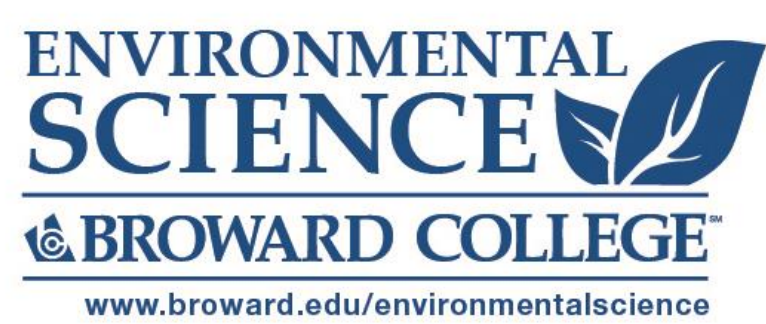# Invasive Tegus Introduced an Exotic Hookworm to Florida

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ABSTRACT

Co-introduction of parasites with their invasive hosts is a lesser studied subject of the ecology of invasive species . We surveyed internal and external parasites of the invasive Argentine black and white tegu *Salvator merianae* (Dumeril). The hookworm-like nematode *Diaphanocephalus galeatus* (Rudolphi) infected 83% of specimens, even occurring in juveniles (14.5 cm snout–vent length). This hookworm occurred primarily in the small intestine of hosts, although specimens sometimes (10%) had low numbers of hookworms in their anterior large intestine. Hookworm numbers increase with snout–vent length, and hosts usually contain more female than male hookworms. Comparing female to male tegus, we failed to find a difference in parasite numbers. *D. galeatus* has a highly aggregated population structure as indicated by a high coefficient of dispersion and close fit to a negative binomial distribution; infected hosts contained 1–57 (35 ± 78, n = 58) hookworms. We suspected that heavily infected hosts might have lower than normal mass, however our data did not support this hypothesis. High infection rates in our samples are comparable to those in the native range of *S. merianae*, suggesting that this parasite-host relationship has been little affected by transplantation to South Florida.

Specimens of *Salvator merianae,* The Argentine black and white tegu, in traps. (Tegu photos courtesy of E. Gati, UF Croc Docs)
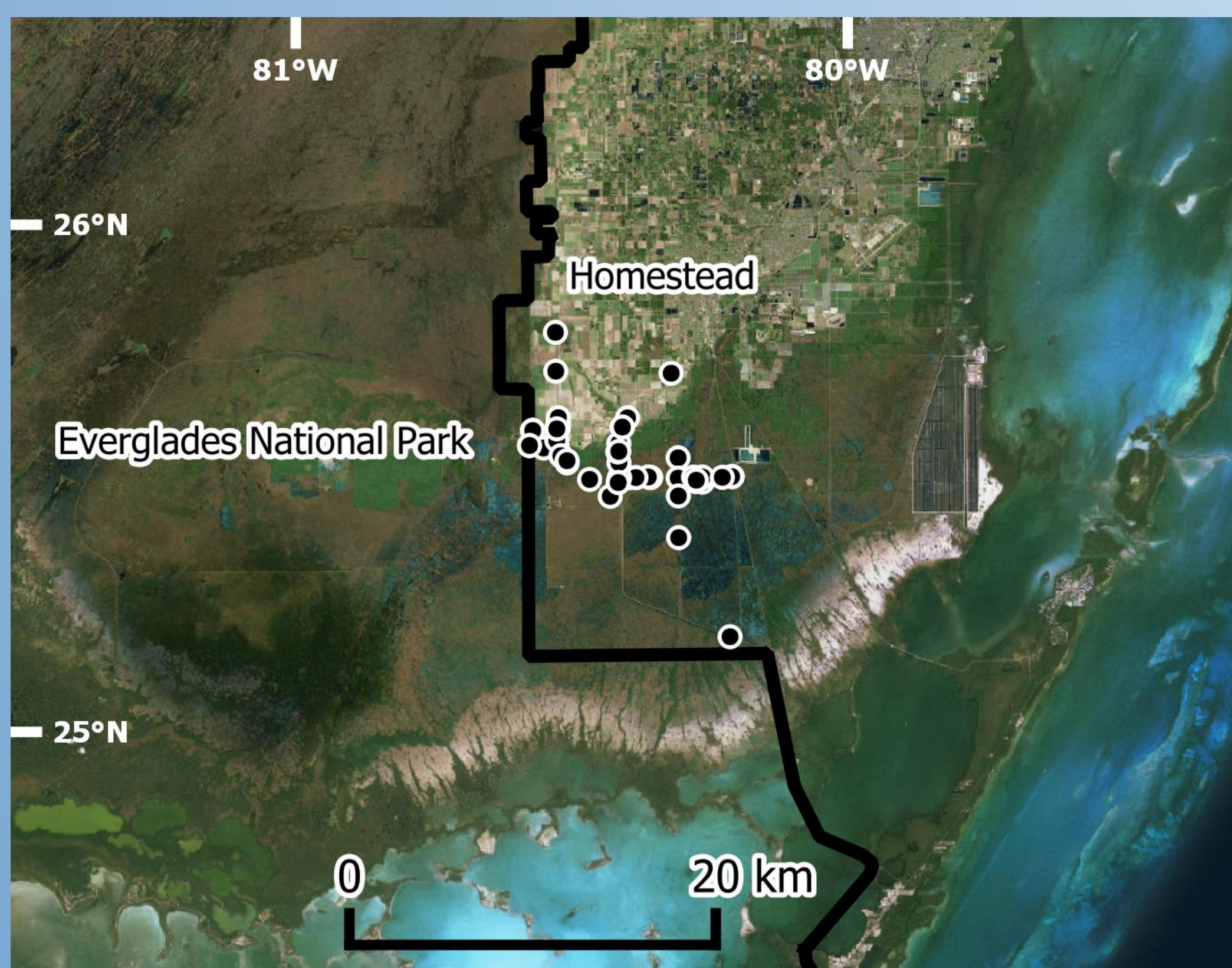
Each dot indicates a tegu used for our study. The black boundary represents the boundary of Everglades National Park.

BACKGROUND

The Argentine black and white tegu is an omnivore with a broad diet consisting of fruits and foliage, eggs of birds and reptiles, insects, and small animals. Thriving as an invasive species in South Florida, the tegu has established a substantial breeding colony in Miami-Dade County. Diet and behavior may predispose an organism to certain parasites, particularly those with intermediate hosts (heteroxenous) or a broad host range.

Known parasites from South America (Ávila & Silva, 2010):
Nematodes: *Diaphanocephalus galeatus, Physaloptera tupinambae, Cruzia travassosi, Spinicauda spinicauda*
Cestodes: *Oochoristica sp.*
No record of acanthocephalans, pentastomes, or ticks.

D. galeatus is highly prevalent in South American tegus but little has been done to describe its population structure or explore its effect on hosts.

METHODS

Laboratory Protocols

For our study we necropsied 58 *Salvator merianae* individuals that were collected between August 2017 and October 2018 from trap lines just outside the eastern boundary of Everglades National Park in Miami-Dade County. We euthanized tegus by pithing which allowed us to collect blood from the puncture produced. We created blood smears, which we stained with Geimsa for viewing under light microscopy to check for the presence of protozoan parasites. We analyzed the body of each tegu for the presence of ectoparasites. We then opened the body cavity, examining the lungs, esophagus, stomach, and small and large intestines for endoparasites. We prepared parasites using standard protocols outlined by Pritchard & Kruse (Manter Lab, University of Nebraska). We fixed specimens of *D. galeatus* in AFA and preserved them in 70% ethanol. We deposited all parasites in the Manter Parasite Collection (Lincoln, Nebraska).

Statistical Analysis

To test whether parasite load affected host mass we first log-transformed SVL and body weight. We then regressed the residuals on number of parasites. We conducted a correlated *t*-test to determine if males and females parasites occur in equal numbers within hosts.

To investigate population structure of *Diaphanocephalus galeatus* we compared parasite load to random (Poisson) and aggregated (Negative Binomial) models of population assemblage. We used Fisher's Exact Test as a goodness of fit test for our observed parasite frequencies instead of the Chi-Square Test because many cells in the contingency table were zero. We also report a coefficient of dispersion to quantify population structure.

For most statistics, we use subscripts to report samples sizes. For Fisher's Exact Test, our subscripts represent "rows, columns" in the contingency table.

RESULTS

We necropsied 58 tegus for our study. Our sample includes 30 males and 27 females; the sex of one tegu was not recorded. The snout–vent lengths of *Salvator merianae* ranged from 12.1 cm–42.5 cm (23.3 ± 6.5 cm, n = 58). From the 58 *Salvatore merianae* individuals necropsied, we collected 2,171 individual parasites. The nematode *Diaphanocephalus galeatus* was substantially prevalent occurring in 82.8% of *S. merianae* necropsied. Infected hosts contained 1–557 (35 ± 78, n = 58) hookworms.

Prevalence, density, and intensity of parasitic infections in sampled *Salvator merianae*:

Parasite	Total Individuals collected	Prevalence[1]	Density[2]	Intensity[3]
Diaphanocephalus galeatus	2027	82.8%	35	42
Physaloptera tupinambae	76	27.6%	1	5
Cruzia fulleborni	6	5.2%	0	2
Centrorhyncus sp.	3	1.7%	0	3
Macracanthorhynchus cf. ingens	1	1.7%	0	1
Cestoda (cysticercus)	2	1.7%	0	2
Pentastomes	50	20.7%	1	4
Acari (larvae)	6	3.4%	0	3

1 Prevalence – Percentage of individuals hosting a parasitic infection in a sample
2 Density – (Mean abundance) Average number of parasites across all sampled individuals.
3 Intensity – Average number of parasites per infected host

Parasites and their ecology that we found in tegu hosts:
- *Diaphanocephalus galeatus* – monoxenous nematode, small intestine, collected from the large intestine in 10% of hosts usually in addition to the small intestine. First recorded in U.S.
- *Physaloptera tupinambae* – heteroxenous nematode, collected primarily from the stomach, one from the small intestine of a single tegu, and one from the large intestine in addition to the stomach of the same tegu
- *Cruzia fulleborni* – monoxenous nematode, collected from the large intestine and stomach
- *Centrorhynchus* – heteroxenous acanthocephalan, cystacanth collected from small intestine
- *Macracanthorhynchus* – heteroxenous acanthocephalan, cystacanth collected from stomach wall
- Cestoda sp. – heteroxenous tapeworm, cysticercus encysted in the mesentery
- Pentastome – *Raillietiella sp.* (possibly multiple species) heteroxenous, collected from the lungs
- Acari – *Amblyomma sp.* Tick larvae collected from the groin

## DESCRIBING PARASITE POPULATION STRUCTURE

We found that load of the hookworm *Diaphanocephalus galeatus* increases with snout–vent length of its host *Salvator merianae*. Using linear regression on all the data we violated assumptions of homoscedasticity (Breusch-Pagan statistic 3.93, $P = 0.047$), linearity (Durbin Watson statistic 0.85, $P = 0.000$), and normality of errors of prediction ($W_{58} = 0.55$, $P = 0.000$). Moreover, log transformation of snout–vent length failed to alleviate these problems. Accordingly, we did not use a *t* -test to determine if nematodes are correlated with SVL. However, a bootstrapped confidence interval around the slope of the regression does not include zero (0.17, 9.27). One specimen contained 557 hookworms, whereas all others contained fewer than 200. Because of the considerable leverage of this datum, we removed this outlier and reran the analysis. The new confidence interval also excluded zero (1.29, 4.94).

Comparing female to male tegus, we failed to find a difference in parasite load (Welch's $t_{27,30} = 0.98$, $P = 0.335$).

We did not detect a relationship between parasite load and mass ($t_{58} = 0.16$, $P = 0.872$).

In tegus, female *Diaphanocephalus galeatus* are more numerous than males ($t_{49} = 2.82$, $P = 0.007$).

Diaphanocephalus galeatus has a highly aggregated population structure as indicated by a high coefficient of dispersion (variance/mean = 175) and close fit to a negative binomial distribution ($k = 0.90$, $P_{11,2} = 0.158$). Additionally, We were able to reject the alternative hypothesis of random distribution (Poisson model, Fisher's Exact Test $P_{11,2} = 0.000$).
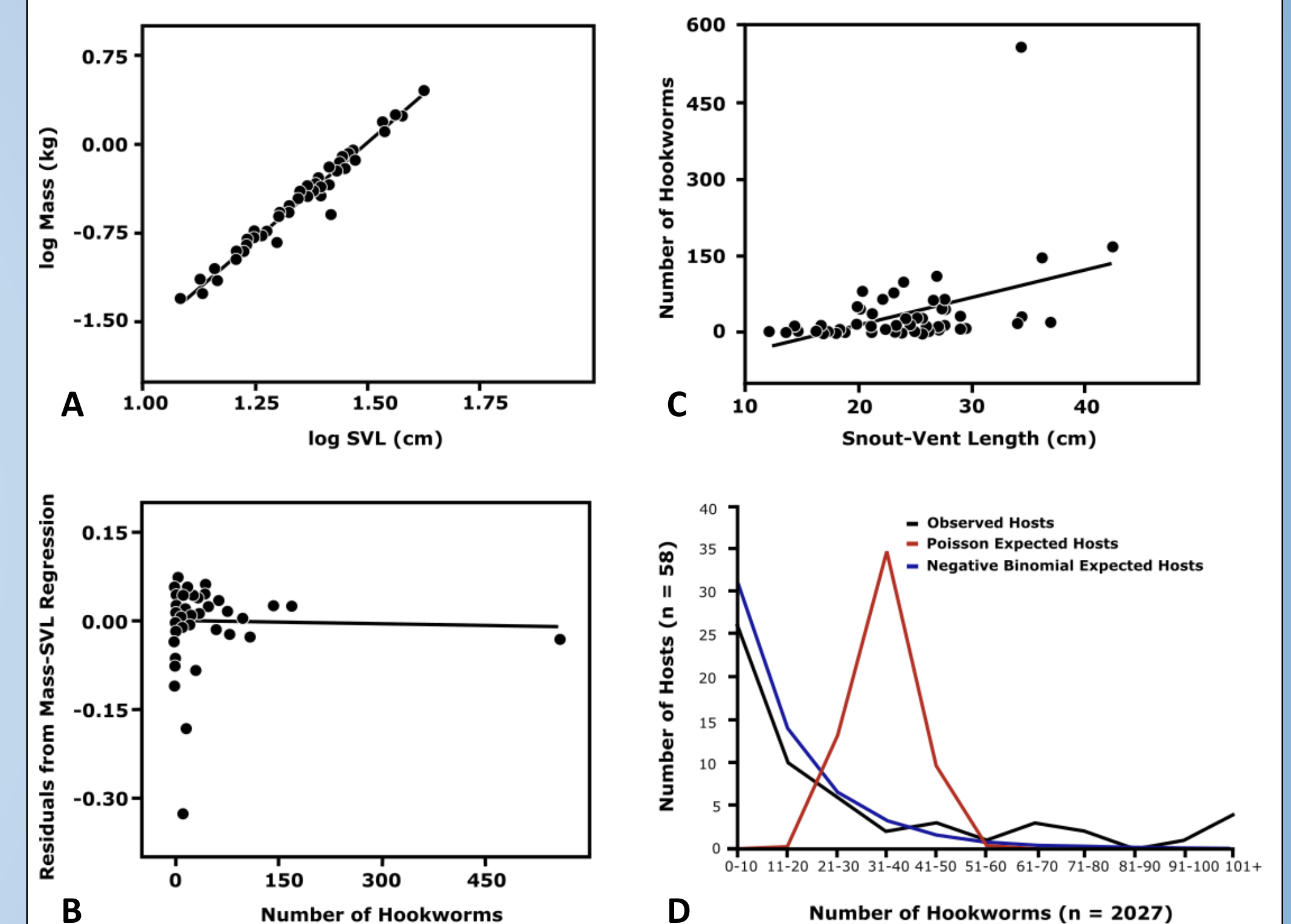
Left: Investigating the effect of *Diaphanocephalus galeatus* on mass of *Salvator merianae* **A.** Correlation between mass and snout–vent length of *S. merianae*. Values are log-transformed. We used residuals from this analysis to generate graph b. **B.** We then regressed the residuals on number of parasites. Right: **C.** Correlation between number of *Diaphanocephalus galeatus* and snout–vent length of its host *Salvator merianae*. **D.** Observed parasite load compared to loads predicted by aggregated (negative binomial) and random (Poisson) population distribution models.
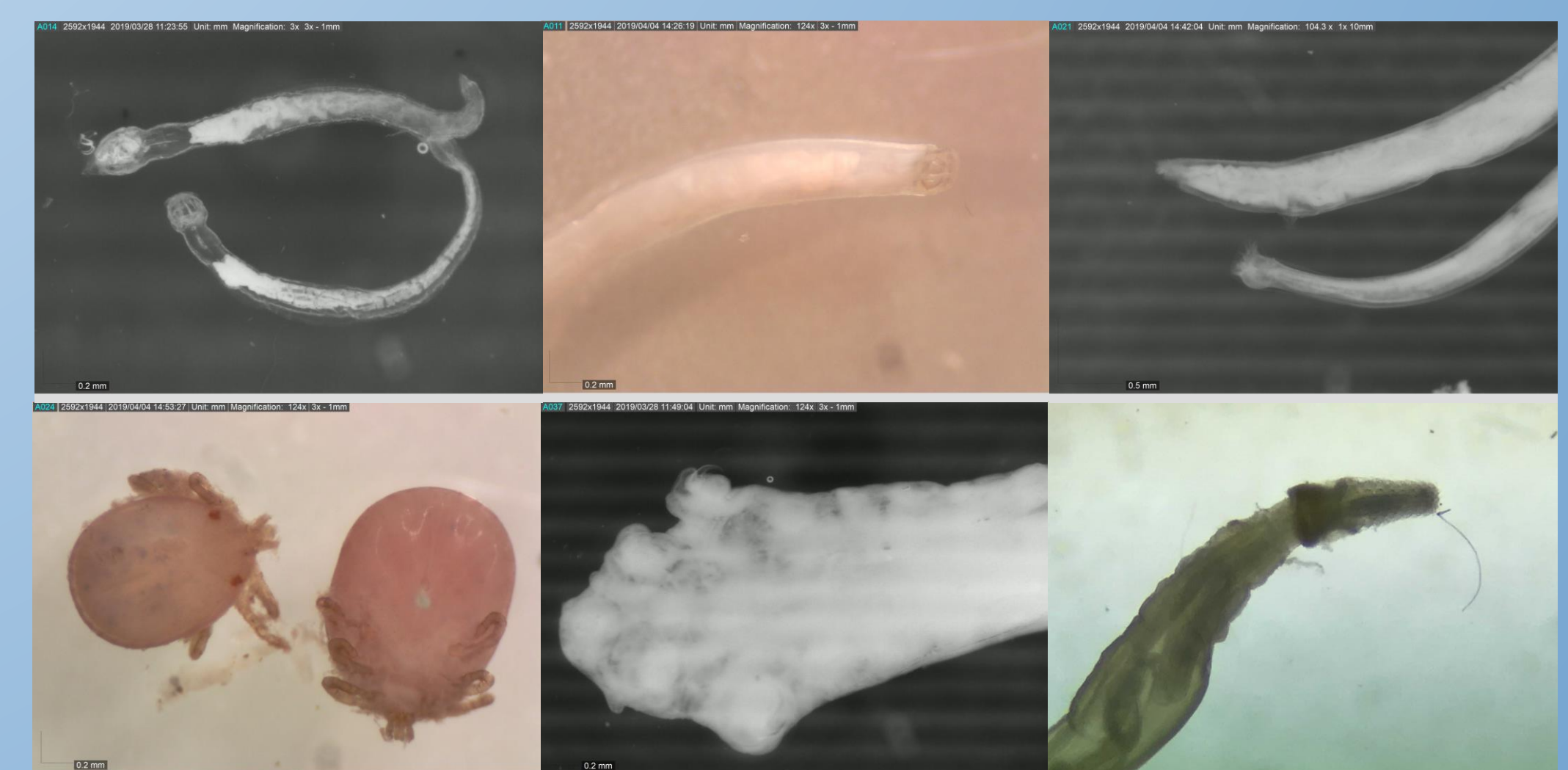
Clockwise from top left: 1. Copulating pair of *D. galeatus,* female above, male below. 2. Anterior of *D. galeatus*. 3. Posterior of *D. galeatus,* female above, male below (note the male's fan shaped copulatory bursa).
4. Acanthocephalan with proboscis everted. 5. Anterior of pentastome *Raillietiella sp.* 6. Larval ticks of *Amblyomma sp.* left: dorsal, right: ventral.