

[10.1071/ZO23010](https://doi.org/10.1071/ZO23010)

*Australian Journal of Zoology*

### Supplementary Material

#### **Changes in parasite species distributions could be driven by host range expansions: the case of hybridisation between two Australian reptile ticks**

*Bridgette Barnden<sup>A</sup>, Amy L. Slender<sup>A,B</sup>, Robert D. Sharrad<sup>A</sup>, and Michael G. Gardner<sup>A,B,\*</sup>*

<sup>A</sup>College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

<sup>B</sup>Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia.

\*Correspondence to: Michael G. Gardner College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia Email: michael.gardner@flinders.edu.au

## Supplementary material

**Table S1:** Tick metadata including life stage, sex and lizard ID where the tick was sampled from as well as the morphological classification, genomic classification, and proportion of ancestry to each *K* from the STRUCTURE result model of *K* = 2. NB: some ticks were sampled from the same lizard on different days.

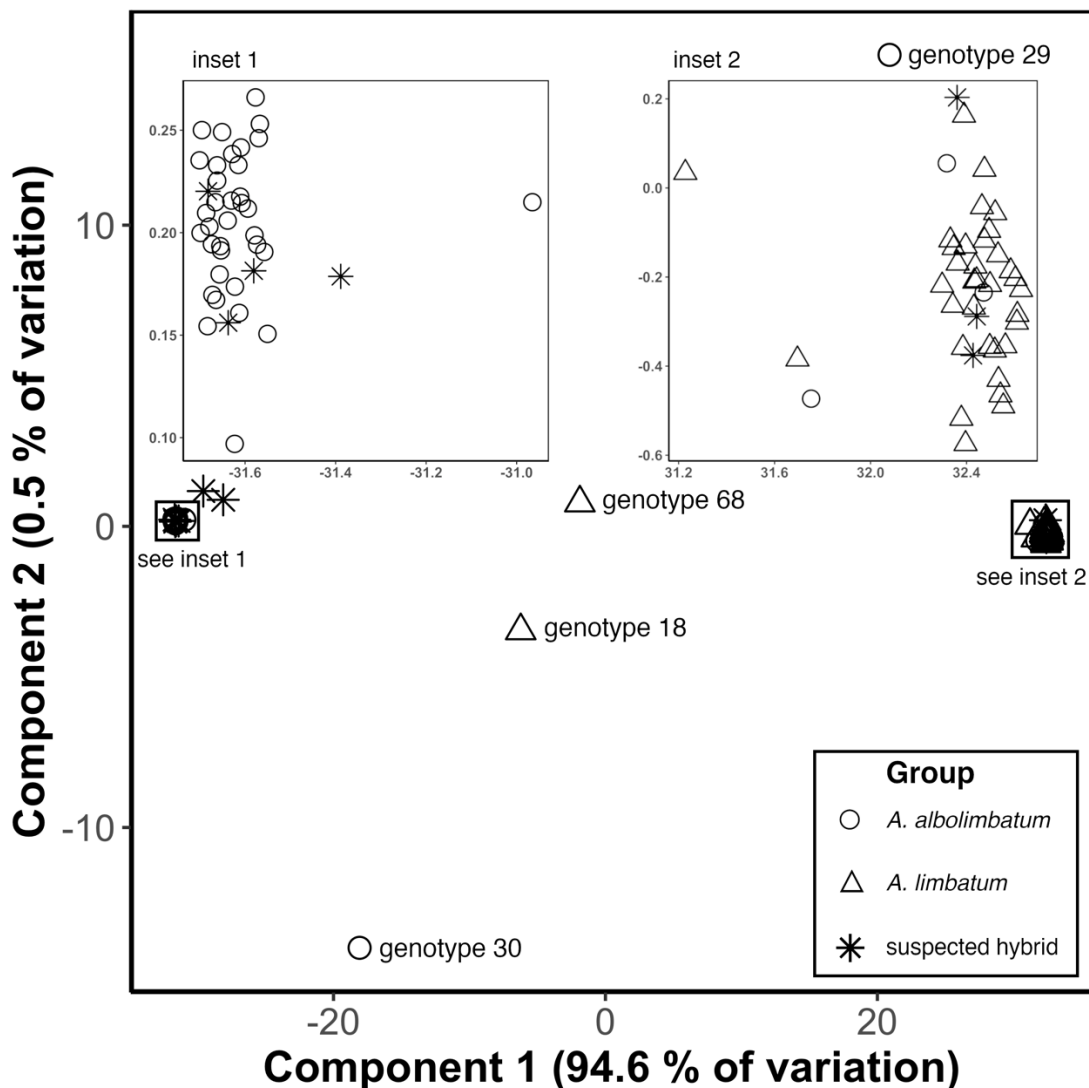
Tick ID	Life stage and sex (if known)	Lizard ID sampled from	Location	Morphological classification	Genomic classification	Proportion of ancestry with <i>A. limbatum</i>	Proportion of ancestry with <i>A. albolimbatum</i>
<b>Genotype 1</b>	Adult F	11	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0.005	0.995
<b>Genotype 2</b>	Adult F	11	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 3</b>	Adult F	11	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 4</b>	Adult F	12	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0.035	0.965
<b>Genotype 5</b>	Adult F	12	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0.058	0.942
<b>Genotype 6</b>	Adult F	1002	Puddle Holes Dam Rd, Cooyerdoo	Suspected hybrid	<i>A. limbatum</i>	1	0
<b>Genotype 7</b>	Adult F	10001	Iron Knob Rd, Middleback Range	Suspected hybrid	<i>A. limbatum</i>	0.9992	0.0008
<b>Genotype 8</b>	Adult F	2010	Nonning Rd, Corunna Station	Suspected hybrid	<i>A. limbatum</i>	1	0
<b>Genotype 17</b>	Adult M	110000	Puddle Holes Dam Rd, Middleback Range	Suspected hybrid	<i>A. albolimbatum</i>	0	1
<b>Genotype 18</b>	Adult M	110	Arid Lands Rd, Port Augusta	<i>A. limbatum</i>	Hybrid	0.403	0.597
<b>Genotype 19</b>	Adult M	DOR	Port Bonython Rd, Bonython	<i>A. albolimbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 20</b>	Nymph	1400	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 21</b>	Adult F	1400	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 22</b>	Adult M	10200	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 23</b>	Adult F	8000	Port Bonython Rd, Bonython	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 24</b>	Adult M	8000	Port Bonython Rd, Bonython	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 25</b>	Adult M	1400	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 26</b>	Adult M	202	Whyalla Rd, Myloa Station	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 27</b>	Adult F	202	Whyalla Rd, Myloa Station	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 28</b>	Adult M	80000	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 29</b>	Adult F	40100	Middleback Rd, Middleback Range	<i>A. albolimbatum</i>	Hybrid	0.823	0.177
<b>Genotype 30</b>	Adult M	40100	Middleback Rd, Middleback Range	<i>A. albolimbatum</i>	Hybrid	0.211	0.789
<b>Genotype 31</b>	Adult M	21000	Puddle Holes Dam Rd, Middleback Range	<i>A. limbatum</i>	<i>A. limbatum</i>	0.982	0.018

<b>Genotype 32</b>	Adult M	11400	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.002	0.998
<b>Genotype 33</b>	Adult F	11400	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 34</b>	Adult F	10002	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 35</b>	Nymph	10002	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 36</b>	Larvae	10002	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 37</b>	Adult M	50000	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 38</b>	Nymph	50000	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 39</b>	Adult M	10100	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 40</b>	Adult M	42	Guidera Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 41</b>	Adult F	101	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 42</b>	Adult M	24	Lincoln Hwy, Midgee	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 43</b>	Adult F	24	Lincoln Hwy, Midgee	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 44</b>	Adult M	90000	Iron Knob Rd, Middleback Range	<i>A. limbatum</i>	<i>A. limbatum</i>	0.9996	0.0004
<b>Genotype 45</b>	Nymph	90000	Iron Knob Rd, Middleback Range	<i>A. limbatum</i>	<i>A. limbatum</i>	0.996	0.004
<b>Genotype 46</b>	Nymph	30000	Iron Knob Rd, Iron Knob	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 47</b>	Adult F	30000	Iron Knob Rd, Iron Knob	<i>A. limbatum</i>	<i>A. limbatum</i>	0.9996	0.0004
<b>Genotype 48</b>	Adult M	2100	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	0.9996	0.0004
<b>Genotype 49</b>	Nymph	2100	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 50</b>	Adult F	210	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 51</b>	Adult M	1020	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 52</b>	Nymph	1020	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 53</b>	Nymph	102	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 54</b>	Adult M	102	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 55</b>	Adult M	17	Hurrels Gap Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 56</b>	Adult M	22	Hurrels Gap Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.002	0.998
<b>Genotype 57</b>	Adult M	11000	Hurrels Gap Rd, Murninnie Beach	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 58</b>	Adult F	11000	Hurrels Gap Rd, Murninnie Beach	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.002	0.998
<b>Genotype 59</b>	Adult F	4100	Hurrels Gap Rd, Murninnie Beach	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 60</b>	Nymph	27	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 61</b>	Larvae	27	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1

<b>Genotype 62</b>	Adult M	27	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 63</b>	Adult M	41	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 64</b>	Adult F	201	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 65</b>	Adult M	11	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 66</b>	Adult F	11	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0	1
<b>Genotype 67</b>	Adult M	120	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 68</b>	Nymph	120	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	Hybrid	0.465	0.535
<b>Genotype 69</b>	Adult M	1001	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	0.9996	0.0004
<b>Genotype 70</b>	Adult M	2200	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 71</b>	Adult M	2001	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 72</b>	Adult M	2002	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	<i>A. limbatum</i>	0.988	0.012
<b>Genotype 73</b>	Adult M	800	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 74</b>	Adult F	800	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 75</b>	Adult M	70020	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 76</b>	Adult F	70020	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 77</b>	Adult F	1010	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 78</b>	Adult M	1010	Puddle Holes Dam Rd, Katunga Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 79</b>	Adult M	70100	Sectus Tanks Rd, Barna	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 80</b>	Adult M	70200	Sectus Tanks Rd, Barna	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 81</b>	Adult M	71000	Middleback Rd, Barna	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 82</b>	Adult F	71000	Middleback Rd, Barna	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.001	0.999
<b>Genotype 83</b>	Adult M	11	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.002	0.998
<b>Genotype 84</b>	Adult M	210	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 85</b>	Nymph	2010	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 86</b>	Adult F	2010	Nonning Rd, Corunna Station	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 88</b>	Adult M	30000	Iron Knob Rd, Iron Knob	<i>A. albolimbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 89</b>	Adult M	101	Schultz Rd, Warren	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.0006	0.9994
<b>Genotype 90</b>	Adult M	1002	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 91</b>	Adult F	1002	Puddle Holes Dam Rd, Cooyerdoo	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0
<b>Genotype 92</b>	Adult M	11	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. limbatum</i>	0.9882	0.0118
<b>Genotype 93</b>	Adult M	11	Puddle Holes Dam Rd, Middleback Range	<i>A. albolimbatum</i>	<i>A. albolimbatum</i>	0.013	0.987
<b>Genotype 94</b>	Nymph	110	Arid Lands Rd, Port Augusta	<i>A. limbatum</i>	<i>A. limbatum</i>	1	0

*Description of fully filtered dataset used to test the sensitivity of the results*

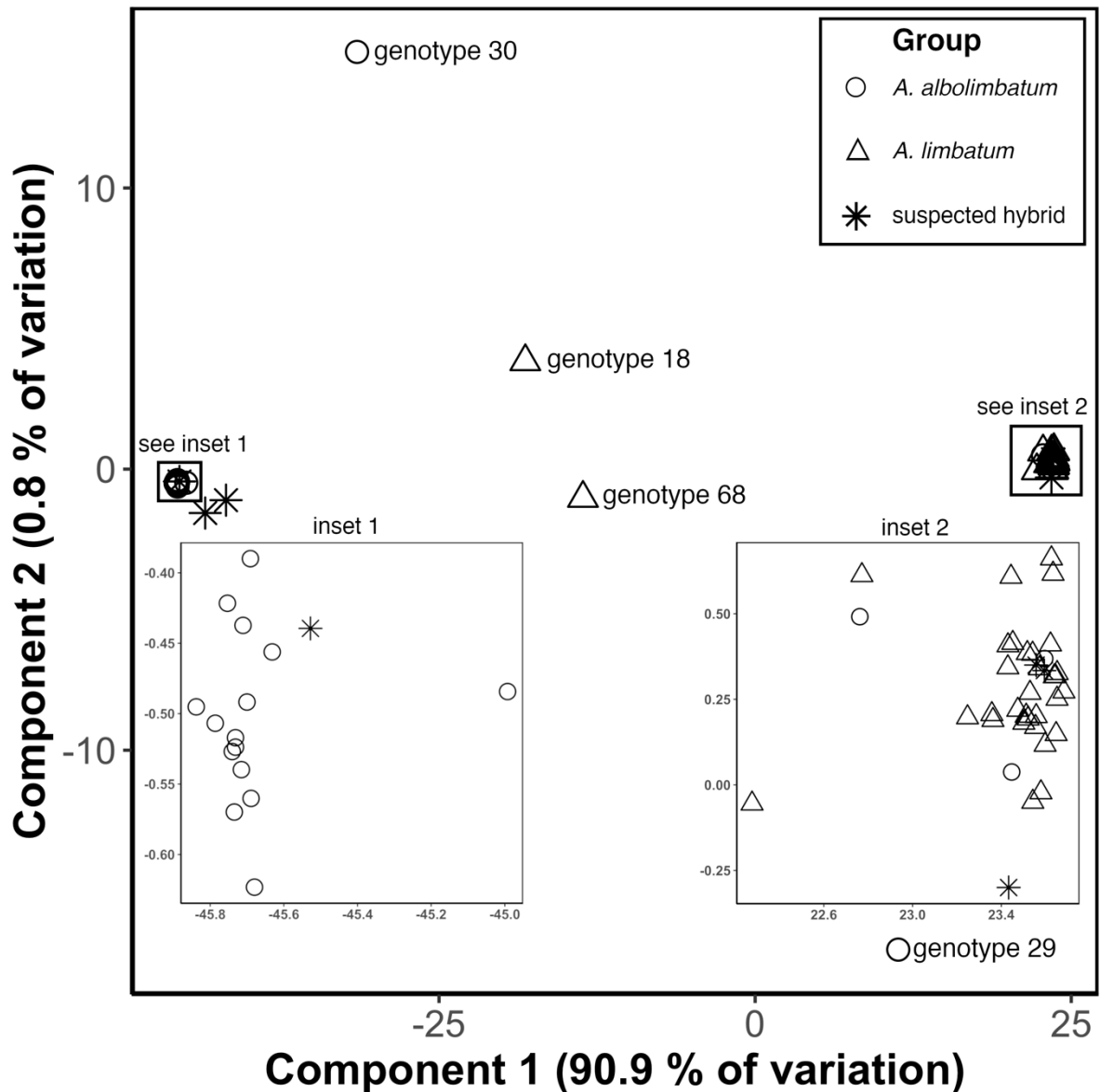
Following SNP calling, DArT produced an initial dataset containing 77,904 SNPs (33.45 % missing data). There were 5,836 loci that had a reproducibility value < 1, with these removed there were 72,068 SNPs remaining. One of each duplicated sample (genotypes 9 - 16) was removed which resulted in the removal of 24 monomorphic loci. When SNPs with any missing data were excluded, 5,491 SNPs remained. Exclusion of secondaries resulted in 5,270 SNPs remaining and finally when SNPs that contained alleles that only appeared in tick body samples were excluded, there were 5,222 SNPs remaining.



**Figure S1.** Plot of principal coordinate analysis that follows the full filtering method to determine how sensitive the results are to locus reproducibility <1, and secondary loci. The group symbol specifies the tick species determined by morphological identification (adults) and the species of other adults present on the same lizard (nymphs and larvae); *A. albolimbatum* (circle), *A. limbatum* (triangle) and suspected hybrid (star).

*Description of method used to interrogate the effect of relatedness between ticks.*

We used loci from the working dataset to create two datasets, one for each of the parental species where the individuals were genomically identified (excluding hybrids). After the removal of monomorphic loci the *A. albolimbatum* dataset contained 41 individuals with 1515 SNPs and the *A. limbatum* dataset contained 40 individuals with 2364 SNPs. We determined relatedness using COANCESTRY (V1.0.1.10). Relatedness estimates were generated using the DyadML method with 100 bootstraps while accounting for inbreeding (Wang 2011). Relatedness was removed from the complete working dataset by 1) identifying individuals that had the greatest number of pairwise comparisons where  $r > 0.4$  and 2) sequentially removing these individuals and recalculating the number of pairwise comparisons where  $r > 0.4$  for each individual for removal until there were no more pairwise comparisons where  $r > 0.4$ . The working dataset contained 59 individuals (37 *A. limbatum*, 18 *A. albolimbatum* and four hybrids – genomically identified) and 6345 SNPs after removal of relatedness and monomorphic loci.



**Figure S2.** Plot of principal coordinate analysis of working dataset to determine how sensitive the results are to relatedness. Individuals have been removed so that those remaining have  $r < 0.4$ . The group symbol specifies the tick species determined by morphological identification (adults) and the species of other adults present on the same lizard (nymphs and larvae); *A. albolimbatum* (circle), *A. limbatum* (triangle) and suspected hybrid (star).

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources* **11**(1), 141-145.