REPTILES & AMPHIBIANS

Establishment of Aquatic Rio Cauca Caecilians, *Typhlonectes natans* (Fischer 1880) (Gymnophiona: Typhlonectidae), in Florida, with Notes on their Relative Abundance, Distribution, and Natural History

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Abstract.—In 2021, the first occurrence of an aquatic caecilian identified as a Rio Cauca Caecilian, *Typhlonectes natans* (Gymnophiona: Typhlonectidae), in the Tamiami (C-4) Canal was documented in Florida, USA. However, whether the species was established at the time was not known. We subsequently trapped for caecilians at nine different sites along the main C-4 Canal and secondary canals between 2021 and 2024. We captured 115 caecilians and received numerous additional reports and observations from multiple individuals. Caecilians of both sexes and all age classes were captured. Twenty-seven preserved specimens were X-ray-imaged to check for pregnancy and stomach contents, and 11 of those specimens were microCT-scanned. Three specimens contained late-stage fetuses, and one contained stomach contents identified as fish bones. We screened 78 specimens for the presence of the amphibian fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), but did not detect *Bd* on any specimens. Results suggest that *T. natans* is well established and locally abundant in at least 16 km of the C-4 Canal system. Preliminary results suggest their diet consists primarily of soft-bodied prey and/or scavenged vertebrate carrion. The caecilians do not appear to be a reservoir for transmission of *Bd*, although further testing is required. Further studies are needed to better understand their distribution, biomass, reproductive cycles, diet, movement patterns, role in disease transmission, risk of spread, and basic biology. Efforts are needed to assess the viability of eradication or control and to understand how the establishment of these aquatic caecilians might affect freshwater ecosystems in southern Florida.

The state of Florida contains more established species of non-native reptiles and amphibians than any other region in the world (Krysko et al. 2011). The majority of these 64+ established species are reptiles. Among amphibians, only four species of established non-native anurans and no established non-native caudates have been documented (Krysko et al. 2019). However, the number of non-native species of reptiles and amphibians that have been introduced in Florida and subsequently became established has been steadily increasing since the late 1800s, largely attributed to Florida's warm, subtropical climate, natural and urban habitats providing favorable conditions for non-native species, and numerous animal importers and dealers operating in the state (Krysko et al. 2019).

A single Rio Cauca Caecilian, *Typhlonectes natans* (Gymnophiona: Typhlonectidae), was captured in 2019 in a portion of the Tamiami (C-4) Canal in Miami-Dade County, southern Florida during a routine fish electroshocking survey conducted by the Florida Fish and Wildlife Conservation Commission (Sheehy et al. 2021). That discovery represented the first time this amphibian order was found in the United States, and it triggered an immediate need for further investigation as to whether the species was established. We subsequently received unconfirmed reports of additional caecilians being found in other parts of the C-4 Canal. Thus, we set minnow traps at multiple sites along the main C-4 Canal and secondary canals in efforts to capture additional individuals. We collected 115 Rio Cauca Caecilians from

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Figure 1. An adult Rio Cauca Caecilian (*Typhlonectes natans*) from the Tamiami (C-4) Canal in Miami-Dade County, Florida. Photograph by Noah Mueller.

three sites along the C-4 Canal (Fig. 1), excluding the site where the original 2019 specimen was taken. In addition, we received numerous reports and observations of caecilians from multiple people, including fishermen and local residents. The capture of mature males, gravid females, and all age classes, along with reported observations of dozens of individuals at multiple locations, clearly demonstrates that this population is established in at least a 16-km section of the C-4 Canal system. We tested specimens for the presence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, and we used X-ray and microCT-scan imaging to investigate aspects of their diet and reproduction. We herein report our findings and comment on the establishment, relative abundance, distribution, and natural history of this species in the Tamiami Canal.

Materials and Methods

Surveys.—We set a total of 27 traps for caecilians at nine different sites along the Tamiami (C-4) Canal in Miami-Dade County, Florida, between 10 August 2021 and 9 March 2024 (Table 1) and numbered them sites 2–10, with site 1 assigned to the original FWC site. These were located north, south, and southwest of the original 2019 location along connected sections or branches of the C-4 Canal (Fig. 2). We primarily used metal wire minnow traps with 2.5-cm concave funnel openings on both ends, but we also used one pyramid-style wire-mesh crab-and-crawfish trap with three small concave funnel openings near the bottom. Unless otherwise noted, we baited all traps with one or two Iberia brand Chicken Vienna Sausages in chicken broth. We also baited a few traps with either bacon, sardines, bread, or catfish bait. In addition to traps, we fished for caecilians using a fishing rod and reel and a small (size 6–8) hook baited with locally caught earthworms (Oligochaeta). We used large dipnets opportunistically to capture caecilians if they were observed close to an accessible edge of the canal. We also conducted visual searches during the day and at night for caecilians that might be swimming near the surface or surfacing to breathe.

We placed traps at different water depths in two main areas of the canal to identify where caecilians were located. Some traps were placed resting on the bottom in shallow water (~15–90 cm deep) in areas containing rocky substrate and an abundance of aquatic vegetation. These traps were located < 60 cm from the shore. We placed other traps in the center and presumably deepest parts of the canal that we could reach (depths ~2–3 m). These traps were not visible on the bottom due to the dark water and details of the substrate are unknown. We secured all traps to small bridges crossing the canal or to objects on the shore using a thin rope.

We placed traps in the water during the day and at night and left them in the canal for varying amounts of time, ranging from 15 min to overnight (ca. 12 h). Sampling for caecilians in various depths of the canals required us to place minnow traps in deep water. To mitigate the risk of bycatch and mortality by drowning, traps were initially checked for bycatch frequently (every 15 min) and gradually extended to checking after several hours and finally overnight. The only bycatch we experienced were small fishes captured in three traps placed in shallow water at three sites (see results below).

Caecilians were identified to species based on diagnostic morphological characters following Sheehy et al. (2021),

Table 1. Locations where minnow traps were set for Rio Cauca Caecilians (*Typhlonectes natans*) in the Tamiami (C-4) Canal in southern Florida, along with trapping dates and numbers of caecilians captured and observed swimming in the canal at each site. Site 1 was the original 2019 FWC site. Sites 2 and 3 were both resampled at later dates, and those results are listed separately. Sites 11–14 were reported observations from fishermen and are described in the text.

Site	Dates	Coordinates	Caecilians captured	Caecilians observed
1	21 Oct 2019	25.771200, -80.310130	1 adult	0
2	10–11 Aug 2021	25.758327, -80.347533	37 adults and 2 juveniles	0
2	7–8 Dec 2022	25.758327, -80.347533	2 adults	0
3	10–11 Aug 2021	25.755236, -80.344022	48 adults	ca. 5 adults
3	7–8 Dec 2022	25.755236, -80.344022	9 adults	0
3	9 Mar 2024	25.755236, -80.344022	6 adults	0
4	8 Dec 2022	25.74714, -80.36569	11 adults	3 adults
5	10–11 Aug 2021	25.840830, -80.305556	0	0
6	10–11 Aug 2021	25.746693, -80.380259	0	0
7	10–11 Aug 2021	25.740811, -80.309890	0	0
8	10–11 Aug 2021	25.808033, -80.312821	0	0
9	10–11 Aug 2021	25.750601, -80.365779	0	0
10	10–11 Aug 2021	25.838905, -80.305285	0	0
11	29 Dec 2023	25.774864, -80.384102	—	Dozens of adults
12	Mid-Dec 2023	25.755036, -80.350092	—	ca. 10 neonates; dozens of adults
13	Mid-Dec 2023	25.761600, -80.369283	—	Dozens of adults
14	22 Aug 2019	25.761284, -80.410082	1 adult	0

and their sex was determined by the size and shape of the cloacal disc (Tapley at al. 2022). Caecilians were euthanized immediately following capture by submersion in a buffered 5g/L aqueous Tricaine methanesulfonate (MS-222) solution following the protocol approved by the University of Florida IACUC. Specimens were fixed using 10% buffered formalin and subsequently preserved in a solution of 70% ethanol and 30% water. Museum acronyms are as follows: UF (University of Florida, Florida Museum of Natural History) and UMMZ (University of Michigan, Museum of Zoology).

Specimen imaging.—We X-ray scanned 27 preserved adult specimens (see Appendix 1) regardless of sex to visualize dense internal structures, such as the skeletons of adults and fetuses and the dense components of any stomach contents. Eleven of those specimens were microCT-scanned using a General Electric Phoenix V|tome|x m 240 scanner at the Nanoscale Research Facility at the University of Florida. Information on microCT-scanning parameters and links to datasets are available in supplementary Table S1.

Batrachochytrium dendrobatidis screening.—Non-native species often influence pathogen dynamics, sometimes to the detriment of local wildlife communities (Crowl et al. 2008). The global spread of *B. dendrobatidis* is partially attributed to introduced amphibian species like the American Bullfrog (*Rana catesbeiana*), which has established feral populations in parts of Europe and serves as a vector, aiding *Bd* persistence and transmission to native amphibians (Garner et al. 2006). *Typhlonectes natans* is known to be susceptible to *Bd* and has tested positive in captivity, including cases at the Bronx Zoo in New York (Churgin et al. 2013). In our study, we screened *T. natans* specimens to assess their *Bd* status and explore the potential role of this non-native caecilian in pathogen dynamics in southern Florida. At the time of *Bd* screening, all specimens had previously been fixed in 10% buffered formalin and were subsequently stored in 70% ethanol in the herpetology collection at the Florida Museum of Natural History. Our *Bd* sampling protocol followed the noninvasive sampling of formalin-preserved specimens described by Cheng et al. (2011). We screened 78 specimens of *T. natans* that had been in ethanol for less than two weeks and were stored in multiple jars containing ten or more specimens each. We also conducted a visual inspection of each individual animal to look for potential skin lesions such as hyperkeratosis or hyperplasia often associated with chytridiomycosis in caecilians (Gower et al. 2013). We handled specimens while wearing nitrile gloves and changed gloves between animals to minimize cross

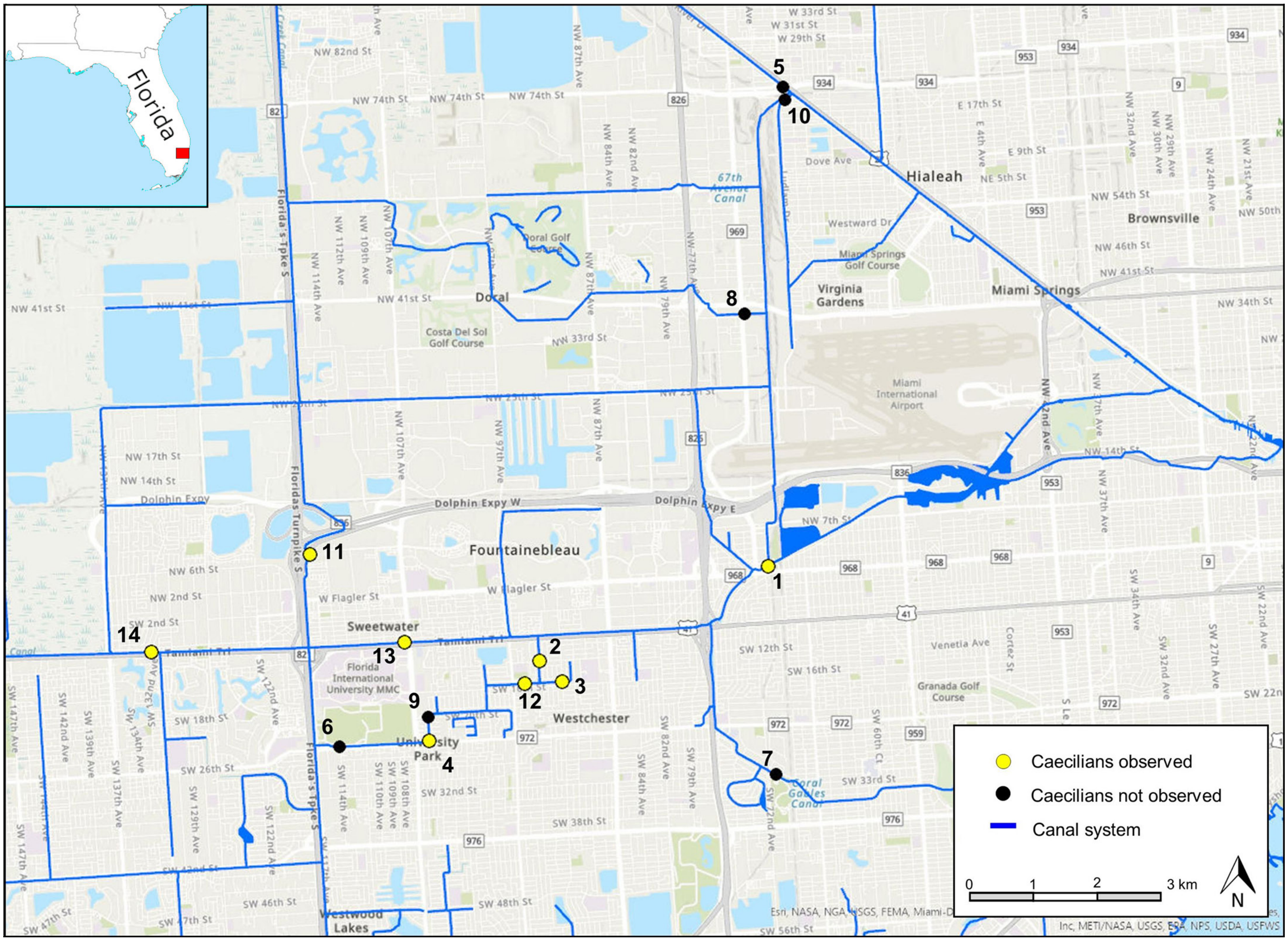

Figure 2. Locations of traps and observations of Rio Cauca Caecilians (*Typhlonectes natans*) in the Tamiami (C-4) Canal in Miami-Dade County, Florida, USA. Yellow dots show sites where at least one caecilian was captured or observed, whereas black dots show sites where no caecilians were captured or observed. Numbers 1–14 refer to site locations, which are explained in Table 1 and in the main text. Site 1 is the location where *T. natans* was first reported by Sheehy et al. (2021). The map was created using the HYDROEDGE and AHED Waterbodies dataset layers within the South Florida Water Management District Geospatial Open Data (https://geo-sfwmd.hub.arcgis.com/) to show water connectivity between canal sites (ArcGIS Pro 3.1.2).

contamination. Upon removal from the jars, specimens were consecutively rinsed with water and ethanol and then swabbed, which consisted of firmly running a sterile cotton swab (MW 113, Medical Wire and Equipment, Corsham, Wiltshire, UK; Kriger et al. 2006) along the entire length of the ventral, dorsal, left, and right sides of the specimen for a combined total of 50 times per animal. Swabs were stored individually in snap cap microcentrifuge tubes at -20 °C until DNA extraction.

DNA extraction and qPCR.—We extracted DNA from swabs using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer protocol, with a final elution volume of 40 µl AE buffer (Cheng et al. 2011). We chose this method over other alternatives because it shows high accuracy for detecting *Bd* when it is present (Bletz et al. 2015). To set up our reactions, we used the Fast Advanced Master Mix TaqMan (Thermo Fisher Scientific) and BSA, which is recommended for its counter effect on PCR inhibitors (Kosch and Summers 2013). Our *Bd* detection assay followed standard protocol (Boyle et al. 2004) and we set qPCR plates in duplicate with positive controls using a synthetic *Bd* that we serially diluted (10^4, 10^3, 10^2, 10, and 0.1) and loaded in duplicate on each plate. We also used negative controls containing sterile water. Quantitative PCR was completed on a QuantStudio 3 machine in the Department of Biology at the University of Florida.

Results

The pyramidal trap was used once at a single location and did not capture any caecilians. Thus, capture results reported below are all from the use of wire minnow traps and are summarized in Table 1. Coordinates all use WGS84 as the geodetic reference datum.

Surveys.—Site 2: On 10 August 2021, we placed minnow traps in the middle and presumably deepest part of the canal within reach of the bridge and captured 18 adult caecilians from 1130–1900 h, one adult from 1915–1930 h, and 15 caecilians (14 adults and 1 juvenile) between 1940–2115 h. One adult was caught while fishing with earthworms as bait at 1930 h. We observed with a flashlight one neonate crawling slowly along the edge of the canal in shallow (~15 cm) water after dark at 2115 h, and it was captured with a dipnet. Three additional adults were captured while fishing with earthworms as bait between 2200–2400 h. Of the 39 animals captured, 28 were accessioned into the UF herpetology collection (UF:Herp:192311–338).

On 7 December 2022, we placed one minnow trap on the north side of the canal in the middle and presumably deepest part of the canal within reach of the bridge from 2053–2123 h and captured no caecilians. The trap was placed again in the same location and left overnight (2123–0754 h on 8 December 2022) but captured no caecilians. The trap was again returned to the same location from 0754–1104 h and captured two adults (UMMZ 249308–309).

Site 3: On 10 August 2021, we placed two minnow traps on the north side of the canal in shallow (< 1 m) water from 1930–2230 h containing catfish bait and captured no caecilians. However, one minnow trap baited with a Vienna sausage and placed in the middle and presumably deepest (2–3 m) part of the canal captured 48 adult caecilians (UF:Herp:192401–448) in the same 3-h period. The total mass of the 48 caecilians excluding the mass of the minnow trap was 4.6 kg. Shortly after dark, we observed at least five adults swimming at the surface in open water above the deepest part of the canal.

On 7 December 2022, from 2109–2139 h, we placed one minnow trap on the west side of the bridge in the same location where 48 caecilians were caught on 10 August 2021 and captured no caecilians. The trap was placed again in the same location and left overnight (2139–0806 h on 8 December 2022) and captured five adults (UMMZ 149310–314). The trap was returned to the same location from 0806–1116 h on 8 December 2022 and captured no caecilians, and a third deployment from 1116–1215 h captured two adults. A trap set in shallow water (< 0.75 m) on the bank immediately northwest of the previous three trap deployments just described and baited with bacon from 1120–1227 h captured two adults. A trap set in the center channel on the east side of the bridge and containing sardine bait from 1120–1227 h captured no caecilians.

On 9 March 2024, one minnow trap was placed in deep water from the bridge as described above from 0830–2130 h as part of an opportunistic survey effort. Six adults were captured (UF:Herp:194670–675).

Site 4: On 8 December 2022, we placed one minnow trap baited with bacon in shallow water from 1251–1405 h and captured no caecilians. A second trap placed in shallow water and baited with a Vienna sausage from 1301–1354 h captured 10 adults, and a third trap baited with sardines and bread from 1307–1400 h captured no caecilians, though we observed three adults inspecting the trap in the shallow water where it was set. One adult was caught while fishing with earthworms as bait, after the baited hook was left on the canal bottom from 1307–1400 h (11 total specimens; UF:Herp:193497–507).

Sites 5–10: Between 10–11 August 2021, we placed one minnow trap each at sites 5–9 and five minnow traps at site 10, each baited with one Vienna sausage. The minnow traps at sites 5–9 were placed in shallow (~15 cm) water at the edge of the canal and left overnight but captured no caecilians. The traps at site 10 were placed in deep water and left for two hours (ca. 1000–1200 h) on 11 August 2021 but also captured no caecilians. The trap at site 7 captured one small Mayan Cichlid (*Mayaheros urophthalmus*) and one Bluegill (*Lepomis macrochirus*). Traps at sites 5 and 8 captured one and five African Jewelfish (*Rubricatochromis letourneuxi*), respectively.

In addition to our surveys, we received numerous reports of *T. natans* observations from fishermen along the C-4 Canal system. On 29 December 2023, while fishing along a section of side canal (25.774864, -80.384102; Table 1, site 11), a fisherman reported seeing dozens of adult caecilians within a roughly 30 min period. The caecilians were actively swimming along heavily vegetated sections of canal edge among limestone holes and in open water. However, in mid-December 2023 at a different location in the canal system (25.755036, -80.350092; Table 1, site 12), the fisherman reported seeing about 10 neonatal *T. natans* swimming along the edges of the canal. The fisherman also estimated seeing dozens of adults scavenging on various dead animals in the water at sites 12 and 13 (see section below on diet and feeding behavior). One adult (UF:Herp:193948; photo voucher) was captured at night on 22 August 2019 by a fisherman using earthworms as bait in the main C-4 Canal (25.761284, -80.410082; site 14).

Overall, no bycatch was in any of the minnow traps that contained even one caecilian. Although the possibility exists that nothing else entered the traps containing caecilians, the caecilians could have consumed anything that entered the traps; however, we find this unlikely given our discussion of diet below. Although most of the caecilians captured in traps were alive, a small number died in the traps, presumably from drowning. None of the caecilians we captured showed external signs of injury, suggesting that they were not biting each other in the traps. Following their removal from the minnow traps, caecilians were placed into 5-gallon buckets half-filled with canal water. After about 5 min, the surface of the water in the buckets became slimy and bubbly with thick white mucus, presumably the result of defensive glandular peptides secreted by the skin. None of the captured caecilians attempted to bite in defense.

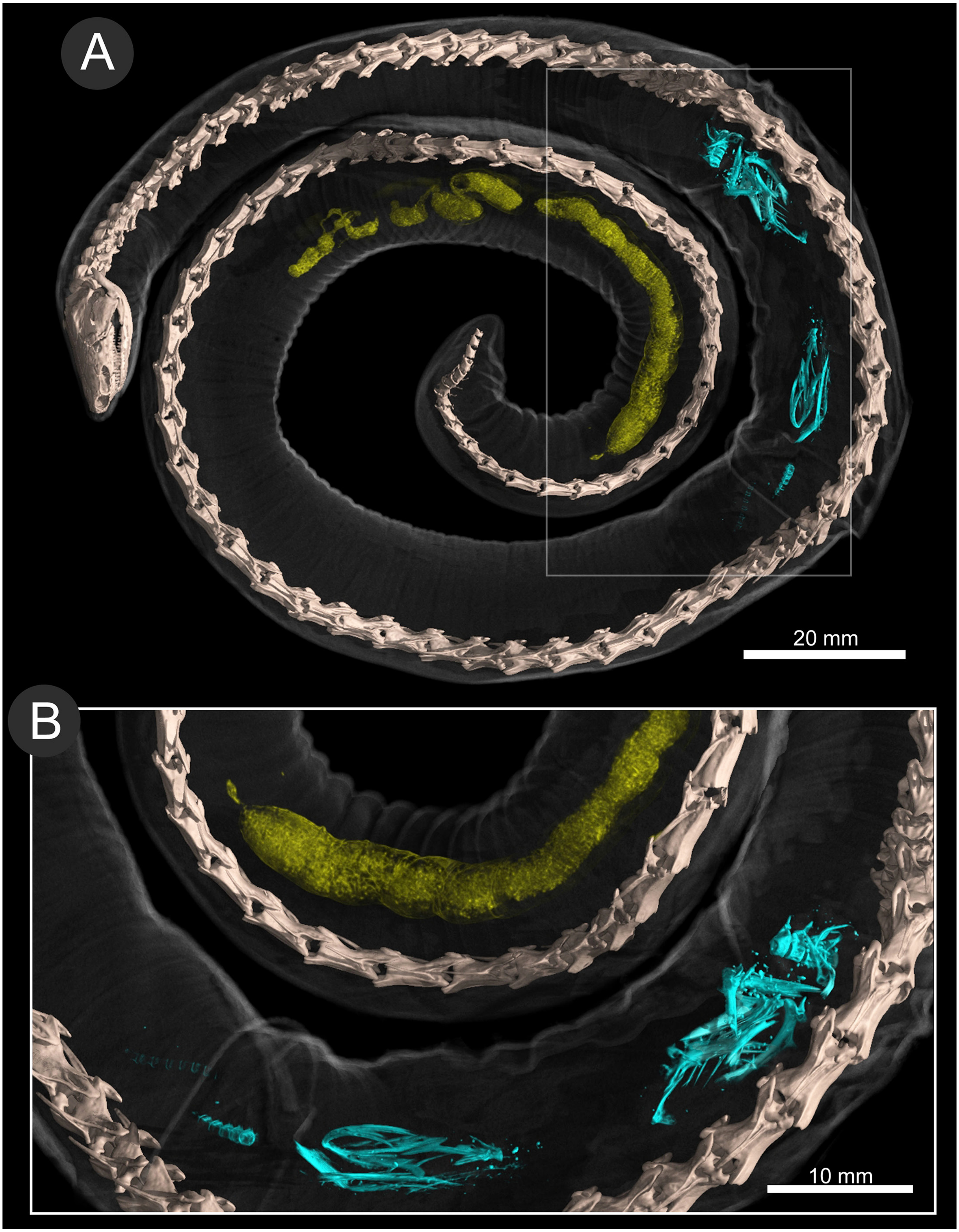

Figure 3. 3D-rendering of a CT-scan of an adult Rio Cauca Caecilian (*Typhlonectes natans*, UF:Herp:192416) collected from the Tamiami (C-4) Canal in southern Florida, USA. The scan shows the skeleton of the caecilian in white, bones from one or more unidentified fish in the stomach of the caecilian in blue, and fecal matter in yellow. Presented are (A) the entire specimen and (B) an insert showing a close-up image of the fish bones.

The rate (caecilians/h) of caecilians captured in 11 minnow traps averaged 4.5 ± 1.62 SE (range = 0.5–16). In a sample of 53 adults housed in the UF herpetology collection at the Florida Museum of Natural History collected over four years from five locations along the C-4 Canal, 29 were females and 24 were males (ratio of 1.21:1).

Diet and feeding behavior.—Three adult *T. natans* that we brought back alive and maintained in an aquarium for a short time enthusiastically consumed live earthworms collected locally and dropped into the aquarium. The aquarium setup consisted of a smooth pea-gravel substrate about 4 cm deep, a cover object for hiding, and a fluorescent aquarium light on top of the lid. The caecilians spent most of the daylight hours under the cover object or buried out of sight in the substrate. After the light was turned off, they would actively swim around the perimeter of the aquarium, but they would quickly hide if the tank or room light was illuminated. At random times during the day when the caecilians were hiding, one of us (CMS) gently placed live earthworms into the aquarium, taking care not to overtly alert the caecilians even though the earthworms were slowly moving in the water. In each case, it took less than 60 s for the caecilians to lift their heads and start swimming vigorously around the aquarium. Upon locating an earthworm, it was immediately bitten and swallowed whole. These observations suggest the caecilians became aware of the presence of the earthworms and subsequently located them using chemoreception, electroreception, mechanoreception, or other nonvisual cues (Jarad et al. 1999).

On 10 August 2021, two of us (CMS and RHR) spoke with a local resident whose home is located on a side canal south of the main C-4 Canal (near collecting site 2; Fig. 2). We showed him caecilians we had captured, and we asked him about seeing them in the canal. He told us that he has seen caecilians in the canal by his house in large numbers for at least five years (as a conservative guess) and that he always assumed they were a type of eel. He said that he was a hunter and that he would put carcasses in the canal for them to scavenge and clean. The caecilians were apparently efficient scavengers and could completely clean the soft tissue from the head of a deer or an alligator in about one or two days. This report is consistent with several other observations reported to us about scavenging behavior by these caecilians. A large group of Rio Cauca Caecilians was observed actively scavenging a carcass of an unidentified freshwater turtle floating at the surface of an undisclosed location on the C-4 Canal during the daytime (A. Werner 2021, pers. comm.). Numerous nighttime observations have been made of caecilians actively scavenging dead animals floating at the surface in both the main C-4 Canal and secondary canals. Two of us (CMS and RHR) spoke with a fisherman who reported observing a group of adult caecilians scavenging on a dead Gopher Tortoise (*Gopherus polyphemus*) in the C-4 Canal (25.761600, -80.369283) in mid-December 2023 (Table 1, site 13). In close proximity within a secondary canal (approximately 25.755036, -80.350092; Table 1, site 12), the fisherman reported seeing dozens of caecilians over several nights scavenging carcasses of a small domestic dog, a domestic Muscovy Duck (*Cairina moschata domestica*), a Hornet Tilapia (*Heterotilapia buttikoferi*), a Green Iguana (*Iguana iguana*), a Raccoon (*Procyon lotor*), and a Virginia Opossum (*Didelphis virginiana*). Caecilians involved in or near scavenging events were not observed actively chasing live prey, although live fish were observed in the same areas.

Of the 27 caecilian specimens that we X-ray scanned, only one (UF:Herp:192416) contained hard-bodied stomach contents dense enough to show in the scans (Fig. 3). This specimen contained the partial skeleton of an unidentified fish. The bones were largely disarticulated and clumped into three separate groups. Only two short sections of vertebrae appeared to remain articulated.

Reproduction.—Three of the 27 caecilian specimens that we X-ray scanned contained late-stage fetuses. Specimen UF:Herp:192402 (Fig. 4A) contained seven fetuses, specimen UF:Herp:192425 (Fig. 4C) contained one fetus, and specimen UF:Herp:192408 (Fig. 4D) contained three fetuses. All three adults were collected on 10 August 2021. The fetuses in all three females were at similar stages of development. No noticeably smaller, earlier-stage fetuses were found in any of the scanned specimens. Females either had fetuses at comparable stages or no fetuses. Eight of the 11 fetuses scanned (73%) in the females had their heads directed posteriorly.

Batrachochytrium dendrobatidis detection assay.—The chytrid fungal pathogen *B. dendrobatidis* was not detected on any of the 78 specimens of *T. natans* in our sample. Our samples all indicated a negative result for the presence of *Bd* against the positive controls. During visual inspections, no skin lesions or other external evidence of infections by the chytrid fungus or other potential pathogens were observed on any of the specimens examined.

Discussion

Between August 2021 and March 2024, we set traps for Rio Cauca Caecilians at nine sites along the C-4 Canal and side canals located north, south, and west of the location where the first caecilian was captured in 2019 (Table 1, Fig. 2; Sheehy et al. 2021). We captured 115 caecilians of all age classes and received one photographic voucher of an adult captured by a fisherman for a total of 116 confirmed captures. We visually observed caecilians actively swimming or crawling along the substrate and aquatic vegetation at two sites, and visual observations were reported from fishermen at three additional sites. At some sites, the density of caecilians was high, exemplified by one minnow trap that captured 48 caecilians in a 3-h period in a deep (2–3 m) part of a canal accessible from a bridge.

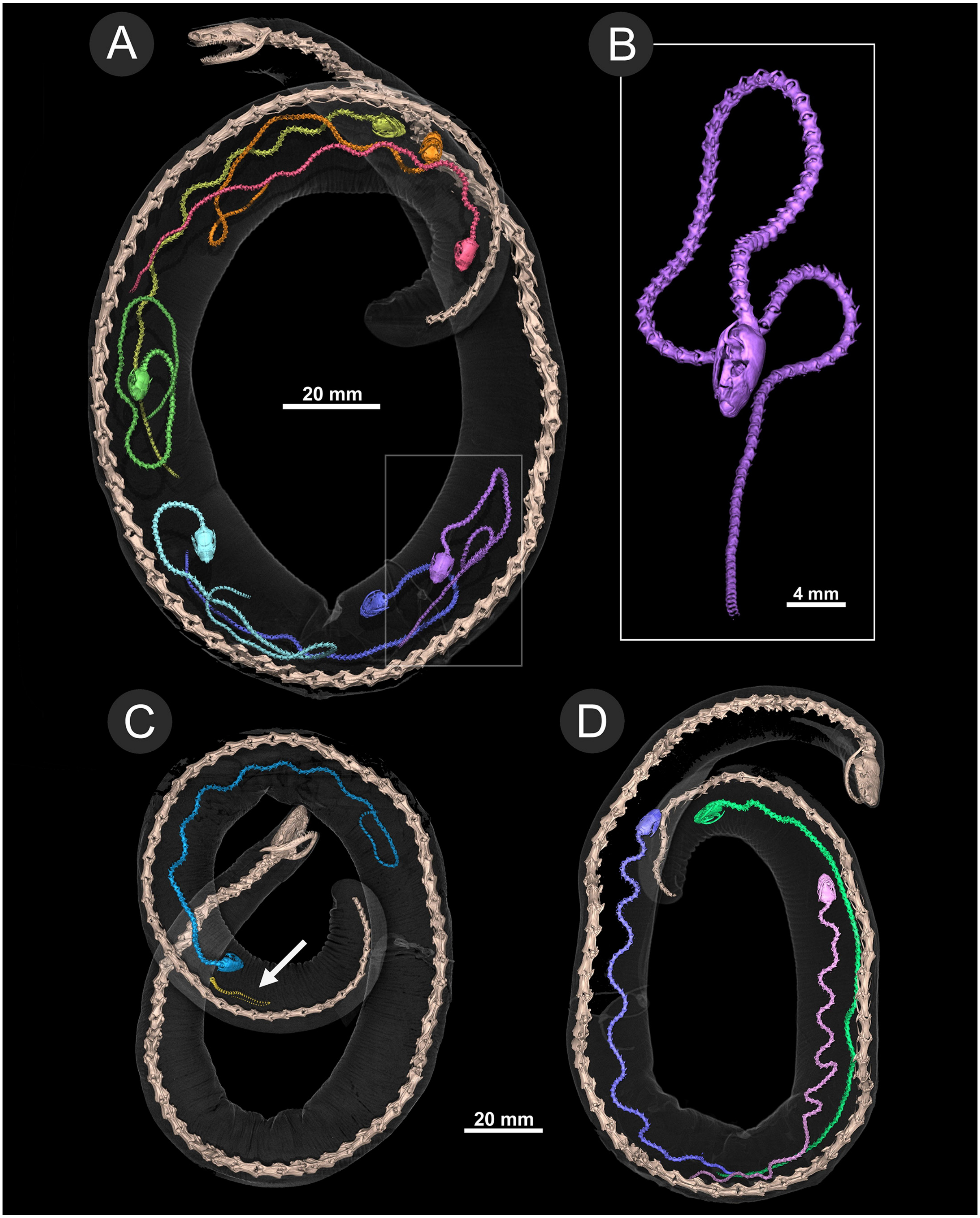

Figure 4. 3D-rendering of CT-scans of three gravid adult female Rio Cauca Caecilians (*Typhlonectes natans*) collected from the Tamiami (C-4) Canal in southern Florida, USA. The scans show the skeletons of the females in white and the skeletons of late-stage fetuses in different colors. Specimen numbers are (A) UF:Herp:192402 containing seven fetuses, (B) with one fetus image enlarged from UF:Herp:192402, (C) UF:Herp:192425 containing one fetus, and (D) UF:Herp:192408 containing three fetuses. Note that specimen UF:Herp:192425 (C) contains a small object in yellow that remains unidentified pending further investigation (see white arrow).

At most sites, we placed baited minnow traps both in shallow (< 1 m) water near the shore and in the deepest parts of the canals to try to assess the water depth where caecilians occurred most frequently. The shallow water traps rarely captured caecilians, even when left overnight. The bait in the shallow water traps showed no signs of disturbance, suggesting that caecilians were not entering the traps, eating the bait, and subsequently leaving. However, the traps placed in the deepest part of the canals would attract and capture caecilians in as little as 15 minutes, and these caecilians were already consuming the bait as evidenced by clearly defined bite marks present on the Vienna sausages. Thus, the caecilians appear to be primarily occupying the deepest parts of the canals at the sites where we found them.

We were able to capture caecilians in the deepest parts of the canals at any time of day using baited minnow traps or on rod and reel with small hooks baited with earthworms. However, we mainly observed caecilians actively swimming at the surface of the water in the evenings. Only occasionally were caecilians observed in shallow water during the day, and they were usually juveniles. Thus, these caecilians appear to be primarily nocturnal when in open or shallow water, but they seem to be foraging, or at least responsive to the presence of food, at any time of day. Juveniles and adults appeared to be partitioning microhabitats with adults primarily occupying deep water and juveniles occupying primarily shallow water. However, our current dataset is not large enough to test this hypothesis. The shallow water microhabitats at our sites generally had rocky substrates interspersed with sand and abundant aquatic vegetation, and the water was clear. Thus, the shallow water microhabitat could offer juveniles ample opportunities to find shelter, food, and easy access to the surface for frequent aerial respiration. The deepest parts of canals were not visible, so we were not able to assess that microhabitat. Temperature differences of at least 3 °C can exist between the bottom and surface water of the canal (EPA 2014), which could influence microhabitat selection if juveniles and adults exhibit different thermoregulatory preferences.

Natural history.—The aquatic habitat in the C-4 Canal system closely matches published descriptions and measured water parameters (i.e., pH and temperature) of habitats within the native range (Tapley and Acosta-Galvis 2010; EPA 2014). *Typhlonectes natans* appears tolerant of poor water quality in its native range, and it apparently can even thrive in such conditions (Hofer 2000; Gower and Wilkinson 2005). Its relatively high hematocrit, high blood volume, and the ability to readily utilize both cutaneous and aerial respiration (Smits and Flanagin 1994; Gardner et al. 2000) further enable this aquatic caecilian to thrive in the variably hypoxic waters of the C-4 Canal system.

Much of the natural history of *T. natans* we have observed in Florida is consistent with observations of these caecilians in their native range and from captive animals. *Typhlonectes natans* has small, paired tentacles that protrude from small openings behind the nostrils. As with all caecilians, these tentacles appear to have olfactory functions that facilitate detection of prey via nonvisual (e.g., chemosensory) cues (Taylor 1968; Schmidt and Wake 1990; Tapley et al. 2019). Observations of *T. natans* in captivity suggest that these caecilians readily consume diverse live invertebrates and carrion, but not live vertebrates (Parkinson 2004; Tapley et al. 2022). For two years, Parkinson (2004) maintained seven wild-caught *T. natans* in an aquarium with a large diversity of live Amazonian fishes and never observed live fish being chased or eaten. However, any fish that died was consumed immediately. Indeed, these aquatic caecilians are well known to consume carrion, including dead fish or fish entrails, and this food source may form a significant portion of their natural diet (Hofer 2000; Parkinson 2004; Tapley and Acosta-Galvis 2010; Tapley et al. 2022). As such, the fish bones identified in the CT-scan of specimen UF:Herp:192416 likely were from a dead fish that was scavenged rather than the result of predation on a live fish. The fact that none of the other 27 specimens we scanned contained dense stomach contents suggests that they are feeding primarily on soft-bodied invertebrates and/or carrion, given that neither of these dietary items would be visible or distinguishable in the scans. Detailed dietary information for these caecilians is needed to assess their potential impacts as scavengers, predators, and competitors in the C-4 Canal system.

The glandular secretions we observed when handling *T. natans* have been documented previously in this and other species of typhlonectid caecilians (Tapley at al. 2022). The mucous-like skin secretions from *Typhlonectes compressicauda* and other typhlonectids have been shown to kill fish housed with them in the same aquaria (Moodie 1978). These secretions cause a burning sensation if they contact the eyes or an open cut (Stebbins and Cohen 1995), and prolonged exposure of the secretions to the hands can cause a tingling sensation (Tapley et al. 2022). In addition to other diverse functions, these dermal secretions likely play an important role in defense (Toledo and Jared 1995). However, predation on *T. natans* is poorly documented. We never observed predation attempts during any of our surveys, and caecilians we captured did not appear to have wounds or scarring, such as those commonly seen in species of aquatic salamanders in the genus *Siren*, that could be interpreted as resulting from failed predation attempts. Certainly, species of predatory fish (e.g., *Cichla ocellaris*, *Mayaheros urophthalmus,* and *Micropterus salmoides*), which are present throughout the C-4 Canal system, are large enough to consume juvenile and potentially subadult *T. natans*, but whether they or any other potential predators (e.g., birds) consume caecilians is currently unknown and in need of further study.

Reproduction in *Typhlonectes compressicauda* varies with seasonal rainfall (Yousef et al. 2018). *Typhlonectes natans* might exhibit a similar pattern, although reports have mixed conclusions. Some authors (e.g., Herman 1994) reported breeding in *T. natans* during the wet season, whereas others (e.g., Parkinson 2004) reported breeding under dry season conditions. Seasonal changes in temperature could trigger reproductive behavior in these caecilians, and they might engage in seasonal movement patterns at all ages (Tapley and Acosta-Galvis 2010). The C-4 Canal system experiences seasonal differences in rainfall and water temperature (EPA 2014), and our limited data suggest that seasonal patterns could exist in *T. natans*. The only three gravid caecilians we scanned contained well developed fetuses at similar stages of development. These three animals were collected the same day (10 August 2021). Further investigation is needed to determine whether *T. natans* reproduces seasonally, whether reproduction is correlated with wet and dry seasons, and whether these caecilians exhibit seasonal movement patterns in the C-4 Canal system.

Densities of *Typhlonectes natans* are reported to be relatively high in several river systems within their native range, especially in areas such as fishing villages where fish entrails are discarded into the water (Hofer 2000; Tapley and Acosta-Galvis 2010). The IUCN SSC Amphibian Specialist Group (2020) listed *T. natans* as a species of Least Concern due to their large distribution, presumed thriving population, and resilience to poor water quality. Results of trapping and visual observations suggest that *T. natans* can be found in high densities in parts of the main C-4 Canal and in many secondary canals. For example, we captured 48 adults in a single minnow trap in a 3-h period. This minnow trap was completely full of caecilians, suggesting that additional individuals were not captured due to limited space in the trap rather than a lack of additional caecilians. We also have reports of dozens of caecilians observed together in several locations in the C-4 Canal system. The distance along the main C-4 Canal system separating the farthest west (UF:Herp:193948) and east (UF:Herp:190000) locations where caecilians have been captured and verified is about 10.5 km. Additionally, they have been confirmed in approximately 5.5 km of secondary canal system. If *T. natans* is found throughout this range of canals at the high densities we have observed, the biomass of caecilians could potentially rival or exceed that of many other species of vertebrates in the canal system.

The actual range of *T. natans* in southern Florida likely exceeds the range of the canal system where we have confirmed their presence. Although *T. natans* is a fully aquatic, freshwater species (Taylor 1968), they are known to occupy floodplains and otherwise flooded land (Tapley and Acosta-Galvis 2010) and this could facilitate dispersal beyond canals or bodies of water in direct contact with the C-4 Canal. The earliest reported sightings we have received of *T. natans* in the C-4 Canal have come from the homeowner at site 2, who conservatively estimated first seeing them in 2016. The specimen (UF:Herp:193948) marking the westernmost datapoint for the distribution of these caecilians was captured in 2019, and this location was located only 8.85 km east of the northeastern boundary of Everglades National Park. Although the C-4 Canal flows west to east, the mean water velocity at that general section of the canal is slow (mean range 0–4.6 cm/s; USGS 2024) and *T. natans* is known to migrate seasonally against the flow of floodplain waters in their native range (Tapley and Acosta-Galvis 2010). Given their dispersal abilities and the length of time they have been present in the C-4 Canal, these caecilians may very well have already expanded westward into Everglades National Park. Furthermore, intense weather events such as the historic rainfall and flooding that affected Miami-Dade County in June 2024 could further facilitate the dispersal of these caecilians throughout parts of southern Florida.

Batrachochytrium dendrobatidis.—Neither visual inspection for skin lesions nor qPCR detected *B. dendrobatidis* in any of the *T. natans* specimens from our southern Florida samples. However, *Bd* has been documented in Miami-Dade County where our sampling took place, with the earliest recorded case in Florida dating back to 1928 from an analysis of museum specimens (Karwacki et al. 2021). Although *T. natans* is known to be susceptible to *Bd* (Churgin et al. 2013), our inability to detect the pathogen could be due to subclinical infection levels in our samples, potentially below the detection threshold of our qPCR assay. The threat of *Bd* has likely been exacerbated by invasive species that can spread the pathogen to native species of amphibians (e.g., Miaud et al. 2016). The ability of *Bd* to survive is significantly reduced at temperatures above 26 °C (Stevenson et al. 2013), and temperatures above 32 °C have been recommended as potential treatment for *Bd* infections in amphibians (Woodhams et al. 2003). Indeed, *Bd* infections in captive-born *Typhlonectes natans* have been reported and successfully treated by exposure to water temperatures of 32.2 °C for 72 h (Churgin et al. 2013).

Throughout the year, median surface and bottom temperatures in the canals of southern Florida range between 27–30 °C and 26–28.5 °C, respectively (EPA 2014). Given that *T. natans* appears to occupy the bottoms of canals diurnally and move to shallow or surface waters nocturnally, we cannot determine if the lack of *Bd* in the specimens we tested is due to lack of exposure to the fungus or due to sufficient exposure to water temperatures warm enough to clear infections. Furthermore, seasonal patterns in temperature and rainfall have been shown to significantly influence *Bd* infections in both anurans (e.g., Longo et al. 2010; Watters et al. 2019) and aquatic caudates (e.g., Basanta et al. 2022). Canals in southern Florida experience seasonal differences in water temperature and rainfall (EPA 2014), but we do not know if *Bd* infections vary seasonally in *T. natans*. As such, we cannot as yet speculate how infected individuals of this aquatic caecilian species could influence the dynamics of the *Bd* pathogen in southern Florida.

In summary, Rio Cauca Caecilians (*Typhlonectes natans*) are now considered established in the C-4 Canal system of southern Florida, and this establishment likely began prior to 2016. Although *T. natans* is susceptible to *Bd*, our results suggest that this species may not be functioning as a reservoir for *Bd* in the C-4 Canal. Long-term testing, however, is needed to better understand their potential role in the transmission of *Bd* and other potential pathogens. Our results provide preliminary information regarding the distribution, biology, and natural history of these caecilians in Florida. However, more information is urgently needed on these enigmatic amphibians to better understand the full extent of their distribution and dispersal capabilities in Florida, their relative biomass per unit area, patterns regarding movement and reproduction, and more detailed aspects of their ecology and basic biology. This information will be crucial for directing efforts to control or eradicate *T. natans* and to understand how freshwater ecosystems in southern Florida are affected by the establishment of these aquatic caecilians.

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Appendix 1. Specimens examined.

X-ray scanned *Typhlonectes natans* specimens (n = 27): UF 192401, 192402, 192406, 192408, 192409, 192410, 192411, 192412, 192415, 192416, 192422, 192423, 192424, 192425, 192427, 192429, 192430, 192432, 192433, 192435, 192436, 192438, 192440, 192442, 192443, 192444, 192446.

MicroCT-scanned *Typhlonectes natans* specimens (n = 11; Table S1): UF 192401, 192402, 192406, 192408, 192416, 192423, 192425, 192436, 192442, 192443, 192444.

Table S1. MicroCT-scan parameters used for specimens of Rio Cauca Caecilians (*Typhlonectes natans*) in this study. Catalog numbers refer to accession numbers for individual specimens and all associated data housed in the Division of Herpetology at the Florida Museum of Natural History. The DOI links in the far-right column link to the individual images in MorphoSource, where the image files and associated datasets are stored and openly available.

Catalog number	scan file name	scan type	Voltage (kV)	Current (uA)	Voxel size (mm)	number of projections	exposure timing	filter	frame averaging	image size	multi-part scan	compressed file size (GB)	DOI
192401	UF-herp-192401-head	high-res	120	200	0.03163245	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	no	0.665	https://doi.org/10.17602/M2/M649662
192401	UF-herp-192401-body	high-res	120	200	0.04524768	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	2x	2.99	https://doi.org/10.17602/M2/M649656
192402	UF-herp-192402-head	high-res	100	200	0.02139322	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	no	0.796	https://doi.org/10.17602/M2/M649675
192402	UF-herp-192402-body	high-res	110	200	0.05635091	2200	200	Al 0.3 mm	3 (skip 1)	2014x2024	2x	1.58	https://doi.org/10.17602/M2/M649671
192406	UF-herp-192406-RAPID	rapid	120	200	0.0631268	2399	200	Al 0.3 mm	1	2014x2024	no	0.754	https://doi.org/10.17602/M2/M649737
192408	UF-herp-192408	high-res	100	200	0.07832415	1560	200	0.1 mm Cu	3 (skip 1)	1300x2024	no	0.663	https://doi.org/10.17602/M2/M649684
192416	UF-herp-192416	high-res	120	200	0.06339812	1680	200	Al 0.3 mm	3 (skip 1)	1400x2024	no		https://doi.org/10.17602/M2/M649688
192423	UF-herp-192423-RAPID	rapid	100	200	0.06693305	2999	200	Al 0.3 mm	1	1500x2024	no		https://doi.org/10.17602/M2/M649693
192425	UF-herp-192425	high-res	100	200	0.07099933	1560	200	0.1 mm Cu	3 (skip 1)	1300x2024	no	0.639	https://doi.org/10.17602/M2/M649700
192436	UF-herp-192436-head	high-res	120	200	0.02673928	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	no	1.34	https://doi.org/10.17602/M2/M649718
192436	UF-herp-192436-body	high-res	120	200	0.0345472	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	2x	3.88	https://doi.org/10.17602/M2/M649706
192442	UF-herp-192442-RAPID	rapid	100	200	0.05329684	2099	200	0.1 mm Cu	1	1600x2024	no	1.16	https://doi.org/10.17602/M2/M649722
192443	UF-herp-192443	high-res	100	200	0.03844307	2200	200	0.1 mm Cu	3 (skip 1)	2014x2024	3x	2.71	https://doi.org/10.17602/M2/M649727
192444	UF-herp-192444-RAPID	rapid	80	200	0.10565364	1499	200	Al 0.3 mm	1	1200x2024	no	0.286	https://doi.org/10.17602/M2/M649733