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Abstract - We collected a single specimen of *Rattus rattus* (Roof Rat) in Highland County, VA, a previously unknown locality for the species. We collected ecto- and endoparasites, including the louse *Polyplax spinulosa*, the tick *Dermacentor variabilis* (American Dog Tick), and the nematode *Aspicularis americana*. which we archived along with the host voucher specimen. Sequencing of the mitochondrial cytochrome b gene confirmed the rodent species identification based on comparisons to *R. rattus* sequences available in the GenBank database. Herein we review specimen records of Roof Rats and *Rattus norvegicus* (Brown Rat). Both species have a deep history in Virginia, but Roof Rats appear to have declined in coastal cities, while Brown Rats have become more common in those areas. Roof Rats apparently persist in rural relictual populations such as that identified in Highland County.

There are 2 species of non-native rats of the genus *Rattus* in Virginia, *Rattus norvegicus* (Berkenhout) (Brown Rat) and *Rattus rattus* (L.) (Roof Rat or Black Rat), though the former is more common and more widely distributed in Virginia than the latter (Linzey 1998). Members of the genus *Rattus* evolved in the Old World, in Asia (Musser and Carleton 2005). The Roof Rat expanded its range into Europe and they, and their flea ectoparasites, have been linked to the bacterial plague epidemics beginning in the 1200s AD. By the 15th century, the Roof Rat was established in the New World. The earliest record of the Roof Rat in North America is from Haiti in 1492 (Armitage 1993). The Roof Rat is said to have come to Virginia "with the first English colonizers and spread to all parts of the state" (Handley and Gordon 1979:516). When the Brown Rat was introduced in Virginia in about 1775, Roof Rat populations declined, and only remnant populations are found today (Handley and Gordon 1979). In a rat-flea survey of the port of Norfolk, VA, 1561 rats were captured, but only 4 were Roof Rats (Hassaltine 1929). The only published record of Roof Rat distribution that cites specimen localities is that of Handley and Patton (1947), who identify specimens examined from Brunswick, Dinwiddie, Nansemond (now City of Chesapeake) and Page counties. We herein report a collection of a Roof Rat from Highland County, VA, in 2019 and its definitive identification using molecular genetic tools. We report the parasites found and give a list of known, specimen-verified occurrences in the Commonwealth of Virginia.

We trapped small mammals on the property of a landowner at an elevation of ~930 m along Back Creek and County Road 600 (38.4438°N, 79.6574°W) for 3 nights (13–15 August 2019) using Sherman live traps (323 trap nights; 7.6 cm x 8.9 cm x 22.9 cm; H.B. Sherman Traps, Tallahassee, FL), Museum Special snap traps (406 trap nights; Woodstream Corp., Lititz, PA), pitfalls (28 trap nights), and rat traps (55 trap nights; Victor Rat Trap, 8.6 cm x 17.7 cm wood base; Woodstream Corp.). Trapping protocols followed standard methods for humane collection of small mammals (Sikes et al. 2016) and were authorized under Northern Michigan University IACUC protocol # 333. The single adult male Roof Rat captured was caught in a rat trap baited with peanut butter and rolled oats. The capture site was near a raised outbuilding with a small, seep-fed stream running beneath it. We placed the rat trap overnight on a runway in a thickly vegetated depression near a ground-

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We brushed the rat over a white pan to collect larger ectoparasites. No mites or fleas were observed. We preserved ectoparasites in 95% ethanol. We recorded body measurements and reproductive data for the rat and preserved its skin, skull, and post-cranial skeleton as museum study specimens. We collected tissue samples (heart, kidney, lung, spleen, liver) that we then flash-froze in liquid nitrogen and removed the gastro-intestinal tract, which we examined for endoparasites (Galbreath et al. 2019). We detected a single nematode and preserved it in 80% EtOH.

We processed the ticks and lice we collected from the rat as follows: partially cleared in 10% KOH, dehydrated in an ethanol series, further cleared in methyl salicylate, and mounted on slides in Canada balsam. Host and ectoparasite voucher specimens are deposited in the collections at Northern Michigan University with the following accession numbers: host – NMU:Mamm:3193; nematode – NMU:Para:31; ticks – NMU:Para:58 to 60; lice – NMU:Para:61 to 70. Additional representatives of the lice are deposited in the Virginia Museum of Natural History collection in Martinsville, VA, with the temporary searchable number RIM 22-2021.

Because it is sometimes difficult to distinguish between *R. rattus* and *R. norvegicus*, we used molecular genetic methods to make a definitive identification. We extracted DNA from liver tissue subsamples using a Qiagen™ DNeasy Tissue Kit® (Hilden, Germany) according to the manufacturer's protocol. We performed PCR amplification of a portion of the cytochrome b gene using primers LGL 765 and LGL 766 (Bickham et al. 2004). Reaction conditions included a 3-minute initial denaturation (94 °C); 35 cycles of 15 seconds denaturation (94 °C), 30 seconds annealing (50 °C), and 30 seconds extension (72 °C); and a single final 10-minute extension (72 °C). Products were sequenced in both directions, and forward and reverse sequences were assembled and checked by eye using Geneious® v6 (Dotmatics, Boston, MA). We compared the resulting consensus sequence to *R. rattus* and *R. norvegicus* cytochrome b sequences in the GenBank database using BLAST.

To characterize the distribution of *Rattus* in Virginia across geographic space and over time, we surveyed museum collections for *R. rattus* and *R. norvegicus* using the VertNet data portal (vertnet.org) and by querying databases of the National Museum of Natural History (Washington, DC) and the Virginia Museum of Natural History (Martinsville, VA). We also queried the wildlife database of the Virginia Department of Wildlife Resources, and iNaturalist. We assume that all museum specimens and unvouchered records were correctly identified, but note that only vouchered records can be confirmed.

The standard measurements taken from the rat are as follows: 361 mm total length, 190 mm tail length, 39 mm hind-foot length, 25 mm ear length, and 142.9 grams weight. A single female nematode of the species *Aspicularis americana* Erickson was collected from the large intestine, and ectoparasites present included 3 ticks, all larvae of *Dermacentor variabilis* (Say) (American Dog Tick), and 12 adult and 4 nymphs of the sucking louse species *Polyplax spinulosa* (Burmeister).

The partial cytochrome b sequence (1067 base pairs; GenBank accession # OK167012) recovered from the specimen was identical to sequences reported for *R. rattus* sampled from the Lesser Antilles, Mexico, Egypt, and elsewhere in the United States (geographically widespread and prevalent haplotype Rr1 identified in Lack et al. [2013]).

Our survey of natural history collections recovered 41 Virginia records of specimens identified as Roof Rat (Fig. 1, Table 1). Specimens were collected from 14 counties or independent cities, with the earliest dating to 1855. In comparison, our survey revealed 75 vouchered specimens of Brown Rat, representing 13 counties or independent cities. The Virginia Department of Wildlife Resources database includes 3 dated county records for Roof Rat and 12 records for Brown Rat. In addition, the database reports "resident

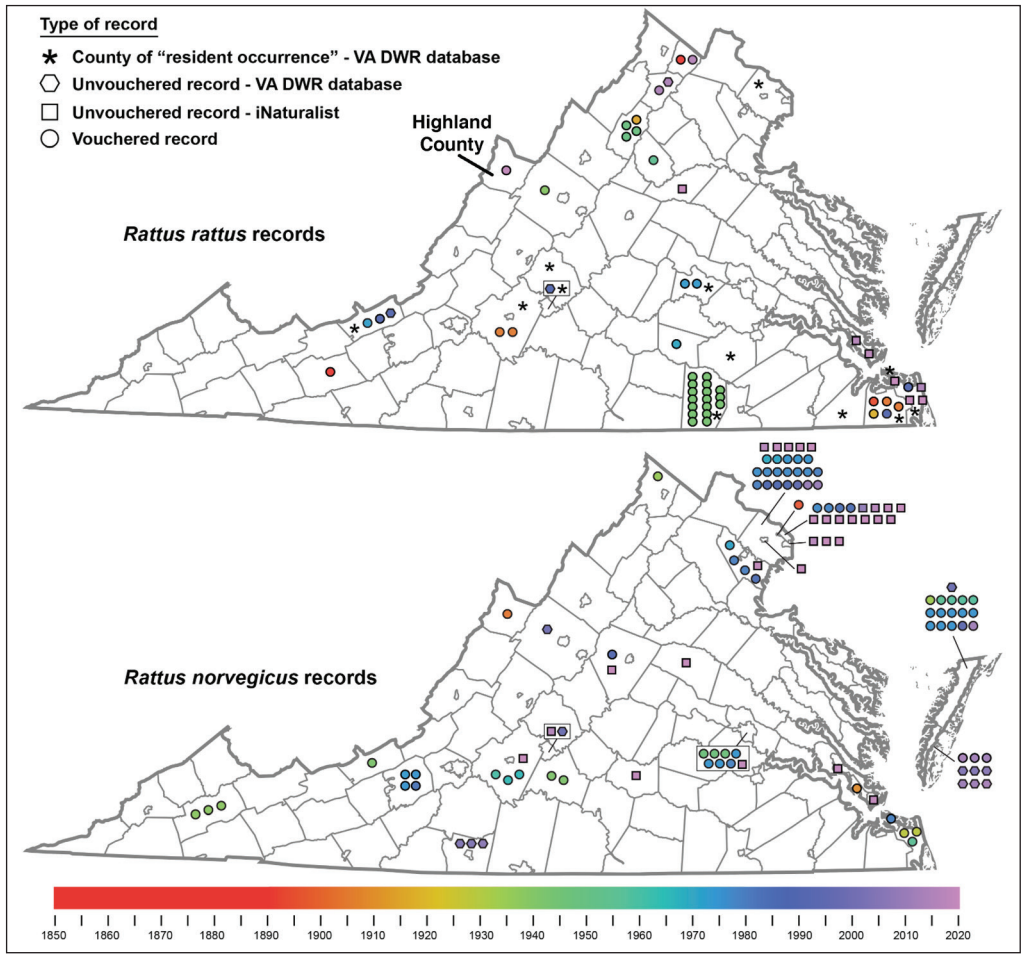

Figure 1. *Rattus rattus* (Roof Rat) and *Rattus norvegicus* (Brown Rat) records for Virginia counties. Circles denote individual specimen records from specific counties for which vouchers are reported in museum collections. Squares denote individual unvouchered records collected via iNaturalist ("research grade" records). Hexagons denote individual unvouchered records found in the Virginia Department of Wildlife Resources wildlife database. Fill color of circles and squares indicates approximate year of sampling according to the color spectrum scale. Asterisks denote counties for which Roof Rat is listed as being of "resident occurrence". Highland County, where the Roof Rat specimen reported in this study was found, is noted on the upper map. Placement of symbols within county boundaries solely denotes county occurrence. It does not represent the specific locality for the record.

Table 1. List of specimens of *Rattus rattus* (Roof Rat) recorded from Virginia. Museum archives include the Northern Museum of Zoology (NMU), Virginia Museum of Natural History (VMNH), National Museum of Natural History (USNM), Museum of Southwestern Biology (MSB), Michigan State University (MSU), Cornell University Museum of Vertebrates (CUMV), American Museum of Natural History (AMNH). * indicates the specimen reported in this study. An "-" indicates no data. [Table continued on following page.]

Museum no.	Collection date	Sex	County/city	Locality
NMU:Mamm:3193*	15 Aug 2019	M	Highland County	Back Creek
VMNH 39624	13 Nov 1991	M	Chesapeake	-

Table 1, continued.

Museum no.	Collection date	Sex	County/city	Locality
VMNH 39625	14 Nov 1991	F	Giles County	Newport
VMNH 39626	01 Dec 1991	M	Virginia Beach	3 km S Pungo
VMNH 130417	26 Sep 1970	M	Nottoway County	Blackstone
USNM 010708	Nov 1855	-	Clarke County	-
USNM 075225	09 Oct 1895	F	Chesapeake	Dismal Swamp
USNM 140774	17 May 1905	F	Chesapeake	Dismal Swamp
USNM 140775	23 May 1905	F	Chesapeake	Dismal Swamp
USNM 143964	29 Jul 1906	M	Bedford County	Peaks of Otter
USNM 143965	30 Jul 1906	F	Bedford County	Peaks of Otter
USNM 217258	12 Nov 1916	F	Page County	20 km E Luray
USNM 251524	09 Aug 1939	M	Augusta County	Stuarts Draft
USNM 273919	20 Jul 1943	F	Brunswick County	Triplet
USNM 273920	27 Jul 1943	F	Brunswick County	Triplet
USNM 273921	28 Jul 1943	F	Brunswick County	Triplet
USNM 273922	28 Jul 1943	M	Brunswick County	Triplet
USNM 273923	05 Aug 1943	F	Brunswick County	Triplet
USNM 273924	05 Aug 1943	M	Brunswick County	Triplet
USNM 273925	10 Aug 1943	F	Brunswick County	Triplet
USNM 274583	21 Mar 1943	M	Brunswick County	Triplet
USNM 290479	07 Sep 1949	F	Page County	Shenandoah NP
USNM 290508	14 Dec 1949	M	Page County	Shenandoah NP
USNM 290515	04 Mar 1950	F	Page County	Shenandoah NP
USNM 293733	18 Aug 1943	M	Brunswick County	Triplet
USNM 293734	20 Aug 1943	F	Buckingham County[†]	Triplet
USNM 526751	05 Aug 1943	F	Brunswick County	Triplet
USNM 526752	20 Aug 1943	F	Brunswick County	Triplet
USNM 526753	20 Aug 1943	F	Brunswick County	Triplet
USNM 526754	03 Dec 1943	F	Brunswick County	Triplet
USNM 526755	18 Dec 1943	F	Brunswick County	Triplet
USNM 534056	24 Feb 1973	F	Powhatan County	Powhatan
USNM 534057	24 Feb 1973	F	Powhatan County	Powhatan
USNM 587987	18 Dec 1943	F	Brunswick County	Triplet
USNM 589429	13 Aug 1972	M	Giles County	Mtn. Lake
AMNH MS-3186	5 June 1889	M	Wythe County	Wythville
MSB:Mamm:311160[#]	8 Sep 2015	M	Warren County	Smithsonian Conservation Biology Institute
MSB:Mamm:311308[#]	21 July 2017	F	Clarke County	Blandy Experimental Farm
MSU MR.2552	6 July 1955	M	Madison County	Shenandoah National Park
UMMZ 53150	16 July 1918	-	Chesapeake	Dismal Swamp, Lake Drummond
CUMV 3779	24 Dec 1943	F	Brunswick County	Triplet, Seward Forest

[†]This county record from the US National Museum of Natural History seems likely to be an error, as other records from the same collector, date, and specific locality identify Brunswick County, not Buckingham County, as the area of collection.
[#]These MSB specimens are a fecal sample and an ear clip rather than a conventional skin and skull.

occurrence" of Roof Rats in 12 counties (Susan Watson, Virginia Department of Wildlife Resources, Henrico, VA, pers. comm.), but these reports are not supported by specific observations and cannot be confirmed. Additional unvouchered "research grade" records of Roof Rat (n = 7) and Brown Rat (n = 29) are reported from Virginia on iNaturalist.org with accompanying photographs.

Highland County is a new county distribution record for the Roof Rat, and to our knowledge, this locality is the highest-elevation capture site in the state to date. Whitaker and Hamilton (1998) show a map of Roof Rat distribution that does not include rats in western Virginia, though our review of archived *Rattus* specimens reveals individuals collected from several western counties both historically and more recently. Since the mid-19th century, the Roof Rat appears to have been widely distributed across rural and urban, coastal and upland portions of Virginia (Table 1, Fig. 1). By comparison, the Brown Rat has a geographic distribution that has consistently been concentrated in coastal zones and areas of high human population density.

Despite the historically widespread distribution of Roof Rats, collection data suggest that they are currently less common than they once were. Of the 41 vouchered records (including the present report) we assembled for Virginia, 29 were collected prior to 1950, and 12 were collected in the time since (Table 1). The inverse pattern is evident for the Brown Rat, for which only 15 of 75 dated collection records fall prior to 1950. Sampling patterns could reflect differences in sampling effort across space and time, but it seems likely that effort would be relatively similar between collections of Roof Rats and Brown Rats.

The negative relationship between the 2 species is consistent with the idea that while both have a deep history in the region, Brown Rats have replaced Roof Rats as the dominant *Rattus* species. The Brown Rat is larger and more aggressive, and therefore may be more successful than the Roof Rat at competing for resources (Barnett and Spencer 1951, Yom-Tov et al. 1999). The remnant population of Roof Rat that we identified in Highland County has probably been able to persist due to the rural nature of the county and its distance from the coastal cities where the Brown Rat dominates.

The parasites collected from the Highland County specimen were not unexpected. The American Dog Tick is geographically widespread and routinely feeds on diverse vertebrate species in the Appalachian region. It is the principal vector of the bacterium that causes Rocky Mountain Spotted Fever in Eastern United States (Nicholson et al. 2009). The louse *Polyplax spinulosa* is a characteristic parasite of *Rattus* and has been reported previously from Virginia (Hasseltine, 1929, Kim et al. 1986). Likewise, *A. americana* is a widely distributed oxyurid parasite of rodents in North America and was reported from nearby Giles County, VA, from *Peromyscus leucopus* (Rafinesque) (White-footed Mouse) and *P. maniculatus* (J.A. Wagner) (Eastern Deer Mouse) (Walsh et al. 2013). Both those mice species were collected by us at the trapping location, though *A. americana* was not collected from either species.

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