Morphological Identification of Ticks and Molecular Detection of Tick-Borne Pathogens from Bare-Nosed Wombats (Vombatus Ursinus)

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Abstract

**Background** Ticks are obligate hematophagous ectoparasites of vertebrate hosts and transmit the widest range of pathogenic organisms of any arthropod vector. Seven tick species are known to feed on bare-nosed wombats (*Vombatus ursinus*), in addition to the highly prevalent *Sarcoptes scabiei* mite which causes fatal sarcoptic mange in most bare-nosed wombat populations. Little is known about the pathogens carried by most wombat ticks or how they impact wombats affected by sarcoptic mange.

**Methods** Wombat ticks were sourced from wildlife hospitals and sanctuaries across Australia and identified to species level using taxonomic keys. Genomic DNA (gDNA) was extracted from a subsample, and following the amplification of the bacterial 16S rRNA gene V3-V4 hypervariable region, next generation sequencing (NGS) on the Illumina MiSeq platform was used to assess the microbial composition.

**Results** A total of 447 tick specimens were collected from 47 bare-nosed wombats between January 2019 and January 2020. Five species of ticks were identified comprising *Bothriocroton auruginans* (n = 420), *Haemaphysalis bancrofti* (n = 10), *H. longicornis* (n = 1), *Ixodes tasmani* (n = 12), and *I. holocyclus* (n = 4). Tick infestations ranged from 1 to 73 ticks per wombat. *B. auruginans* was the most prevalent tick species comprising 94% of the total number of samples and was present on 97.9% (46/47) of wombat hosts. NGS results revealed the 16S rRNA gene diversity profile was predominantly *Proteobacteria* (55.1%) followed by *Firmicutes* (21.9%) and *Actinobacteria* (18.4%). A species of *Coxiella* sharing closest sequence identity to *C. burnetii* (99.07%), was detected in 72% of *B. auruginans* and a *Rickettsiella* endosymbiont dominated the bacterial profile for *I. tasmani*.

**Conclusions** A new host record for *H. longicornis* is bare-nosed wombats. One adult male and two engorged adult female specimens were found on an adult male wombat from Coolagolite in NSW and more specimens should be collected to confirm this host record. The most prevalent tick found on bare-nosed wombats is *B. auruginans* confirming previous records. Analysis of alpha-diversity showed high variability across both sample locations and instars, similar to previous studies. The detection of various *Proteobacteria* in this study highlights the high bacterial diversity in native Australian ticks.
Background
Ticks (Acari: Ixodidae) are obligate ectoparasitic arachnids that are classified into three families; Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae. Each of the three families have evolved unique biological, physiological and ecological disparities which have resulted in different abilities and capacities to transmit pathogens [1]. However, ticks can transmit the widest range of pathogens of any arthropod vector and are the primary cause of vector-borne diseases in livestock and domestic animals [2]. Ixodids transmit the widest number of pathogens worldwide and are responsible for the majority of tick-borne infections [3].

In addition to pathogens, the tick microbiome comprises a community of commensal and symbiotic obligate endosymbionts which make up the majority of the tick microbiome and reside both inside and outside the body of ticks [4]. The effect of these organisms has been somewhat neglected in studies, but may present various detrimental, neutral, or beneficial effects to their tick hosts, and also contribute to driving the transmission of tick-borne pathogens [5]. Non-pathogenic microorganisms are typically transovarially transmitted [6] and may impact tick growth, reproduction, fitness, nutritive adaptation and defence against environmental stresses [7, 8]. The functional role and relationship between tick microorganisms may provide further insight into the pathogenicity and evolution of tick pathogens. For example, it has become increasingly clear since the advancement of molecular barcoding techniques that many species of Rickettsia, Francisella, and Coxiella, which are generally considered pathogens of medical and veterinary importance, have evolved as non-pathogenic endosymbionts of ticks [9].

While tick-borne bacteria have been relatively well studied in the Northern hemisphere, very little is known about the presence or diversity of bacteria in Australian ticks [10]. The microbiome and pathogenicity of Australian ticks are unique when compared to other species, and so is the response to ticks and tick-borne pathogens (TBPs) from native vertebrate hosts [11]. Recently unique Australian species of Anaplasma, Ehrlichia and Neoehrlichia [12, 13] and the first native Borrelia species were characterised in native ticks [14]. Other novel microbial species have also been reported in Australian ticks [12, 15, 16], however the focus has largely been on ticks of human, domestic
animal and livestock importance, and few have surveyed ticks associated with wildlife [17, 18].

Bare-nosed wombat (Vombatus ursinus) populations are significantly impacted by the ectoparasite Sarcoptes scabiei which causes sarcoptic mange [19], however little is known about other wombat ectoparasites or their associated pathogens. Australian fauna have coevolved with native tick species and healthy wombats regularly carry large burdens of ticks which would otherwise affect humans and domestic animals [20]. However, wombats affected by sarcoptic mange, orphaned or injured wombats released from captivity and wombats raised in a comparatively parasite-free captive environment before release are likely at an increased risk of contracting tick-borne diseases. Managing wild species in captivity may induce stress, impair immunity and expose hosts to novel parasites to which the immune system is naïve [21]. Population density is also often atypical in captivity which may result in higher than usual parasite burdens. Additionally, the use of anti-parasitic medications on captive animals affects both host-parasite relationships and individuals are at an increased disease risk once released having not developed an acquired immunity [22].

Seven species of tick have previously been recorded feeding on bare-nosed wombats including the wombat tick B. auriginans [23], the wallaby tick Haemaphysalis bancrofti [24], Australian paralysis tick I. holocyclus [25], Tasmanian paralysis tick I. cornuatus [26], I. phascolomyis [27], common marsupial tick I. tasmani and I. victoriensis [28]. The relationship between S. scabiei and other known wombat ectoparasites, their pathogens, ability to co-infect hosts, and their overall impact on wombat hosts has not yet been investigated. There is also very little known about the lifecycles of wombat ectoparasites and their level of host-specificity. Coxiella burnetti has been found in B. auriginans collected from bare-nosed wombats as well as a Rickettsia species closely related to R. massiliae which causes human disease [29]. These are the only pathogens to be detected in ticks taken from wombat hosts and utilised specific targeted methods.

The development of Next Generation Sequencing (NGS) technologies has enabled the microbial communities of ticks to be explored in a fast and cost-efficient manner [15], however very little is known about the microbiome of native Australian ticks [10] and no studies have focussed on wombat ticks or TBPs. Bare-nosed wombats are already significantly affected by a known ectoparasite, so it
would be beneficial to understand the other parasitic and pathogenic threats that wombats may need to overcome simultaneous to or following the treatment of sarcoptic mange. It is also important to identify potential zoonotic threats to wombat handlers and domestic animals that may come into contact with wombats or their burrows. This study aimed to identify the species of ticks associated with bare-nosed wombats and to use next generation sequencing and metabarcoding to investigate the bacterial diversity associated with these ticks.

Methods
Tick Collection and Identification
All ticks were collected directly from wombat hosts between January 2019 and January 2020 throughout eastern Australia (Fig. 1) including from live animals being rehabilitated for release, as well as opportunistic collections from road killed wombats, and placed into 70% ethanol. Location where ticks were collected, date, and habitat type for the wombat hosts were recorded. Temperature and rainfall were obtained from the Bureau of Meteorology for the date and GPS coordinates where ticks were submitted. Ticks were identified morphologically to species and life stage using existing taxonomic keys [30, 31] and a Nikon SMZ445 stereomicroscope. Species, sex and instar were recorded for each specimen except for (i) two nymphaal specimens (ii) where specimens were damaged during removal. There is a lack of detailed morphological keys for some Australian native larval and nymphaal ticks [32], and so some of these specimens were only able to be identified to genus level. Damaged ticks were identified to instar and genus. Photographs of tick specimens were taken using an Olympus DP72 stereomicroscope with an external Euromex fibre optic light source EK-1 and cellSens Standard 1.5 software. Ticks were stored in sterile tubes containing 70% ethanol between identification and molecular analysis.

Sample Mapping
The locations of tick sample collection were geo-referenced using the open source software QGIS version 3.12.1 [33] with the latest Australian coordinate system Geocentric Datum of Australia 2020 (GDA2020) incorporated through the ICSM NTv2 Transformer plugin [34]. Layers were styled with a categorised renderer and layer symbology was characterised according to tick species. To visualise overlapping points, a point displacement renderer was used around a centre symbol on rendering
circles for tick distribution, and a point cluster renderer was used to visualise overlying pathogen
distribution [35].

**Molecular Methods**

Samples were sent to the Australian Genome Research Facility (AGRF) in Urrbrae, Adelaide Australia. DNA was extracted using the DNeasy PowerSoil Pro DNA Extraction Kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s instructions. A total of 79 whole tick specimens were then sequenced on an Illumina MiSeq platform [36]. Based on previous studies [37], the presence of bacteria in tick samples was detected using the primer pair 341F (5’-CCTAYGGGRBGCASCAG-3’) and 806R (5’-GGACTACNNGGGTATCTAAT-3’) to amplify the V3-V4 region of the 16S rRNA gene, generating a 300 bp fragment.

The bioinformatics analysis involved demultiplexing, quality control, OTU (operational taxonomic unit) clustering, and taxonomic classification. Image analysis was performed in real time using MiSeq Control Software (MCS) version 2.6.2.1 and Real Time Analysis (RTA) version 1.18.54 (Illumina, Inc., San Diego, CA, USA), running on the instrument computer. Then the Illumina bcl2fastq 2.20.0.422 pipeline was used to generate the sequence data. Paired-ends reads were assembled by aligning the forward and reverse reads using PEAR version 0.9.5 [38], and primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME) version 1.8.4 [39], USEARCH version 8.0.1623 [40], and UPARSE [41] software. Using USEARCH tools sequences were then quality filtered, full length duplicates were removed and sequences were sorted by abundance.Singletons or unique reads were discarded, sequences were clustered and chimeric sequences were filtered using the “rdp_gold” database as a reference. To obtain the number of reads in each OTU, reads were mapped back to OTUs with a minimum identity of 97%, taxonomy was assigned using QIIME and taxonomies were confirmed using the NCBI MegaBLAST. Non-bacterial (eukaryote, unidentified) OTUs were removed and samples with < 100 assigned OTUs were not considered a positive identification.

**Data Management And Statistical Analyses**

Tick collection and identification details were recorded in Microsoft Excel version 2002. Quality
assurance was ensured prior to statistical analyses by reviewing all physical data and data entries. Statistical analyses and data visualisation were performed using R Commander version 2.6-2 [42], RStudio version 1.2.5033 [43] with the addition of packages Vegan version 2.5-6 [44] and phyloseq [45], and Geneious Prime 2020.1.1 (https://www.geneious.com). Alpha diversity was assessed by richness (Inverse Simpson and ACE index) and diversity (Shannon and Simpson index).

Results

Tick Species

A total of 447 tick specimens were collected from 47 bare-nosed wombats in NSW and Tasmania between January 2019 and January 2020 (Table 1). Five species of ticks comprising three genera were morphologically identified (Table. 2); Bothriocroton auruginans (n = 420; Fig. 2d), Haemaphysalis bancrofti (n = 10; Fig. 2f), H. longicornis (n = 3; Fig. 2a,e), Ixodes holocyclus (n = 4; Fig. 2b), and I. tasmani (n = 12; Fig. 2c). Approximate tick infestation ranged from one to 73 ticks per wombat with a total mean infestation of 9.8 ± 3.9 ticks per host. Joey wombats exhibited higher mean infestation rates (25.3 ± 20.4), followed by adult female wombats (7.1 ± 4.5) and adult male wombats (6.6 ± 3.6).

B. auruginans was the most prevalent tick species comprising 94% of the total number of samples and was present on 97.9% (46/47) of wombat hosts. Approximate tick diversity ranged from one to four tick species per wombat. The highest tick diversity was from an adult male wombat in Coolagolite in NSW, an adult male wombat from Dalgety NSW and a wombat of unknown age and sex at Quaama NSW with three tick species identified each. Females were the most abundant instar identified (n = 164), followed by males (n = 129), nymphs (n = 115), and larvae (n = 39). The majority of females (89%), nymphs (96.5%) and larvae (100%) were engorged or semi-engorged from a bloodmeal (Fig. 3). Larvae were identified to genus level but informally assumed to be B. auruginans as all larval specimens shared their host with other B. auruginans instars and no other tick species. In addition to ticks, there were also incidental collections of nine unidentified fleas and six lice (all identified as Boopia tarsata). The majority of ticks were collected in winter (58%), followed by spring (25%), autumn (6%) and summer (6%) with the remaining ticks being older specimens only having the year of collection recorded.
| Collection location                        | GPS Coordinates    | No. of hosts | No. of ticks |
|-------------------------------------------|--------------------|--------------|--------------|
| Cedar Creek, NSW                          | -32.825089, 151.150619 | 7 (6♂, 1♀)  | 32 (17♂, 15♀) |
| Quaama, NSW                               | -36.43, 149.18     | 2 (1♂, 1♀)  | 18 (2N, 16♀)  |
| Bells Line of Rd, NSW                     | -33.51, 150.48     | 1♀           | 5 (2♂, 3♀)     |
| Murrabrine Forest Rd, Yowrie, NSW         | -36.345160, 149.759200 | 2♂           | 9 (1N, 1♂, 7♀) |
| Bridge over Colombo Creek, Bemboka, NSW   | -36.63384, 149.57734 | 1♀           | 2♀            |
| Rilys Rd, Coolagolite, NSW                | -36.382956, 150.014975 | 2 (1♂, 1♀)  | 4♀            |
| Wolgan Valley, NSW                        | -33.228605, 150.181693 | 1♀           | 6 (1♂, 5N)   |
| Wagga Wagga, NSW                          | -35.115186, 147.375704 | 1♂           | 1♂            |
| The Rock, NSW                             | -35.268098, 147.112130 | 1♀           | 1♂            |
| Werombi Rd, Orangeville, NSW              | -34.023298, 150.656308 | 1♀           | 6 (4N, 2♀)    |
| West Parade, Thirlemere NSW               | -34.221387, 150.557375 | 1♀           | 10 (4N, 3♂, 3♀) |
| West Parade, Couridjah, NSW               | -34.227402, 150.553900 | 1♀           | 9 (7N, 2♀)    |
| Picton, NSW                               | -34.169246, 150.609028 | 1♀           | 61 (28N, 7♂, 6♀) |
| Spring Creek Rd, Mount Hunter NSW         | -30.086604, 150.6295028 | 1♀           | 5 (4♂, 1♀)   |
| Eastview Dr, Orangeville NSW              | -30.0150208, 150.5866525 | 1♂ (joey)   | 16 (6♂, 10♀) |
| Silverdale Rd, The Oaks NSW               | -30.0689341, 150.5737696 | 1♀           | 10 (5♂, 5♀)  |
| Moulders Rd, Orangeville NSW              | -34.045689, 150.573187 | 1♀           | 11♀           |
| Courijah, NSW                             | -34.2319118, 150.5494526 | 1♀           | 9 (2♂, 7♀)   |
| Pheasants Nest Rd, Pheasant Nest NSW      | -34.2542546, 150.6299937 | 1♂ (pinky)  | 2N            |
| Mowbray Park Rd, Mowbray Park NSW         | -34.1609750, 150.5483730 | 1♂           | 4 (1♂, 3♀)   |
| Buxton Rd, Buxton NSW                     | -34.2512528, 150.5261583 | 1♀           | 26 (2N, 13♂, 3♀) |
| Kangaroo Valley, NSW                      | -34.742151, 150.552230 | 1♀ (joey)   | 73 (32N, 15♂, 26♀) |
| Bellmount Forest, NSW                     | -34.899662, 149.248122 | 2 (1♂, 1♀)  | 23 (6N, 16♂, 1♀) |
| Holbrook Road, Gelstone Park              | -34.226111, 147.337308 | 1♂           | 37♂           |
| Ryliss Rd, Coolagolite NSW                | -36.832979, 150.015045 | 2♂           | 13 (3N, 1♂, 9♀) |
| Captains Flat Rd, Primrose Valley NSW     | -35.454129, 149.4189036 | 1♂           | 3 (1♂, 2♀)   |
| Hard Rd, Burra NSW                        | -35.557901, 149.222036 | 1♀ (joey)   | 44 (38L, 5N, 1♂) |
| Ironmongie Rd, Dalgety NSW                | -36.364893, 148.918758 | 1♀           | 7♀            |
| Gidleigh Lane, Bungendore NSW             | -35.295460, 149.455922 | 1♀ (joey)   | 14N           |
| Unknown                                   | Unknown            | 1♀           | 10 (2N, 8♀)   |
| Cradle Mountain Rd, Cradle Mountain Tas   | -41.523131, 146.075733 | 1♀ (joey)   | 3 (1♂, 2♀)   |

Ngs Analysis And Bacterial Composition Of Wombat Ticks

A total of 5,890,950 bacterial sequences and 1,759 OTUs (average length 414.3 bases) were assigned, however only 745 OTUs had greater than 100 total sequences from all tick samples. Ticks had an average of 74,569 assigned sequences each (males 63,397 sequences, females 92,827 sequences, nymphs 56,470 sequences and larvae 57,701 sequences). Engorged females had an average of 99,550 assigned sequences in comparison to unfed females which had an average of 40,723 sequences. The closest match for bacterial isolates as determined through GenBank for taxa of interest are shown in Table 3. *Proteobacteria* comprised the majority of the bacterial phyla.
composition (55.1%) followed by *Firmicutes* (21.9%) and *Actinobacteria* (18.4%) as shown in Fig. 4. At the genus level *Coxiella* comprised 40.3% of the total composition followed by *Staphylococcus* (13%). *Coxiella* was the most dominant genus detected in larvae with a mean prevalence of 81.6%. Nymphs were less likely to be infected with one dominant phyla of bacteria than other instars and often exhibited equal frequencies of three phyla. Male and female adult ticks were predominantly associated with *Proteobacteria* (Table 4).

**Table 3**
Bacterial composition of ticks parasitising bare-nosed wombat (*Vombatus ursinus*) hosts between January 2019 and January 2020. Only taxa of interest are shown and number of positive samples was based on samples with >100 with assigned OTUs.

| Tick Species                  | Locality | Closest match in GenBank (% similarity) | No. positive | Length | Bit-score |
|------------------------------|----------|----------------------------------------|--------------|--------|-----------|
| *Bothriocroton auriginans*    | NSW      | *Coxiella burnetii* (99.07%) Staphylococcus sciuri (100%) Brevibacterium luteolum (99.76%) Corynebacterium amycolatum (100%) Dermacoccus nishinomiyaensis (97.80%) Macroccus brunensis (100%) Planomicrobium glaciei (100%) Lysinibacillus sp. (100%) Brachybacterium paraconglomeratum (100%) Escherichia coli (100%) Acinetobacter sp. (100%) Pseudomonas sp. (100%) Candidatus Borrelia ivorensis (99.53) uncultured Anaplasma sp. (98.51) | 56/78 16/78 8/78 9/78 12/78 20/78 4/78 8/78 13/78 16/78 19/78 7/78 1/78 4/78 | 429 bp 429 bp 409 bp 410 bp 409 bp 429 bp 428 bp 426 bp 409 bp 429 bp 426 bp 409 bp 429 bp | 771 793 750 758 706 793 791 787 756 793 793 793 793 713 |
| *Ixodes tasmani*              | NSW      | Rickettsiella endosymbiont (100%)      | 1/1          | 429 bp | 793       |

**Table 4**
List of wombat tick samples sequenced on the Illumina MiSeq platform and absolute OTU counts for each sample

| Sample name | Species                  | Sex     | Instar | Total abundance *Coxiella burnetii* | Total abundance *Staphylococcus agnetis* | Total abundance *Rickettsiella* |
|-------------|--------------------------|---------|--------|------------------------------------|----------------------------------------|-------------------------------|
| 39a         | *Bothriocroton auriginans* | Female  | Adult  | 96850                              | 240                                    | 0                             |
| 39b         | *Bothriocroton auriginans* | Female  | Adult  | 38832                              | 617                                    | 0                             |
| 40a         | *Bothriocroton auriginans* | Male    | Adult  | 424                                 | 86                                     | 0                             |
| 40b         | *Bothriocroton auriginans* | Male    | Adult  | 499                                 | 37                                     | 0                             |
| 40c         | *Bothriocroton auriginans* | Male    | Adult  | 0                                   | 52                                     | 0                             |
|   | Bothricroton auruginans |   |   |   |   |
|---|-------------------------|---|---|---|---|
| 40d | Bothricroton auruginans | Male | Adult | 245 | 33 | 0 |
| 41a | Bothricroton auruginans | Female | Adult | 118871 | 23 | 0 |
| 41b | Bothricroton auruginans | Female | Adult | 135810 | 1 | 0 |
| 41c | Bothricroton auruginans | Female | Adult | 151567 | 0 | 0 |
| 41d | Bothricroton auruginans | Female | Adult | 35440 | 0 | 0 |
| 42a | Bothricroton auruginans | Female | Adult | 121194 | 0 | 0 |
| 42b | Bothricroton auruginans | Male | Adult | 11701 | 0 | 0 |
| 43a | Bothricroton auruginans | Female | Adult | 42 | 49 | 0 |
| 44a | Bothricroton auruginans | Female | Adult | 22187 | 3 | 0 |
| 44b | Bothricroton auruginans | Female | Adult | 150092 | 74 | 0 |
| 46a | Bothricroton auruginans | Female | Adult | 8363 | 0 | 0 |
| 47b | Bothricroton auruginans | Female | Adult | 0 | 0 | 0 |
| 48a | Bothricroton auruginans | Female | Nymph | 7320 | 0 | 0 |
| 48b | Bothricroton auruginans | Female | Adult | 694 | 0 | 0 |
| 49a | Bothricroton auruginans | Female | Nymph | 56494 | 0 | 0 |
| 49b | Bothricroton auruginans | Male | Adult | 82607 | 155 | 0 |
| 49c | Bothricroton auruginans | Male | Adult | 28893 | 51956 | 0 |
| 49d | Bothricroton auruginans | Female | Nymph | 0 | 0 | 0 |
| 49f | Bothricroton auruginans | Male | Adult | 0 | 1158 | 0 |
| 50a | Bothricroton auruginans | Female | Adult | 87091 | 160 | 0 |
| 51a | Bothricroton auruginans | Female | Adult | 6965 | 328 | 54 |
| 51b | Bothricroton auruginans | Female | Adult | 172 | 100 | 0 |
| 51c | Bothricroton auruginans | Male | Adult | 0 | 194 | 0 |
| 51d | Bothricroton auruginans | Male | Adult | 0 | 293 | 69 |
| 52a | Bothricroton auruginans | Female | Adult | 47707 | 1719 | 0 |
| 52b | Bothricroton auruginans | Female | Adult | 36035 | 1682 | 0 |
| 52c | Bothricroton auruginans | Male | Adult | 107270 | 195 | 0 |
| 52d | Bothricroton auruginans | Male | Adult | 47213 | 432 | 0 |
| 53a | Bothricroton auruginans | Female | Adult | 12669 | 520 | 0 |
| 53b | Bothricroton auruginans | Female | Adult | 3819 | 1029 | 0 |
| 53c | Bothricroton auruginans | Female | Adult | 2535 | 1130 | 0 |
| 53d | Bothricroton auruginans | Female | Adult | 2314 | 3475 | 0 |
| 54a | Bothricroton auruginans | Female | Adult | 47 | 2612 | 0 |
| 54c | Bothricroton auruginans | Female | Adult | 6442 | 137 | 0 |
| 55a | Bothricroton auruginans | Female | Adult | 76233 | 6706 | 0 |
| 55b | Bothricroton auruginans | Male | Adult | 4771 | 119 | 0 |
| 56a | Bothricroton auruginans | Male | Adult | 72344 | 0 | 0 |
|      | Bothricroton auruginans |      |      |      |      |
|------|-------------------------|------|------|------|------|
| 56b  | Male Adult              | 0    | 0    | 0    | 0    |
| 56d  | Male Adult              | 20493| 0    | 0    | 0    |
| 56e  | Male Adult              | 2468 | 0    | 0    | 0    |
| 56f  | Male Adult              | 0    | 0    | 0    | 0    |
| 57a  | Bothricroton auruginans | Nymph | 0  | 39411| 0    |
| 57b  | Bothricroton auruginans | Nymph | 2  | 106880| 0    |
| 57c  | Bothricroton auruginans | Nymph | 0  | 5956 | 0    |
| 57d  | Bothricroton auruginans | Nymph | 0  | 5700 | 0    |
| 57e  | Bothricroton auruginans | Nymph | 0  | 12343| 0    |
| 57f  | Bothricroton auruginans | Nymph | 0  | 9720 | 0    |
| 58a  | Bothricroton auruginans | Male Adult | 107406| 44 | 0    |
| 58b  | Bothricroton auruginans | Male Adult | 1859| 348 | 0    |
| 58c  | Bothricroton auruginans | Male Adult | 9  | 0    | 0    |
| 58d  | Bothricroton auruginans | Nymph | 449 | 0    | 0    |
| 58e  | Bothricroton auruginans | Nymph | 9918| 0    | 0    |
| 58f  | Bothricroton auruginans | Nymph | 8  | 0    | 0    |
| 59b  | Bothricroton auruginans | Nymph | 93668| 0  | 0    |
| 60a  | Bothricroton auruginans | Male Adult | 8398| 1543| 0    |
| 60c  | Bothricroton auruginans | Male Adult | 128271| 30 | 0    |
| 60d  | Bothricroton auruginans | Male Adult | 92622| 99  | 0    |
| 60e  | Bothricroton auruginans | Male Adult | 99967| 3503| 0    |
| 60f  | Bothricroton auruginans | Male Adult | 129252| 5470| 0    |
| 60g  | Bothricroton auruginans | Male Adult | 55074| 903 | 0    |
| 61c  | Bothricroton auruginans | Nymph | 1709| 1    | 0    |
| 62a  | Bothricroton auruginans | Female Adult | 131882| 1  | 0    |
| 63a  | Bothricroton auruginans | Male Adult | 101 | 3536| 0    |
| 64b  | Bothricroton auruginans | Larvae | 37394| 1684| 0    |
| 64c  | Bothricroton auruginans | Larvae | 61504| 14953| 0  |
| 64d  | Bothricroton auruginans | Larvae | 61897| 466  | 0  |
| 64e  | Bothricroton auruginans | Larvae | 27290| 0    | 0    |
| 65a  | Bothricroton auruginans | Female Adult | 147715| 143| 14   |
| 65b  | Bothricroton auruginans | Female Adult | 0  | 1453| 1    |
| 66a  | Bothricroton auruginans | Nymph | 118704| 969 | 0    |
| 66c  | Bothricroton auruginans | Nymph | 0  | 17181| 0    |
| 66d  | Bothricroton auruginans | Nymph | 0  | 3741 | 0    |
| 67b  | Bothricroton auruginans | Nymph | 0  | 6881 | 0    |
| 67c  | Ixodes tasmani          | Female Adult | 0  | 173 | 85653|
Table 2
List of the tick species collected and identified from bare-nosed wombats (Vombatus ursinus) hosts between January 2019 and January 2020. L: Larvae, N: Nymph

| Tick Species          | Common Name          | Quantity | Instar       | Locality                                      |
|-----------------------|----------------------|----------|--------------|-----------------------------------------------|
| Bothriocroton auruginans | Wombat tick         | 420      | 128♂, 141♀, 112N, 39L | NSW; Coolagolite, Yowrie, Bellmount Forest, Bilpin, Bemboka, Buxton, Primrose Valley, Courijah, Orangeville, Bungendore, Burra, Gelstone Park, Dalgety, Kangeroo Valley, Mowbray Park, Pheasant Nest, Picton, Quaama, The Oaks, Mount Hunter, The Rock, Wagga, Thirlmere, Wolgan Valley |
| Haemaphysalis bancrofti  | Wallaby tick        | 10       | 8♀, 2N       | NSW; Coolagolite, Dalgety, Picton, Quaama |
| Haemaphysalis longicornis | Bush tick            | 3        | 1♂, 2♀       | NSW; Coolagolite                            |
| Ixodes tasmani        | Common marsupial tick | 12       | 11♀, 1N     | NSW; Dalgety Tas; Cradle Mountain            |
| Ixodes holocyclus     | Australian paralysis tick | 4        | 4♀          | NSW; Coolagolite, Quaama                     |

Four OTUs (OTU_1, LC464975, 99% identity; OTU_977, LC464975, 94.41% identity; OTU_1383, LC464975, 98.51% identity; and OTU_1806, CP014561, 93.26% identity ) were identified as a species of Coxiella closest matched to C. burnetii and were detected in 72% of B. auruginans (86% of females, 68% of males, 39% of nymphs and 100% of larvae) but not detected in I. tasmani. Females had a mean prevalence of 51.7%, males 30.7%, nymphs 19.6% and larvae 82.3% for C. burnetii. The distribution of C. burnetii infected ticks detected in this study is shown in Fig. 5.

OTU_9 was assigned to a Rickettsiella endosymbiont of Ixodes tasmani (KP994859, 100% identity) and comprised 94.5% of the bacterial diversity in the single female I. tasmani sample. This tick was collected from a wombat in Dalgety NSW which is 100 km from the collection location of the wombat in Coolangubra NSW from which this sequence was originally isolated [6]. OTU_79 was assigned to Candidatus Borrelia ivorensis (KT364340, 99.53% identity) and was detected in only one engorged adult female B. auruginans (2051 sequences) from Mowbray Park in NSW. An uncultured Anaplasma sp. (OTU_29, MK041546, 98.51% identity) was detected in four female B. auruginans from Quaama, Coolagolite and The Oaks NSW.

The genus Staphylococcus was identified in six OTUs and was present in 66% of samples. OTU_14 (MT214233, 100% identity) was assigned to S. sciuri and was present in 21% of B. auruginans
samples (29% of females, 27% of males, no nymph or larvae) but not in *I. tasmani*. OTU_2 (MN314593, 100% identity), OTU_1817 (MN314593, 96.50% identity) and OTU_1923 (MN314593, 94.87% identity) had a top BLAST hit of *S. agnetis* and were present in 56% of samples (60% of females, 41% of males, 56% of nymphs and 75% of larvae) including *I. tasmani*. Two additional OTUs were assigned to miscellaneous *Staphylococcus* spp. (OTU_15, MH549514, 100% identity; and OTU_1791, MG572712, 99.53% identity) but were represented in very low numbers of sequences. Eight OTUs were assigned to the genus *Streptococcus*, however only three were present in more than 100 sequences in any ticks. *S. dysgalactiae* (OTU_5, CP044102, 100% identity) was detected in very high sequence numbers in four female *B. auruginans* collected in Orangeville NSW. Nine *B. auruginans* (2 female, 3 male, 3 nymph and 1 larvae) had *S. salivarius* (OTU_51, MN559932 100% identity), and *S. didelphis* (OTU_1504, NR_115730, 99.53% identity) detected in a low number of sequences (<200) in one female and one nymph *B. auruginans*. *Escherichia coli* (OTU_4, NZ_CP045277, 100% identity) was identified in 21% of *B. auruginans* ticks (14% of females, 50% of nymphs, 50% of larvae and no males) but not in *I. tasmani*. OTUs that had a taxonomic identity associated with environmental bacteria such as *Acidobacteria*, *Bacteroidetes* and *Cyanobacteria* comprised <4% of the total composition. Skin and soil-associated bacteria that occurred in high sequence numbers included *Corynebacterium ulcerans* (OTU_20, 100% identity), *C. amycolatum* (OTU_6, MK465377, 100% identity), *Macrococcus brunensis* (OTU_8, MK097326, 100% identity), *Comamonas serinivorans* (OTU_11, 9 CP021455, 9.77% identity), *Paraburkholderia caffeinilytica* (OTU_17, MN150516, 100% identity), and *Dietzia timorensis* (OTU_43, MN511783 100% identity). Discussion This study aimed to record the species of ticks that feed on bare-nosed wombats and identify the bacteria associated with them. Five tick species were collected and included the first record of *H. longicornis* on bare-nosed wombats. A very high number of bacterial sequences were detected in wombat ticks, highlighting the effectiveness of NGS and the diversity of microorganisms in Australian ticks. *Proteobacteria*, *Firmicutes* and *Actinobacteria* dominated the bacterial profile, and the bacterial
composition of the ticks studied supports similar investigations into these species [14, 17, 29].

The wombat tick *B. auruginans* is consistently the most prevalent tick found on bare-nosed wombats [46, 47, 48] and all instars were represented in this study. Heavy tick infestation has been associated with anaemia and poorer health parameters in other native marsupials [49, 50] and at least two of the wombats in this study were diagnosed with anaemia as a result of their tick burden (D. Kerr, personal communication). While it has been suggested that *B. auruginans* occurs throughout most of the bare-nosed wombat range in NSW [51], the only confirmed localities in the state are from Burrawang [46], Tooloom, Armidale [30] and Wee Jasper [17]. This study provides additional locality reports for *B. auruginans* and highlights the abundance of this species on bare-nosed wombats.

Despite the host specificity of *B. auruginans*, it has been suggested that it is likely a three-host tick like other *Bothriocroton* sp. which parasitise reptiles [30] however further research on the life cycle and seasonality of this species is needed.

Known as the bush tick in Australia and the cattle tick or Asian longhorned tick elsewhere, *H. longicornis* is an introduced three-host tick distributed from south-east Queensland to Victoria [52]. The adult male *H. longicornis* found in Coolagolite NSW is particularly unusual considering this species are obligate parthenogens in Australia resulting in males being quite rare [53]. Cattle, sheep and horses are the preferred hosts for this species but have also been collected from humans, domestic animals, various species of birds, black-striped wallabies (*Wallabia dorsalis*), northern brown bandicoots (*Isoodon macrourus*) and common wallaroos (*Macropus robustus*) [30, 31]. The three specimens collected in this study were from a free-ranging wombat on a 100-acre property with no active livestock, however access to properties with livestock is possible across dried creek beds at certain times of the year (D. Ondinea, personal communication). The bush tick has been extensively studied overseas and is considered a vector of bacteria, viruses and protozoa, in particular *C. burnetii* [54], *Ehrlichia chaffeensis*, *Borrelia* spp. [55], and *Theileria orientalis* [56], however transmission has not been shown to occur in Australian specimens [57].

The Australian paralysis tick is well known for causing tick paralysis in domestic animals and humans [58]. Native Australian marsupials and eutherians have however coevolved with *I. holocyclus*, are the
natural hosts for this tick and are typically immune to tick paralysis [59]. Found along the entire east coast of Australia, *I. holocyclus* is an eclectic feeder and has been found on many different bird and mammal species, however in certain areas it is dependent upon bandicoots to survive between seasons [30]. All specimens collected in this study were engorged females, however adult males are rarely seen as mating occurs off the host and adult male ticks feed on adult female ticks as opposed to the mammalian hosts [60]. With the use of targeted blocking primers a relapsing fever *Borrelia* sp. was recently isolated from a single *I. holocyclus* collected from an echidna [13], highlighting the hidden pathogenic potential of this species.

Like *I. holocyclus* the common marsupial tick *I. tasmani* is similarly indiscriminate in its feeding habits having been found on various wildlife, domestic animals and humans, however it is the most widespread *Ixodes* species in Australia and has been associated with various pathogens such as *Rickettsia, Rickettsiella, Bartonella, Theileria*, nematodes and hepatozoons [29, 61, 62, 63, 64, 65, 66]. Regularly found on bare-nosed wombats in low numbers [48, 67], *I. tasmani* is a nidicolous species that detaches from its nocturnal vertebrate hosts during the day and are therefore likely associated with wombat burrows. Given the fast reproductive rate, three-host lifecycle and variety of pathogenic organisms typically harboured, this species is likely to pose a disease threat to wombats and wombat handlers, however more research needs to be conducted to determine the extent.

An endemic tick that primarily feeds on macropods, *H. bancrofti* is predominantly distributed throughout coastal Queensland and northern NSW, and apart from a disjunct population on Raymond Island Victoria, the southernmost report of this species is from a bare-nosed wombat, a red-necked wallaby (*Macropus rufogriseus*) and a swamp wallaby (*Wallabia bicolor*) in the Nadgee State Forest NSW [24]. The specimens collected in the present study were from Dalgety and Quaama NSW which are located approximately two hours north of Nadgee. These new specimens further confirm the presence of *H. bancrofti* in the far south NSW region and provide the second account of this species feeding on bare-nosed wombats [24]. Although macropods are the native host for *H. bancrofti*, there are more records of this species from cattle than native animals [24] and it is one of the main vectors of *Theileria orientalis* that impacts cattle in Australia [57, 68].
Analysis of alpha-diversity (Fig. 6) showed high variability across both sample locations and instars, similar to previous studies [17, 69], however there was some similarity between the same instars from the same collection location. Diversity can vary greatly between tick studies depending on extraction methods, the quality of filtering and bioinformatic analysis. All samples in this study underwent identical extraction, library preparation and bioinformatic analysis so it is possible that this affected sequencing depth of samples. It has been noted that some species of native ticks including *B. auruginans* require a larger number of sequences to produce an accurate representation of bacterial diversity [17]. The most abundant and diverse phylum was the *Proteobacteria* which is consistent with similar studies of native hard ticks [29, 70].

Pathogens previously isolated from *B. auruginans* include *C. burnetii*, *Rickettsia massiliae* and *R. typhi* [29] and varying levels of *Proteobacteria* and *Firmicutes* [14, 17]. The very high prevalence of *C. burnetii* found in all *B. auruginans* instars in this study is similar to previous findings in this species [29]. *Coxiella*-like organisms are known to be highly efficient at transovarial transmission between tick hosts [71], and their presence within Malpighian tubules may suggest the organisms role in nutrition for ticks [7]. Different strains of *C. burnetii* have been shown to be highly related (> 99%) based on 16S rRNA, highlighting that the species recently evolved from an ancestral symbiont of ticks [6]. Because *B. auruginans* exhibits such remarkable host specificity, it is unlikely that this species is a significant vector for *C. burnetii* in humans. It is unknown however what impact this pathogen has on both healthy and sarcoptic mange-affected wombats. Blood and urine samples taken from wombats have failed to detect the presence of *C. burnetii* [72] where other native marsupials such as the koala, bandicoots and macropods are regularly found to be seropositive [73, 74, 75]. Further studies to investigate the presence of *C. burnetii* in wombat faeces, blood and other parasites may be beneficial to determine the importance, role and impact of this pathogen in wombats and wombat ticks.

The presence of *Borrelia* in Australian ticks is a recent discovery [13], and targeted approaches using blocking primers and highly conserved housekeeping genes have provided insights into potential reservoirs and vectors of novel *Borrelia* sp. in Australia [14]. A species of *Borrelia* had the closest
match to Candidatus *Borrelia ivorensis* and was detected in a single *B. auruginans* from NSW. The original isolate for this sequence was from *Amblyomma variegatum* in western Africa and is more closely related to the relapsing fever *Borrelia* group than the Lyme group [76]. The uncultured *Anaplasma* sp. detected was originally isolated from an echidna tick (*Bothriocroton concolor*). All recognised *Anaplasma* spp. are obligate intracellular tick-borne mammalian pathogens [77], and as transovarial transmission between ticks has not yet been shown, it is believed that this genus persists solely through infected mammalian hosts [78].

A commensal bacterium of the mammalian gastrointestinal tract, *Escherichia coli* is commonly found in native mammals, with the highest prevalence in herbivorous mammals with larger body masses [79]. Some species of *E. coli* are zoonotic and impact human health [80]. One study found Northern hairy-nosed wombats (*Lasiorhinus krefftii*) to have an *E. coli* prevalence of 80% and southern hairy-nosed wombats (*L latifrons*) to have 86% [79], however another study found no zoonotic *E. coli* in all three species of wombats [81]. While a species of *E. coli* was fairly prevalent in *B. auruginans*, it has been shown that ticks exhibit various innate immune responses to this bacterium [82, 83] and it is destroyed in the body of the tick rather than harboured and transmitted.

Ticks are often found to have large quantities of bacteria associated with the soil environments in which they spend most of their lives, in addition to bacteria associated with the skin of their mammalian hosts [13, 84]. Some pathogenic environment and skin-associated bacteria were detected in both *B. auruginans* and *I. tasmani* that may have potential implications for wombats with sarcoptic mange or could even be a result of ticks feeding on wombats with sarcoptic mange-associated bacteria. Both ticks and *S. scabiei* can potentially be coinfected with multiple pathogens [85] and the implications of coinfection with both ticks and mites with the addition of clinical signs of mange on wombat hosts is unknown. When affected by sarcoptic mange, the invasion of microorganisms into the body of a wombat is facilitated by a combination of host scratching and the burrowing of mites under the skin [86]. Hence wombats with sarcoptic mange are highly likely to be more susceptible to simultaneous infection of multiple pathogenic organisms, in addition to ticks being likely to encounter mite or sarcoptic mange-associated bacteria. Most ticks in this study were coinfected with multiple
genera of environment or skin-associated bacteria including *Staphylococcus, Streptococcus, Corynebacterium, Mycobacterium, Bacillus, Pseudomonas, Stenotrophomonas* and *Acinetobacter*. The genus *Staphylococcus* is typically a commensal of mammalian skin, commonly found in ticks of native Australian wildlife [84, 87] and some species such as *S. aureus* are associated with Sarcoptes mites and responsible for causing scabies-associated pyoderma in humans [86]. Two species of *Staphylococcus* were detected in this study; *S. agnetis* which is typically associated with clinical disease in cattle and poultry [88, 89], and *S. sciuri* which is a skin-associated bacterium acquired through contact with host skin [84]. *S. sciuri* has also been detected in fleas from bandicoots and dogs in Australia, various lice and tick species including *I. holocyclus* and *H. longicornis* [87].

Further skin-related bacteria found included *Corynebacterium ulcerans* which causes a zoonotic infection similar to diphtheria [90], *Dolosigranulum pigrum* which is associated with pneumonia in humans [91, 92], and *Macrococcus brunensis* which is phylogenetically similar to a species of *Macrococcus* responsible for causing skin infection in dogs [93]. At least three distinct species of *Streptococcus* were detected of which *S. dysgalactiae* and *S. didelphis* are important pathogens of humans and animals causing skin infection [94, 95]. Other species of *Streptococcus* such as *S. pyogenes* from Sarcoptes mites are responsible for causing skin infection in humans [86]. The pathogenicity and consequences of these skin-associated bacteria on both healthy and sarcoptic mange-impacted wombats may therefore be important.

Endosymbiotic bacteria are an important component of the tick microbiome and often play a role in tick reproductive and nutritional fitness [15]. Some tick endosymbionts found in this study include *Rickettsiella, Acinetobacter* and *Pseudomonas*. The genera *Acinetobacter* and *Pseudomonas* have previously been isolated in wombat fleas [87], found across all species of *Ixodes*, and believed to play an important role in the physiological processes of ticks [96]. Despite *I. tasmani* exhibiting a very high prevalence of a *Rickettsiella* endosymbiont, some known tick endosymbionts such as *Wolbachia* and *Francisella* were not detected in this study.

It is believed that bacterial endosymbionts are dominant in the majority of ixodid ticks [9], and there are examples of endosymbiotic bacteria so abundant they mask other microbes including pathogens,
for example Candidatus *Midichloria mitochondrii* in the paralysis tick *Ixodes holocyclus* [13]. DNA extracted from whole tick specimens, in particular those which have fed from their vertebrate hosts, will contain tick DNA, host DNA and microbial DNA (i.e. bacteria, virus, eukaryote). The presence of host DNA in engorged ticks has been known to cause difficulties due to inhibitory properties in mammalian blood [97] and so a targeted approach is required when examining bacterial communities. Popular genetic markers used for molecular identification of ticks and tick bacteria include the cytochrome c oxidase subunit 1 (COI) protein coding gene, the 16S ribosomal RNA gene (rRNA), 12S rRNA and 18S rRNA [98]. Each has its advantages and limitations, for example COI offers an extensive existing library of universal primers as it is the standard marker for barcoding of animal species, however is limited in its ability to distinguish certain groups of organisms such as the Ixodidae to species level. 16S is the most commonly used marker because it can accurately distinguish between most prokaryotic taxa, but some microbial groups such as *Rickettsiales* may be difficult to distinguish due to their inter-species 16S similarity [99]. There are nine hypervariable regions of bacterial 16S rRNA genes that can be effectively targeted to identify bacterial taxa (V1-V9) and regions V1-V4 have been most commonly used in ticks [15].

The management of sarcoptic mange in wombats is likely to have an impact on tick-wombat relationships. The use of anti-parasitic medications such as Cydectin® to treat *S. scabiei* mites are non-targeted and will kill various different endoparasites and ectoparasites. While the benefits are clear in reduced parasite burdens and eventual recovery from sarcoptic mange which is otherwise fatal, it is not known how eliminating some internal and external parasites for captive-raised or joey wombats may impact their immunity or ability to cope with future parasites and their pathogens once released. It has been recommended that animals intended to be re-introduced back into the wild are exposed to low levels of parasites in order to acquire immunity [100], however it is equally a concern to release animals into an environment with novel parasites that have been acquired from humans, domestic animals or elsewhere in captivity [21]. Parasites are essential for the effective development of host immune systems, and the loss of one parasite species may increase disease risk by altering competitive pressure for other parasites [101]. Wombats have increased immunological and thermal
costs as a direct consequence of sarcoptic mange, often having anaemia and lower metabolic rates than healthy wombats [19, 102, 103]. As a result, it would be beneficial to understand more about the impact of ticks and TBPs on immunocompromised wombats with sarcoptic mange and immune-naïve wombats released from captivity or treated with anti-parasitic drugs.

The nature of the sampling method in this study was both economical and allowed for a fair assessment of tick infestation rates on wombat hosts, however it can be assumed that in some cases smaller nymphal and larval tick instars were likely overlooked. It is also likely that some ticks had left road killed wombats which were opportunistically sampled, despite Skerratt et al (2004) finding no difference between tick density on live or road-killed wombats. The high abundance of female instars is likely indicative of some collection bias due to their larger size. The collection of ticks from animals in care limits the assessment of the origin of tick species and species of microorganism due to the uncertainty of whether the ticks attached in the location of rehabilitation or the original habitat where the wombat was collected from. Three species of ticks collected from wombats (H. bancrofti, H. longicornis and I. holocyclus) were unable to be processed for bacterial presence in this study, however these species are known vectors of significant pathogens to domestic animals and humans, and as a result have been extensively studied. All ticks collected in this study except I. tasmani were non-nidicolous hard ticks and presumably obtained by the wombat hosts whilst grazing. Many of the wombats used in this study were also in an atypical environment and had not had recent exposure to burrows. Considering that the majority of soft ticks are nidicolous and feed for very short periods of time, further investigation into the ticks associated with wombat burrows would provide a broader perspective of all the tick species associated with wombats.

Conclusions
This study builds upon recent wildlife tick research and provides the first focused investigation into the ticks and tick-associated bacteria of bare-nosed wombats. The detection of various Proteobacteria in this study highlights the high bacterial diversity in native Australian ticks that was unrecognised prior to the development of NGS. Furthermore, the detection of C. burnetii in a large proportion of wombat ticks highlights the need for further investigation into wombat ectoparasites and their
associated pathogens, in addition to the ability for wombats to cope with these pathogens and tick burdens in the presence of sarcoptic mange. The complex and dynamic relationships between vertebrate wildlife hosts, ticks and pathogens are continuously highlighted in the northern hemisphere [104, 105]. The unique evolutionary history of Australian fauna and tick species is shown in the distinct diversity yet taxonomic difference of these tick-host-pathogen relationships from those overseas. With the advancement of molecular methods the extent of these unique evolutionary relationships will become clearer, and may lead to potential improvements in the management of vector-borne diseases such as sarcoptic mange.

List Of Abbreviations
16S 16S ribosomal ribonucleic acid (rRNA) gene
NGS Next generation sequencing
TBP Tick-borne pathogen

Declarations

Ethics approval and consent to participate
The collection of invertebrates described did not require ethics approval

Consent for publication
Not applicable

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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The authors declare that they have no competing interests

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Authors’ contributions
Conceived the idea for the study
Conducted the systematic review of the literature and compiled the data
Contributed to the interpretation of data and writing

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Figures
Figure 1

Geographic distribution of ticks collected from bare-nosed wombats (Vombatus ursinus) hosts between January 2019 and January 2020. Each point represents a unique collection location for the corresponding tick species. Overlapping points were displaced with a point displacement renderer around a centre symbol (denoted in legend); point displacement distance was defined by number of map units (kilometres).
Figure 2

a) Haemaphysalis longicornis female i) dorsal, ii) ventral, b) Ixodes holocyclus female i) dorsal, ii) ventral, c) Ixodes tasmani female i) dorsal, ii) ventral, d) Bothriocroton auruginans female i) dorsal, ii) ventral, e) Haemaphysalis longicornis male i) dorsal, ii) ventral, f) Haemaphysalis bancrofti female i) dorsal, ii) ventral
Species of tick and frequency of each instar collected from bare-nosed wombat (Vombatus ursinus) hosts between January 2019 and January 2020
Figure 4

Taxonomic summary of bacterial phyla found in wombat ticks between January 2019 and January 2020.
Figure 5

Geographic distribution of Coxiella burnetii detected in ticks from bare-nosed wombat (Vombatus ursinus) hosts between January 2019 and January 2020. Each point corresponds with the collection location of the tick(s) which were positive (>100 sequences) for C. burnetii. A point cluster renderer was used to group nearby points into a single rendered marker symbol. Point cluster distance was determined by point units.
Figure 6

Alpha diversity of bacterial composition in ticks collected from bare-nosed wombats (Vombatus ursinus) between January 2019 and January 2020 assessed by diversity (Shannon, Simpson) and richness (ACE, Inverse Simpson).

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