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CALICOTYLE CALIFORNIIENS N. SP. AND CALICOTYLE UROBAT I N. SP. (MONOGENEA: CALICOTYLINEI) FROM ELASMOBRANCHS IN THE GULF OF CALIFORNIA

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ABSTRACT: Two new species of Calicotre (Monocotylidae: Calicotreline) are described from elasmobranchs in the western Gulf of California. Calicotre californiensis n. sp. is described from a single specimen collected from a gray smoothhound shark (Mustelus californicus, Carcharhiniformes: Triakidae). It is distinguished from its congeners by the combination of having vaginal pores opening outside the intercel space, and a relatively long male copulatory organ recurving 3 times and passing between the distal penis bulb and the seminal vesicle. Calicotre urobati n. sp. is described from 16 specimens collected from at least 2 cloaca and rectum of the round rays Urobatis halleri and Urobatis maculatus (Rajiformes: Urolophidae). It is distinguished from its congeners by the combination of having vaginal pores opening outside the intercel space and proximal regions of the vaginae terminating at the level of the ceca. Members of Calicotre have not been reported previously from the eastern Pacific Ocean or from these hosts. In the past, species of Calicotre have been distinguished based primarily on the shape and size of the male copulatory organ and hamuli. Divisions of the vaginae and the positions of the vaginal pores are also useful in distinguishing members of the genus.

In the most recent review of Calicotreline Monticelli, 1903 (Monocotylidae: Taschenberg, 1879), Chisholm et al. (1997) identified 14 species of Calicotre Diesing, 1850. Members of this genus infect the cloaca, rectum, rectal gland, spiral intestine, and oviducts of chimaeras, rays, and sharks (Chisholm et al., 1997). In the present paper, we describe 1 new species from the body cavity of a gray smoothhound shark (Mustelus californicus) and another from the cloaca and rectum of the round rays Urobatis halleri and Urobatis maculatus. All were collected in the western Gulf of California.

MATERIALS AND METHODS

The shark host (M. californicus) was captured with a gill net and identified according to Compagno (1984). The round rays (U. halleri and U. maculatus) were captured with spear gun, trident, or gill net and identified using the key of MacEachran and Notarbortolo di Scia (1996). Parasites were removed carefully with forceps, heat-killed without pressure using hot water, and fixed in 10% neutral buffered formalin. All specimens but 1 were stained overnight in a solution of Van Cleave’s hematoxylin with several additional drops of Ehrlich’s hematoxylin and dehydrated to 70% ethanol. Several drops of aqueous saturated lithium carbonate were added first, followed by several drops of 6% butylamine solution to keep the specimens basic. Specimens were then fully dehydrated in an ethanol series, cleared in clove oil, and mounted in neutral Canada balsam. The remaining worm was transferred to and cleaned with 70% ethanol, brushed to remove debris, postfixed in osmium tetroxide, rinsed in 0.1 M sodium cacodylate buffer, dehydrated through an ethanol series, placed in a critical point drier, sputter-coated with gold, and viewed under a JEOL JSM-T330 scanning electron microscope to search for haptor hooklets. Drawings were made with the aid of a drawing tube. Whole-mounted specimens were measured with an ocular micrometer. Measurements are reported in micrometers. Where applicable, measurements are given as ranges, followed by the number of specimens measured in parentheses. As close as possible, measurements follow the curves of structures. Specimens of related species were loaned from the United States National Parasite Collection (USNPC), USDA, Beltsville, Maryland, and the Harold W. Manter Laboratory (HWML) of The University of Nebraska State Museum, Lincoln, Nebraska. Holotypes and a paratype were deposited in the Institutio de Biologia, Universidad Nacional Autonoma de Mexico, Mexico City (IBUNAM), and other paratypes were deposited in the USNPC and HWML.

DESCRIPTIONS

Calicotre californiensis n. sp. (Figs. 1–6)

Based on 1 preserved, stained, whole-mounted adult specimen. Body 5,483 long (including haptor and accounting for slight folding of anterior and posterior ends of specimen), 2,470 wide at widest portion just anterior to midbody (Fig. 1). Haptor oval, 810 long, 1,031 wide; septa muscular, dividing ventral surface of haptor into 1 central and 7 peripheral loculi. Hamuli single pair (not drawn in profile) 228 and 218 long (Fig. 2), in septa along sides of posterior median loculus (Fig. 1). Marginal hooklets not evident. Mouth not visible. Pharynx bulbous, 346 in diameter. Esophagus indistinct. Esophageal gland cells numerous, weakly stained in holotype, posterolateral to pharynx, with ducts leading to posterior portion of pharynx. Ceca 2, unbranched, with 1 on each side of body, extending posteriorly approximately parallel with lateral body margins, terminating blindly in posterior region of body; cecal bifurcation immediately posterior to pharynx. Eyespots or dispersed pigment granules not visible.

Testicular mass follicular, 1,449 in maximum width, occupying intercel space from anterior third of body to region just anterior to blind ends of ceca (Fig. 1). Vas deferens emerging from anterosinistral portion of testicular mass, extending parallel to and near sinistral cecum, thickening anteriorly, 55 in maximum width anteriorly (Figs. 1, 4, 6). Seminal vesicle 104 in maximum width, extending posteriorly, narrowing to 32 in width before connecting to proximal penis bulb; penis bulb with distinct proximal and distal portions; proximal penis bulb 99 in maximum width, with 2 subspherical chambers, each 42 in maximum width (Figs. 4, 5); distal penis bulb extending anteriorly, ventral to seminal vesicle; penis bulb tube hollow, sclerotized. 134 long, originating from junction of seminal vesicle and proximal penis bulb, passing within proximal and distal penis bulbs (Figs. 4, 5). Male copulatory organ sclerotized, a hollow tube, 640 long, continuous with penis bulb tube, coiled slightly more than 1 turn, extending anteriorly for short distance before recurving and coiling ventral to distal penis bulb, passing anteriorly between seminal vesicle and distal penis bulb before recurving posteriorly and ventrally at level of proximal penis bulb, terminating at level of ootype (Figs. 4, 5, 6).

Ovary convoluted, possessing numerous germ cells; blind end lobed in sinistral half of body, looping over right cecum dorsally (Fig. 1). Vitellarium dense, in extracecal bands, not confluent anteriorly or posteriorly, consisting of vitelline ducts and vitelline cells; vitelline ducts dendritic, approximately 37 in diameter, located along each side.
Figures 1–6. Holotype of *Calicotyle californiensis* n. sp. from *Mustelus californicus*. 1. Ventral view of body (correcting for folding of anterior and posterior ends of specimen). Bar = 2,000 μm. 2. Hamulus (not in profile). Bar = 100 μm. 3. Diagrammatic representation of female reproductive system to show relative position of vaginal pore (VP), common genital pore (GP), ootype (OT), distal region of vaginae (DV), ovary (OV), proximal region of vagina (PV), and seminal receptacle (SR). 4. Ventral view of seminal vesicle, proximal and distal portions of penis bulb, spherical chambers, penis bulb tube (dashed lines), and male copulatory organ. Bar = 100 μm. 5. Diagrammatic representation of male reproductive system to show relative position of seminal vesicle (SV), proximal penis bulb (PPB), spherical chambers (SC), distal penis bulb (DPB), penis bulb tube (PBT), and beginning of male copulatory organ (P). 6. Partial ventral view of male and female reproductive systems. Bar = 250 μm.

of body between body margin and ceca from level of pharynx to posterior end of ceca (Fig. 1); transverse vitelline ducts in anterior fourth of body; left transverse vitelline duct ventral to vas deferens (Fig. 6); vitelline cells irregularly shaped, approximately 3–4 (n = 5) long, interspersed within vitelline ducts. Vaginae 2, with each consisting of distinct proximal and distal regions; proximal regions meeting to form a common kidney bean–like pouch ventral to transverse vitelline ducts medially, 224 wide and 109 long; distal regions approximately 400 (n = 2) long, 10 (n = 2) wide, following transverse vitelline ducts until midway to cecum, then turning anteriorly; pore of each vagina within intercecal space, opening ventrally at level of or immediately anterior to common genital pore, surrounded by glandular cells; glandular cells approximately 1-cell thick, surrounding each distal region from junction of proximal and distal region to approximately 2/3 total length of distal
region, bound by a membrane; membrane thin, weakly stained in holotype (membrane not illustrated in Fig. 6); common genital pore immediately posterior to proximal penis bulb (Figs. 1, 6). Seminal receptacle 117 wide, 141 long, dorsal to transverse vitelline ducts (Figs. 3, 6). Mehlis' gland dispersed, located between ovary and transverse vitelline ducts at level of seminal receptacle, connecting to base of ootype by collecting ducts (not drawn) (Fig. 1). Ootype 136 wide, 174 long, with triangular lumen, leading to common genital pore anteriorly (Figs. 1, 3, 6); egg apparently tetrahedral, collapsed, 154 long (2 presumed germ cells and 1 egg in specimen).

**Taxonomic summary**

*Type host:* *Mustelus californicus* Gill, 1864, gray smoothhound shark (Carcharhiniformes: Triakidae).

*Site of infection:* Body cavity (may be erroneous, see Discussion).

*Type locality:* Western Gulf of California (Bahia de los Angeles), Mexico.

*Type specimen:* Holotype, IBUNAM CNHE 3907.

*Intensity and prevalence of infection:* One worm in 1 of 20 specimens of *M. californicus*.

**Etymology:** The specific name *californiensis* refers to the Gulf of California, the type locality.

**Remarks**

The following specimens of related species were examined for comparison: 1 voucher specimen of *Calicothoe asterii* (Szidat, 1970) from the cloaca of *Mustelus norrisi* collected by T. Hanskecht in the northern Gulf of Mexico (USNPC 87188); holotype of *Calicothoe ramsayi* Robinson, 1961 from the cloaca of Squalus acanthias (Squaliformes: Squaleidae) (as *Acanthias lebruni*) collected in Cook Strait, New Zealand (USNPC 39429); 3 voucher specimens of *Calicothoe stossichi* Braun, 1899 from the rectal gland of *M. norrisi* plus 1 from the rectal gland of Mustelus musculus collected in the eastern Atlantic Ocean by T. Hanskecht (USNPC 87193 and 87194). The species of *Calicothoe* reported from sharks in the genus *Mustelus* Linck, 1798 (*C. asterii*, *Calicothoe palombi* Euzet and Williams, 1960, and *C. stossichi*) are morphologically more similar to *Californiella* n. sp. and to each other than they are to any other species in the genus in that they all possess an elongate body form, a small haptor and small hamuli relative to body size, U-shaped vaginae, proximal regions of the vaginae that join to form a compact medially located structure, and vaginal pores, which one stands out within the intercecal space. The voucher specimens of *C. stossichi* concurred with the description by Euzet and Williams (1960) in that the vaginal pores occurred within the intercecal space. *Calicothoe californiensis* is easily distinguished from *C. asterii*, *C. palombi*, and *C. stossichi* by the structure formed by the junction of the proximal regions of the vaginae medially being kidney bean-shaped and by its possession of a vitelliferous duct (640 μm in length before recurving and extending posteriorly approximate parallel to lateral body margin) and a relatively long male copulatory organ (110–210 long, sclerotized, tubelike, recurving once, initially running anteriorly and extending approximately 40% of its total length before recurving and extending posteriorly along ventral surface of distal penis bulb, ending roughly at level of proximal penis bulb (Figs. 8, 9). Penis bulb tube not evident.

Ovary convoluted, looping around right cecum dorsoventrally at midbody, with blind end smooth in sininal half of body (Fig. 7). Vitellarium located between ceca and body margin from immediately anterior to level of pharynx to posterior end of body, not confluent anteriorly or posteriorly, consisting of vitelline cells and vitelline ducts; vitelline cells spherical, approximately 2–4 (n = 12) in diameter, densely packed within vitelline ducts; vitelline ducts dendritic, 114–204 (n = 14) long, 85–148 (n = 10) wide. Egg tetrahedral, with longest edge 109–129 (n = 5), 75–104 (n = 5) wide, with filament forming 2 pairs of small distinct eyespots when concentrated (Fig. 7). Haptor circular, 908–1,277 (n = 13) in diameter; septa muscular, dividing ventral surface of haptor into 1 central and 7 peripheral loculi (Fig. 7). Hamuli 1 pair, 208–308 (n = 25) long, varying slightly in shape among specimens, with each hamulus occurring within both sides of posterior median loculus; hamulus guard 90–164 (n = 16) long (Figs. 7, 10). Marginal hooklets not evident. Mouth ventral, subterminal (Fig. 7). Pharynx bulbous, 109–209 (n = 12) long and 149–214 (n = 13) wide. Esophagus 25–75 (n = 5) long. Esophageal gland cells encircling esophagus (Fig. 7). Ceca 2, unbranched and without diverticula, with 1 on each side of body, extending posteriorly approximately parallel with lateral body margin until turning medially to the third of body and then abruptly turning posteriorly and ending blindly about 10–27% of length of body proper from junction of haptor (Fig. 7).

Testicular mass follicular, 445–1,129 (n = 9) wide, occupying intercecal space immediately posterior to midbody, not extending laterally beyond ceca (Fig. 7). Vas deferens extending anteriorly and medially to sinistral cecum (Figs. 7, 8). Seminal vesicle 27–55 (n = 11) in maximum width, running posteriorly before connecting to penis bulb (Fig. 8). Penis bulb with proximal and distal portions; proximal portion 45–65 (n = 12) wide, 32–50 (n = 8) long, with 2 subspHERALLY shaped chambers (Figs. 8, 9); distal portion 22–35 (n = 8) wide, 22–32 (n = 8) long, connecting to male copulatory organ; male copulatory organ 110–210 (n = 16) long, sclerotized, tubelike, recurving once, initially running anteriorly and extending approximately 40% of its total length before recurving and extending posteriorly along ventral surface of distal penis bulb, ending roughly at level of proximal penis bulb (Figs. 8, 9). Penis bulb tube not evident.
to the cecum (Fig. 12). Calicotyle kroyeri and C. australis have vaginae with a proximal region that terminates ventral to the ceca. Calicotyle niensis, the species of Calicotyle reported from sharks in Mustelus, is the most similar among members of the genus, as are their respective hosts. Calicotyle urobati occurs in the round rays U. halleri and U. maculatus from the Gulf of California, and C. urolophi is reported from the stingarees U. bucculentus, U. cruciatus, and U. paucimaculatus from off the coast of southeast Tasmania, Australia (Chisholm et al., 1991). The hosts for C. urobati and C. urolophi are morphologically similar and were once grouped into the single genus Urolophus. Rays in both Urobatis and Urolophus are shallow-water coastal species that probably are not capable of trans-Pacific migration. Despite the geographic distance separating these parasite populations, C. urobati and C. urolophi have diverged only slightly, as have their respective hosts.

**DISCUSSION**

Based on the suite of morphological features for C. californiensis, the species of Calicotyle reported from sharks in Mustelus form a natural group distinct from other members of the genus reported from nontriakid hosts. Szidat (1970) erected Paracalicotyle Szidat, 1970 based on body shape and haptor size. He established C. stossichi as the type species and included C. asterii and C. palombi. Our observations on specimens of C. asterii, C. californiensis, and C. stossichi (see Remarks section for C. californiensis) and the published description of C. palombi suggest to us that Paracalicotyle should be resurrected. However, we herein continue to place the species in Calicotyle until more is known about the female reproductive anatomy of other species of Calicotyle and until more species of Calicotyle are collected from shark hosts.

Members of Calicotyle may provide insight into host phylogeny. Calicotyle ramsayi from Squala acanthias lacks many of the morphological characters shared among members of Calicotyle reported from species of Mustelus. The holotype and only reported specimen of C. ramsayi collected from S. acanthias is in poor condition; however, several of its general features (e.g., pyriform body, extracecal vaginal pores, large haptor relative to body size) are more similar to those of species of Calicotyle reported from rays than to those from species of Mustelus. The phylogenetic hypothesis of Shirai (1996) separated neoselachians into 2 superorders: Galea and Squalea. Galea was comprised of sharks included in Heterodontiformes, Orectolobiformes, Lamniformes, and Carcharhiniformes (which includes Mustelus). Squalea was comprised of the remaining sharks, i.e., Chlamydoselachiformes, Hexanchiformes, Echinorhiniiformes, Dalatiiformes, Centrophoriformes, and Squaliformes (which includes members of Squallus), and all rays (Squatiniiformes, Pristiphoriiformes, and Rajiformes). The low degree of host specificity reported for some members of Calicotylinae (Chisholm et al., 1997) discourages the formulation of coevolutionary hypotheses among members of this group and their hosts. However, the morphological similarities we observed among C. ramsayi and its congeners from rays are concordant with the system of Shirai (1996), in which S. acanthias is grouped with rays and squalean sharks rather than with gal-eans.

Calicotyle urobati and C. urolophi are morphologically the most similar among members of the genus, as are their respective hosts. Calicotyle urobati occurs in the round rays U. halleri and U. maculatus from the Gulf of California, and C. urolophi is reported from the stingarees U. bucculentus, U. cruciatus, and U. paucimaculatus from off the coast of southeast Tasmania, Australia (Chisholm et al., 1991). The hosts for C. urobati and C. urolophi are morphologically similar and were once grouped into the single genus Urolophus. Rays in both Urobatis and Urolophus are shallow-water coastal species that probably are not capable of trans-Pacific migration. Despite the geographic distance separating these parasite populations, C. urobati and C. urolophi have diverged only slightly, as have their respective hosts.

The existence and shape of the penis bulb tube may help distinguish species of Calicotyle. This structure previously had not been described and was not evident in other specimens that were examined. However, observations on additional specimens of congeneric species are needed so that the comparative shape of the penis bulb tube may be understood in greater detail.

Our observations suggest that the age of the worm may influence the presence and shape of some features. Chisholm et al. (1997) reported that 14 marginal hooklets were present in all species of Calicotyle. We did not see a marginal hooklet using light microscopy on fixed adult specimens of C. californiensis or C. urobati and using scanning electron microscopy on a specimen of C. urobati; however, hooklets could have dissolved in the fixative or been present in juveniles worms. Living specimens or highly flattened juvenile and adult specimens mounted in Hoyee’s or De Paules medium should be examined for hooklets. Rohde et al. (1992) showed that the number of coils in the male copulatory organ of C. australiensis increased with body length and that the organ became longer until specimens reached maturity at 1–2 mm total body length. As stated previously, the holotype of C. californiensis represents a relatively large (nearly 5.5 mm in total body length, to ceca and transverse vitelline ducts in 3 species of Calicotyle. 12. Calicotyle urolophi (paratype, HWML 31747). Bar = 300 µm. 13. Calicotyle kroyeri (voucher specimen, HWML 38527). Bar = 300 µm. 14. Calicotyle australis (voucher specimen, USNPC 80510). Bar = 500 µm.
including haptor), sexually mature adult specimen. Because of this development, we are confident that the shape and length of the male copulatory organ and the kidney bean–shaped structure formed by the junction of the proximal portions of the vaginae medially in the adult reliably distinguish the species from its apparently closely related congeners reported from other species of Mustelus.

The body cavity and pericardial cavity are unusual sites for specimens of Calicotyle spp., if indeed they are accurate. The sites were based on labels occurring with the specimens. If these sites are correct, we cannot explain how the specimens gained access to those sites and why only 1 of 47 total specimens of C. urobatis would occur in the pericardial cavity. Several species of Calicotyle have been reported from the rectal gland and oviduct, and none has been reported from the body cavity or pericardial cavity (Chisholm et al., 1997). In contrast, specimens of Dictyocotyle coeliaca Nybelin, 1941 (Calicotyliinae) attach to the coelom and body cavity wall of several species of Raja (see Lawler, 1981). If our specimen of C. californiensis was attached similarly, the utilization of the same or of a similar microhabitat within M. californicus, e.g., the body cavity, could serve as a shared ecological characteristic that might support the hypothesized phylogenetic link between D. coeliaca and species of Calicotyle.

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