 (70; n = 10); hook VII 49–56 (52; n = 10). Vagina not observed. MCO comprising copulatory tube and accessory piece; k = 70–79 (74; n = 10). Copulatory tube a broad slightly curved tube with spoon-like base and subterminal flange; l = 66–71 (69; n = 10). Accessory piece articulated to the base of the copulatory tube, with constricted medial part and hook-shaped terminal portion.

Fig. 7 Quadriacanthus mandibulatus n. sp. ex H. bidorsalis (Sudan). Sclerotized structures. Abbreviations: Va, ventral anchor; Vb, ventral bar; Da, dorsal anchor; Db, dorsal bar; HI-HVII, hooks; Mco, male copulatory organ
Differential diagnosis
Based on the comparative morphology of the haptoral sclerites, *Q. mandibulatus* n. sp. resembles *Quadriacanthus thyzi* described on the gills of *Heterobranchus longifilis* (Agnéby River, Ivory Coast) by N’Douba et al. [36]. The new species differs from the latter species by possessing a lightly sclerotized (poorly differentiated or needle-like) distal part of the supporting membrane of the dorsal bar (the distal part of the supporting membrane is fimbriated in *Q. thyzi*) and from all other congeneric species by having a comparatively broad copulatory tube with subterminal flange.

Molecular characterization
The sequence of the 18S-ITS1 region of *Q. mandibulatus* n. sp. was 879 bp long, of which 500 bp corresponded to the partial 18S rDNA region and 379 bp corresponded to the ITS1 region. The sequence of the partial 28S region was 777 bp long. No intraspecific variability was found in the 18S-ITS1 and 28S sequences.

**Quadriacanthus pravus** Francová & Řehulková n. sp.

**Type-host:** *Clarias gariepinus* (Burchell) (Clariidae).
**Type-locality:** Nile River Basin, Sudan (locality 5).
**Other locality:** Nile River Basin, Sudan (locality 4).

**Type-material:** Holotype: MNHN HEL638. Paratype: IPCAS M-636 (1 specimen).
**Voucher material:** MNHN HEL639 (1 specimen; locality 4). Hologenophore: MNHN HEL645 (locality 5).
**Comparative material examined:** Voucher specimen of *Quadriacanthus numidus* Kritsky & Kulo, 1988 (MNHN 146 HF).

**Site in host:** Gill lamellae.

**Representative DNA sequences:** 18S-ITS1 rDNA (GenBank acc. no. KX713997) and 28S rDNA (GenBank acc. no. KX685955) (see also Table 2).

**ZooBank registration:** To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [37], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:ADCB9E56-E8F1-48B6-AD21-BBA5E52D0B39. The LSID for the new name *Quadriacanthus pravus* n. sp. is urn:lsid:zoobank.org:act:11D623F6-CA7D-434F-AB7C-28D44D17C0A5.

**Etymology:** The specific name is derived from Latin (*pravus* = crooked, distorted, deformed) and refers to the shape of the ventral anchor point.

**Description**
[Based on 4 flattened and 2 unflattened specimens in GAP; Fig. 8] Body length 580–589 (585; n = 2); greatest width
98–144 (121; n = 2). Haptor 78–108 (93; n = 2) long, 111–162 (136; n = 2) wide. Ventral anchor with small base, long evenly curved shaft; doubly recurved (waved) point; accessory sclerite small, wings unequal; a = 39–40 (40; n = 4); b = 8–9 (8; n = 4); c = 8–10 (9; n = 3); x/y = 1.4–1.6 (1.5; n = 4). Dorsal anchor with relatively broad base, bent shaft; tiny point; accessory sclerite triangular; a = 41–44 (43; n = 4); b = 2–3 (2; n = 4); c = 15–17 (16; n = 4); x/y = 1.0–1.0 (1.0; n = 4). Ventral bar composed of two rapidly tapering components; d = 38–44 (41; n = 4). Dorsal bar with small anterior shield; mid-posterior process triangular, with uneven margin: e = 47–53 (50; n = 4); f = 25–26 (26; n = 4); g = 11–14 (12; n = 4); h = 11–15 (13; n = 4). Hooks: 7 pairs, dissimilar in size; i = 12–29 (17; n = 2); hook I 19–21 (20; n = 3), hooks II–V 12–14 (13; n = 2); hook VI 28–29 (28; n = 4), hook VII 16–17 (17; n = 4). Vagina not observed. MCO comprising copulatory tube and accessory piece; k = 29–32 (30; n = 4). Copulatory tube a short lightly curved tube; length 27–30 (28; n = 4). Accessory piece articulated to base of the copulatory tube; proximal part rod-shaped (usually lightly sclerotized); medial part complex; distal part with terminal hook and subterminal pestle.

Differential diagnosis
Quadriacanthus pravus n. sp. resembles the following species by its ventral anchor having a doubly recurved point: Q. ashuri Kritsky & Kulo, 1988; Q. numidus Kritsky & Kulo, 1988; Q. papernai Kritsky & Kulo, 1988; and Q. gourenei N’Douba, Lambert & Euzet, 1999 [5, 36]. It differs from Q. gourenei and Q. papernai by the ventral bar possessing longer (rapidly tapering) components, and is easily differentiated from Q. ashuri by having a ventral anchor with a longer shaft. The new species most closely resembles Q. numidus in the morphometry of the haptoral sclerites; in particular, the ventral anchor of both species is characteristic by having a relatively small base and markedly long evenly curved shaft, and by lacking a sclerotized vagina. However, Q. pravus n. sp. differs from Q. numidus in the shape of the accessory sclerite of the dorsal anchor (triangular in Q. pravus vs wing-shaped in Q. numidus), and in having an MCO characterized by an accessory piece with a terminal hook and subterminal pestle (an accessory piece lamellate in Q. numidus). Douellou & Chishawa [28] reported that the accessory piece of the MCO of their specimens, identified as Q. numidus, was slightly different from that described by Kritsky & Kulo [5]. According to Douellou & Chishawa’s [28] characterization and depiction, it seems that their specimens are conspecific with our specimens (Q. pravus n. sp.) rather than with the Q. numidus specimens of Kritsky & Kulo [5]. However, because of the poor condition of the slide (MNHN 146 HF), the MCO could not be observed in any of the two voucher specimens. Thus, we hesitate to formally synonymize Q. numidus of Douellou & Chishawa [28] with Q. pravus n. sp. at this time.

Molecular characterization
The sequence of the 18S-ITS1 region of Q. pravus n. sp. was 919 bp long, of which 514 bp corresponded to the partial 18S rDNA region and 405 bp corresponded to the entire ITS1 region. The sequence of the partial 28S region was 799 bp long. No intraspecific variability was found in the 18S-ITS1 and 28S sequences.

Quadriacanthus zuheiri Francová & Řehulková n. sp.

Type-host: Clarias gariepinus (Burchell) (Clariidae).
Type-locality: Nile River Basin, Sudan (locality 5).
Other locality: Nile River Basin, Sudan (locality 4).
Type-material: Holotype: MNHN HEL640.
Voucher material: MNHN HEL639 (1 specimen; locality 4); IPCAS M-637 (2 specimens; locality 4). Hologenophore: MNHN HEL646 (locality 5).
Comparative material examined: Type-specimens of Quadriacanthus agnebiensis N’Douba, Lambert & Euzet, 1999 (holotype and two paratypes MNHN 572 HF Tk 89).
Site in host: Gill lamellae.
Representative DNA sequences: 18S-ITS1 rDNA (GenBank acc. no. KX713998) and 28S rDNA (GenBank acc. no. KX685956) (see also Table 2).
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [37], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:ADCB9E56-E8F1-48B6-AD21-BBA5E52D0B39. The LSID for the new name Quadriacanthus zuheiri n. sp. is urn:lsid:zoobank.org:act:A725FAF7-9AAF-4156-9647-6AE3EB7A0BCC.
Etymology: This species is named in honour to Prof. Zuheir N. Mahmoud of the Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan, for his valuable and kind assistance during our field campaigns in Sudan.

Description
[Based on 6 flattened and 2 unflattened specimens in GAP; Fig. 9] Body length 600–690 (645; n = 2); greatest width 124–140 (132; n = 2). Haptor 95–113 (104; n = 2) long, 124–125 (124; n = 2) wide. Ventral anchor with moderate base, slightly curved shaft, long point; accessory sclerite small, with subequal wings; a = 37–38
(37; n = 6); b = 10–11 (11; n = 6); c = 8–11 (9; n = 6); x/y = 1.1–1.6 (1.4; n = 6). Dorsal anchor with large base, shaft bent proximally, tiny point; accessory sclerite large, triangular; a = 43–47 (45; n = 6); b = 2–3 (3; n = 6); c = 18–22 (20; n = 6); x/y = 1.0–1.3 (1.1; n = 6). Ventral bar composed of two elongated components; d = 39–44 (41; n = 5). Dorsal bar with small anterior shield; mid-posterior process tongue-like, with uneven margin; e = 42–51 (46; n = 5); f = 24–32 (29; n = 6); g = 13–18 (15; n = 6); h = 11–18 (14; n = 5). Hooks: 7 pairs, dissimilar in size; i = 12–31 (17; n = 5); hook I 17–18 (18; n = 6); hooks II–V 12–14 (13; n = 5), hook VI 27–31 (29; n = 6), hook VII 19–19 (19; n = 6). Vagina not observed. MCO comprising copulatory tube and accessory piece; k = 30–34 (32; n = 6). Copulatory tube straight to slightly curved; base with thickened margins; l = 28–30 (30; n = 6). Accessory piece basally articulated to the copulatory tube; medial part formed as a clamp jaw; hook-shaped termination serving as a guide for distal portion of the copulatory tube.

**Differential diagnosis**

Based on the comparative morphology of the haptoral sclerites, *Quadriacanthus zuheiri* n. sp. most closely resembles *Q. aegypticus* El-Naggar & Serag, 1986, and may also be confused with *Q. agnebiensis* N’Douba, Lambert & Euzet, 1999, a parasite of *Heterobranchus isopterus* from Ivory Coast [27, 36]. *Quadriacanthus zuheiri* n. sp. differs from *Q. aegypticus* by having a noticeably smaller MCO composed of a copulatory tube without basal flange (with flange in *Q. aegypticus*) and simpler accessory piece (i.e. without two medial diverticula and distal hooks). Examination of the holotype and two paratypes of *Q. agnebiensis* showed that *Q. zuheiri* n. sp. differs from the latter species by possessing: (i) a longer ventral anchor with less arched shaft; (ii) a larger accessory sclerite on the part of the dorsal anchor; (iii) shorter and less robust hooks VI and VII; and (iv) an accessory piece with more complex medial part (formed as a lightly sclerotized clamp jaw) and hooked (double hooked in *Q. agnebiensis*) distal termination.

**Molecular characterization**

The sequence of the 18S-ITS1 region of *Q. zuheiri* n. sp. was 877 bp long, of which 469 bp corresponded to the 18S rDNA region and 408 bp corresponded to the ITS1 region. The sequence of the partial 28S region was 772 bp long. No intraspecific variability was found in the 18S-ITS1 and 28S sequences.

**Interspecific genetic relationships within genus Quadriacanthus**

No intraspecific variability was detected for the 18S-ITS1 and 28S regions. The overall K2P mean genetic distance was 10.34% for the 18S-ITS1 sequences and
3.32% for the 28S rDNA sequences. The pairwise genetic distances are presented in Table 3. Among the Quadriacanthus species, Q. clariadis exhibited the lowest genetic divergence from Q. bagrae (1.89% for 18S-ITS1, 0.92% for 28S). Q. mandibulatus n. sp. and Q. fornicatus n. sp. exhibited the greatest genetic distances (5.87%) for 28S rDNA sequences, and Q. mandibulatus n. sp. and Q. bagrae represented the most divergent species pair for 18S-ITS1 sequences (13.95%; Table 3).

An unambiguous alignment of 18S-ITS1 sequences spanned 799 positions, of which 275 positions were variable. The 28S alignment contained a total of 725 bp with 246 variable characters. The phylogenetic trees of 18S-ITS1 sequences (13.95%; Table 3).

**Discussion**

The geographical distributions and host preferences of species of Quadriacanthus suggest an interesting evolutionary history of the group. Species of Quadriacanthus have been confirmed as parasites of fishes representing three families, namely the Claridae, Bagridae (Siluroidae), and Notopteridae (Osteoglossiformes) [2, 3]. Clarid catfishes most likely originated in Asia ~40–50 MY ago but contemporary African and Asian species originated from a common ancestor that was present on the Arabian plate about 15 MY ago [38]. From that moment, the ancestral species came back to Asia and colonized Africa probably through brackish water bridges like lagoons [39]. Species of Quadriacanthus infesting clarids occur in the freshwaters of Africa, India, Malaysia, Thailand, China and Vietnam [2, 7]. Inasmuch as members of dactylogyrid genera are generally considered highly host-specific (usually confined to members of a single host family), the wide geographical distribution of Quadriacanthus spp. on clarid hosts suggests comparatively old host-parasite relationships, i.e. lasting at least 15 MY. On the other hand, formulating a hypothesis on the origin of Quadriacanthus species on bagrids (B. bajad, B. docmak and B. orientalis) in Africa is more problematical. Species of Quadriacanthus have not been found on bagrids in Asia, although these fishes have occasionally been examined for gill parasites [2]. The family Bagridae was poorly defined until its revision by Mo [40] and de Pinna [41], who established the families Austroglanididae, Claridae and Auchenoglanididae for all African genera (except Bagrus) previously considered members of the Bagridae [42]. The well-known Farenholz’ rule states that the natural classification of some parasite groups usually corresponds directly with the natural relationships of their hosts [43]. Indeed, species of claroteids and auchenoglanids are known to harbour species of Protoacynychodiscoides Paperna, 1969 and Bagrodella Paperna, 1969, respectively, while those of Bagrus are known to be infected with one species of Quadriacanthus, i.e. Q. bagrae [2].

Some authors (e.g. Brooks & McLennan [44]) believe that monogeneans possess characteristics that perfectly adapt them for surviving numerous host-switching events. Assuming that members of the Claridae are the ancestral hosts of species of Quadriacanthus, the occurrence of Q. bagrae (while clearly a member of the genus) on African bagrid hosts probably resulted from host switching. Our phylogenetic reconstruction indicates that Q. bagrae is phylogenetically nested within the parasites from Clarias gariepinus at a derived position of the tree (Fig. 10). More specifically, Q. bagrae from Bagrus docmak is a sister species to Q. clariadis from C. gariepinus.

| Table 3 | Pairwise 18S-ITS1 (below diagonal) and 28S (above diagonal) nucleotide divergences for each observed Quadriacanthus spp. using K2P distance (%) |
|---------|----------------------------------------------------------------------------------|
|         | 1     | 2     | 3     | 4     | 5     | 6     |
| 1       | Quadriacanthus bagrae               | 0.92  | 2.93  | 4.88  | 2.12  | 2.94  |
| 2       | Quadriacanthus clariadis            | 1.89  | -     | 2.80  | 4.74  | 2.26  | 2.80  |
| 3       | Quadriacanthus fornicatus n. sp.   | 6.40  | 5.85  | -     | 5.87  | 2.94  | 3.76  |
| 4       | Quadriacanthus mandibulatus n. sp. | 13.95 | 13.32 | 12.57 | -     | 4.45  | 4.73  |
| 5       | Quadriacanthus pravus n. sp.       | 13.48 | 13.01 | 12.58 | 10.05 | -     | 1.58  |
| 6       | Quadriacanthus zuheiri n. sp.      | 13.34 | 12.12 | 11.67 | 10.51 | 4.50  | -     |
The clade is located at a derived position of the tree, suggesting that *Q. bagrae* (or its ancestor) transferred from clariids to species of *Bagrus* and not conversely. Several studies suggested that such lateral transfer (host switch) can occur both between related host species (e.g. [45]) and even between phylogenetically distant host species [46–48]. Recently, Nack et al. [3] hypothesized that the presence of *Quadriacanthus euzeti* Nack, Pariselle & Bilong Bilong, 2015 on *Papyrocranus afer* (Notopteridae, Osteoglossiformes) is probably the result of a lateral transfer from species belonging to *Clarias* or *Bagrus* which live sympatrically with *P. afer* in Lake Ossa (South Cameroon). Although more data are needed to resolve phylogenetic relationships within *Quadriacanthus*, the occurrence of *Q. bagrae* on *Bagrus docmak* may represent a similar lateral transfer from a species of *Clarias*, probably *C. gariepinus*. *Bagrus docmak* inhabits, among other locations, the Nile River, where it lives in sympathy with *Clarias gariepinus* [9].

Although we cannot verify the accuracy of the identification, *Q. bagrae* was also recorded on *C. gariepinus* by some authors [5, 7]. Because the drawings of the MCO provided by these authors are insufficient for detailed
Fig. 11 Phase-contrast photomicrographs of the sclerotized haptoral structures and male copulatory organ of *Quadriacanthus aegypticus* (a, b), *Q. bagrae* (c, d), *Q. claradis* (e, f). Scale-bars: a, c, e, 30 μm; b, d, f, 20 μm
Fig. 12 Phase-contrast photomicrographs of the sclerotized haptoral structures and male copulatory organ of *Quadriacanthus fomicatus* n. sp. (*a, b*), *Q. pravus* n. sp. (*c, d*), *Q. zuheiri* n. sp. (*e, f*). Scale-bars: *a, c, e, 30 μm, b, d, f, 20 μm
Fig. 13 Male copulatory organ of seven representative species of Quadriacanthus (characters of interest indicated by arrows). a Q. mandibulatus n. sp. b Q. aegypticus. c Q. bagrae. d Q. clariadis. e Q. fornicatus n. sp. f Q. pravus n. sp. g Q. zuheiri n. sp.

Fig. 14 Phase-contrast photomicrographs of the sclerotized structures of Quadriacanthus mandibulatus n. sp. a Haptoral structures. b Male copulatory organ. Scale-bars: a, 30 μm; b, 20 μm
comparison with our specimens, confirmation of the records of *Q. bagrae* on *C. gariepinus* will depend on the collection and evaluation (morphological and molecular) of new parasite material from *C. gariepinus*. It will be interesting to see whether *Q. bagrae* on *C. gariepinus* is a genuine *Q. bagrae* (sensu stricto). If they represent two different species of *Quadriacanthus*, then the occurrence of *Q. bagrae* on *Bagrus docmak* may suggest, at this time, a case of host switching with speciation.

Until now, there were no studies on the genetic characteristics of *Quadriacanthus* spp.; thus, the molecular data presented here represent an important advance in the molecular identification and differentiation of this genus. In our study, molecular characterization is presented for six *Quadriacanthus* species (i.e. for all the species recorded in our study, except *Q. aegypticus*). The interspecific genetic relationships among *Quadriacanthus* spp. observed in this study are congruent with the similarity of the basic morphology of the sclerotized structures, especially of those of the MCO (Figs. 11, 12, 13). The separation of *Q. mandibulatus* n. sp. from the other species corresponds with the different morphology of its copulatory tube. The copulatory tube is terminally enlarged and with a subterminal flange in *Q. mandibulatus* n. sp., while the corresponding structure in all other congener species is comparatively small and with an oblique tapering termination (Figs. 13, 14).

**Conclusions**

This study suggests that species of *Quadriacanthus* parasitizing catfishes in the Old World provide useful models for the study of biogeography and coevolution. However, future studies are needed that would have to involve the examination of dactylogyrids from a greater number of host individuals and host species from a larger geographical area, the utilization of other monogenean taxa, and the incorporation of a homologous series of host features into the matrix derived from the parasite cladogram.

**Acknowledgements**

We thank David Lotuliakou, John O. Malala (KWFRI, Lake Turkana Station, Kenya) and Miloslav Jirků (Institute of Parasitology, AS CR, Czech Republic) for logistical support throughout the field sampling. We also are indebted to Matej Polačík (Institute of Vertebrate Biology, AS CR, Czech Republic) for help with fish sampling in Sudan, and to Sárka Maľová, Iva Plíškylvová and Maria Luža Klínčáková (Department of Botany and Zoology, Masaryk University, Czech Republic) for help with monogenean collection. Big thanks go to Ondřej Hájek (Department of Botany and Zoology, Masaryk University, Czech Republic) for making the map with the sampling sites. Our thanks also go to Jean-Lou Justine (Museum National d’Histoire Naturelle, France) and Maarten P. Vanhove (Musée Royal de l’Afrique Centrale, Belgium) for the kind loan of type-material. We are very grateful to Delane C. Kritsky (Idaho State University, USA) who carefully reviewed the submitted manuscript and gave us valuable advice.

**Abbreviations**

GAP: Glycerine and ammonium picrate; ITS1: Internal transcribed spacer 1; K2: Kimura 2-parameter; MCO: Male copulatory organ; MNHN: Muséum National d’Histoire Naturelle; MRAC: Musée Royal de l’Afrique Centrale; PCR: Polymerase chain reaction

**Funding**

This study was financially supported by ECIP (European Centre of Ichthyoparasitology) – Centre of Excellence, Grant Agency of the Czech Republic, No. P505/12/G112.

**Availability of data and materials**

The data supporting the conclusions of this article are included within the article. The parasitological material is deposited in the Helminth collection of the Muséum National d’Histoire Naturelle, Paris, France (MNHN), and the Helminthological collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCAS). The molecular datasets generated during the present study are available in the GenBank repository under accession numbers KX713993–KX713998 and KX685951–KX685956.

**Authors’ contributions**

ER designed this study. KF, ER performed the morphological characterization and described the species. MS performed molecular analyses. EF, KF and MS wrote the paper. RB identified fish species, contributed to fish sampling in Lake Turkana, White and Blue Nile. MG provided scientific background in the field of monogenean research. ZNM and MG revised the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval**

The research was approved by the Ethics Committee of Masaryk University. The approval number which allows us to work with vertebrate animals is CZ01302.

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**Acknowledgements**

We thank David Lotuliakou, John O. Malala (KWFRI, Lake Turkana Station, Kenya) and Miloslav Jirků (Institute of Parasitology, AS CR, Czech Republic) for logistical support throughout the field sampling. We also are indebted to Matej Polačík (Institute of Vertebrate Biology, AS CR, Czech Republic) for help with fish sampling in Sudan, and to Sárka Maľová, Iva Plíškylvová and Maria Luža Klínčáková (Department of Botany and Zoology, Masaryk University, Czech Republic) for help with monogenean collection. Big thanks go to Ondřej Hájek (Department of Botany and Zoology, Masaryk University, Czech Republic) for making the map with the sampling sites. Our thanks also go to Jean-Lou Justine (Museum National d’Histoire Naturelle, France) and Maarten P. Vanhove (Musée Royal de l’Afrique Centrale, Belgium) for the kind loan of type-material. We are very grateful to Delane C. Kritsky (Idaho State University, USA) who carefully reviewed the submitted manuscript and gave us valuable advice.
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