A DEEPER LOOK INTO THE MORPHOLOGY AND RECEPTORS FOUND IN THE TICK (ACARI: IXODIDAE) CHEMOPERCEPTION STRUCTURE, THE HALLER'S ORGAN

BY

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THESIS

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ABSTRACT

Keywords: Haller's organ, ticks, geometric morphometrics, chemoperception expression

The Haller's organ is a sensory structure unique to ixodid ticks that assists in host seeking behaviors. Presented here are the results of a detailed comparative study of the morphology and the chemoperception gene expression of the Haller's organ.
The morphometrics study focuses on the three important North American tick species: *Ixodes scapularis*, *Amblyomma americanum*, and *Dermacentor variabilis*. Possible differences in morphology between and within these species and between males and females for each species were observed using environmental scanning electron microscopy (ESEM). Analyses using geometric morphometrics resulted in low levels of intraspecific, within sex variation in the morphology of Haller's organ and high variation between species. Differences between species may be due to different host seeking behaviors (passive versus active). The differences in Haller's organ morphology of males and females of the same species could be attributed to post-mating behaviors.

The exploration of chemoperception gene expression in the Haller's organ focused on a single species, *Ixodes scapularis*. This study focused on the expression of ionotropic (IR) and gustatory receptors (GR) in the forelegs of male and female ticks. Additionally, two phylogenetic trees were created corresponding to each receptor type. The phylogenetic trees show the orthology between the tick ionotropic and gustatory receptors and the described insect chemoreceptors. There were two *I. scapularis* IRs expressed in the forelegs of these ticks and five GRs of interest. This research aids in providing an increase in our knowledge of the Haller's organ. The Haller's organ is critical to the performance ability of tick activities including host location. Therefore, improved knowledge of the Haller's organ may facilitate tick management.

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INTRODUCTION

Insects and other arthropods have a wide array of physiological mechanisms for finding food, mates, and in the case of hematophagous invertebrates, hosts. Blood feeding insects such as mosquitoes utilize their proboscis, maxillary palps, and antennae to find hosts (Maekawa et al. 2011). However, these structures are not shared among all arthropods. Ticks, unlike insects, have neither a proboscis nor antennae to aid in host finding, instead they use a structure called the Haller's organ (Sonenshine and Roe 2013). The Haller's organ is a small sensory structure on the forelegs of ticks and is considered unique to the superfamily Ixodoidae (Sonenshine and Roe 2013). G. Haller first described the organ in 1881, which he believed to have an auditory function (Haller 1881). Not until 1908 was this conclusion tested for accuracy (Nuttall et al. 1908). It was then noted that ticks lift up and wave their forelegs in a similar manner as insects move their antennae. This observation changed the belief that the organ had olfactory functions as opposed to auditory (Nuttall et al. 1908). Research has since shown that the Haller's organ helps ticks find hosts and mates because it is responsible for chemoreception of carbon dioxide, ammonia, and other chemicals such as pheromones (Soares and Borger 2012, Sonenshine and Roe 2013).

Despite the development of more sensitive and precise microscopy techniques over the last 30 years, the structure and morphology of the Haller's organ, the sensor that likely is key to host location and thus disease spread, has not been characterized in more detail. Due to the small size of the Haller's organ (10-100µm), scanning electron microscopy (SEM) is a good imaging method to observe its morphology (Homsher et al. 1977). In addition, older studies of Haller's organ morphology were qualitative and did not incorporate any of the quantitative analysis tools which can now be employed (Homsher et al. 1977, Homsher et al. 1988, Bookstein et al. 1998, Adams et al. 2004,

Elewa 2010, Sonenshine and Roe 2013). Geometric morphometrics is an analytic technique that allows one to obtain quantitative data for morphological comparisons (Adams et al. 2004). When conducting a geometric morphometric analysis, a set number of landmarks are used to designate key morphological features. These landmarks represent Cartesian coordinates, which are designated homogeneously on all images of specimens (Klingenberg et al. 2011). Image processing programs allow the user to upload images of their specimens and designate landmark coordinates on these images (Abramoff et al. 2004). Landmarks can then be taken from the image processing program into a geometric morphometrics program and statistically compared to the coordinates of other specimens (Kingenberg et al. 2011). The use of geometric morphometrics has been shown to provide quantitative evidence of evolution of biological shape, link the effects of genetics and environment to shape, as well as help build phylogenies (Klingenberg et al. 2010, 2011).

In this study, through the use of ESEM (Environmental Scanning Electron Microscopy) and geometric morphometrics I examined the Haller's organs of three major disease-vectoring ticks of North America: *Ixodes scapularis* (the black-legged tick), *Amblyomma americanum* (the lone star tick), and *Dermacentor variabilis* (the American dog tick). I tested quantitatively if the Haller's organs of these three species are morphologically different. Additionally, I determined if there is within species sexual dimorphism present the morphology of the Haller's Organ. In order to do so, three main structures of the Haller's organ were analyzed because they outline both the shape and internal structures. By using geometric morphometrics for my analyses, I ensured that image size was not a variable affecting my results, which was a drawback to traditional morphometrics previously used (Brookstein et al. 1998).

I show here that morphological differences in the overall structure of the Haller's organ exist between species. Additionally, the Haller's organ of one species (*D. variabilis*) showed sexual dimorphism. I conclude that Haller's organ morphology may help in tick species identification and found that more detailed imaging of the structure can lead to a better understanding of the functional morphology of the Haller's organ in the future.

MATERIALS AND METHODS

Selection and Description of Study Sites

Collection took place from April-May 2014, when adult ticks of all three species were active. Ticks were collected from two locations: Allerton Park (Piatt County, Illinois) and Waterfall Glen County Forest Preserve, (DuPage County, Illinois). Allerton Park (1,517 acres) includes an oak-hickory forest, sugar maples and prairies. Tick species found at this location are *I. scapularis* and *D. variabilis* (Rydzewski 2011). *Ixodes scapularis* adults were collected within an upland young successional forest comprised of oak-hickory and *D. variabilis* adults were collected from a restored tall-grass prairie. Waterfall Glen County Forest Preserve (2,492 acres) contains a large variety of habitats including a large oak-maple woodland area. The adult stage of three adult tick species, *I. scapularis*, *D. variabilis*, and *A. americanum,* were collected within the preserve ("Ticks," 2014). Regardless of location or site, all ticks were collected alongside existing trails.

Tick Collection and Identification Methods

All ticks were collected using dragging and flagging methods. Every 2-3 meters, the 1m x 1m drag cloth was inspected and all ticks on the cloth were removed and kept in vials. Collection continued until at least 10 males and 10 females of each tick species were caught. In the lab, tick species identifications were confirmed and ticks were placed into separate vials filled with 70% ethanol (Keirans et al. 1989). Additionally, five male and three female *A. americanum* were obtained from Oklahoma State University's tick rearing facility to supplement low field collection numbers of *A. americanum*.

Environmental Scanning Electron Microscopy (ESEM)

I chose to use ESEM as opposed to standard scanning electron microscopy (SEM) because the ESEM microscope (Imaging Group Beckman Institute, University of

Illinois at Urbana-Champaign) is equipped with a field emission electron gun (FEG), which allows for a higher imaging resolution ("How ESEM…" 2015). In preparation for ESEM, five ticks of each species and sex were placed on Kimwipes (©Kimberly-Clark) to allow the ethanol to evaporate. Dry ticks were attached to aluminum mounts (25mm diameter) covered with double-sided adhesive carbon tape (all materials from Structure Probe, West Chester, PA, USA). Using forceps, the forelegs of each tick were turned so that the Haller's organ faced upwards. Using a turbo sputter coater (©Denton Vacuum, Desk-1 TSC), the stub was coated with Au-Pt and placed into the microscope (©FEI Company, Philips XL30 ESEM-FEG, at 50kV) for imaging. A micrograph of a complete view of the Haller's organ was captured for both the right and left leg of all five males and five females for all three species. Care was taken to always use the same magnification and view angle for each micrograph (Figure 1.1).

Geometric Morphometrics

All of the ESEM images (Figure A.1) showing the complete view of the Haller's organ for each species and sex were loaded into the open source program ImageJ (Abramoff et al. 2004). Using ImageJ, approximate lengths and widths of each organ, the capsule length, and the number of setae were recorded and averaged (Table A.1). Then with the ImageJ Pointpicker add-on, a total of twelve landmarks were chosen to outline the overall shape and structures of the Haller's organ in all three species (Figure 1.1). I used 3 fixed and 9 semi-landmarks to define the Haller's organ of each species. Landmarks 1-8 were for the pit perimeter, 9-10 were setae locations, and 11-12 were for the capsule aperture (Figures 1.2-1.4). Because of the high variation in the shape and number of setae present my fixed landmarks are defined as the location of the main multiporose sensillum (landmark 9) and the length of the capsule aperture (landmarks 11-12). My semi-landmarks outline the overall shape of the pit (landmarks 1-8) as well as the average location of remaining setae (landmark 10, Figures 1.2-1.4). After the landmarks for every image were placed, their locations were uploaded into the software package MorphoJ (Kingenberg 2011). Using MorphoJ, a Procrustes superimposition was run so that all of the landmark data could be standardized. The Procrustes

superimposition helps insure that size, symmetry, or position of the leg are accounted for and ensures that these additional variables are not factored into the analyses. Overall, this process increases statistical significance validity of subsequent analyses (Rohlf 1994). A covariance matrix was generated and analyzed by principal component analysis (PCA) to understand the overall variation in shape. The final step taken was to run a Procrustes ANOVA to analyze the relationships among the following variables: all ticks, all males, all females, male *I. scapularis* versus female *I. scapularis*, male *A. americanum* versus female *A. americanum*, and male *D. variabilis* versus female *D. variabilis*. To further confirm my results, I ran a canonical variate analysis (CVA) and discriminant analysis (DA), which are multivariate analyses that have been used to identify taxonomic shape differences (Viscosi 2011). The main difference between the two is that a DA is used to compare two groups and a CVA is used to compare three or more groups (Timm 2002, Viscosi 2011)

When analyzing differences in shapes using both the CVA and DA I applied 10,000 permutations, which is considered a reasonable number of permutations for the majority of shape comparisons (Elewa 2010). The permutation test can be used to determine statistical significance because it does not assume any statistical distribution for shape variation (Elewa 2010). These analyses were done in MorphoJ.

I analyzed data for all tick species, all males and all females in both 3-way and pairwise comparisons. I also ran an intraspecific comparison of male and female ticks. Finally, I re-ran all morphometric analyses using only the eight landmarks around the pit of the Haller’s organ to ensure the setae (landmarks 9-10) and capsule aperture (landmarks 11-12) were not the sole source of variation in my 12 landmark analyses.

RESULTS

In total, 60 images were taken of the Haller's organ (Figure A.1) and 54 morphometric analyses were performed. My measurements showed that females of each species had larger Haller's organ than males. *Dermacentor variabilis* ticks had the largest Haller's organ while *I. scapularis* had the smallest Haller's organ (Table A.1).

Principal Component Analysis of Geometric Landmark Data

Figures 1.5-1.11 represent my six main comparisons of the tick species: all tick species, all males, all females, and intraspecific sex comparisons. When looking at all tick species (*I. scapularis*, *D. variabilis*, and *A. americanum*), principal components 1 and 2 accounted for the most variance 73.5% (61.2 % and 12.3%). The Haller's organ of *I. scapularis* is the most unique in this analysis, as it is non-overlapping with the other two species (Figure 1.5). For the male only and female only analyses, principal components 1 and 2 provided most variance 76.4% (66.3% and 10.1%) for males and 78.3% (59.4% and 18.9%) for females. The clusters representing *I. scapularis* are isolated from the other two species for both sex-specific analyses (Figure 1.6-1.7).

When running a PCA for intraspecific, between sex comparisons, I only found significant non-overlap of male and female clusters for *D. variabilis* (Figure 1.8). In this case, both principal components 1 and 2 provided the most variance 70.5% (52.1% and 18.4%). For *I. scapularis*, it was found that principal components 1 and 2 provided the most variance 68.7% (57.2% and 11.5%). I found no clear separation between male and female clusters (Figure 1.9). In the case of *A. americanum,* principal components 1, 2, 3, and 4 provided the most variance 69.4% (29.5%, 15.1%, 13.7%, and 11.1%). There was no clear separation of males and females in either graph for these analyses (Figure 1.10-1.11). The wireframe diagrams in all of my analyses show the locations of variance in PC1 and PC2 (Figure 1.5-1.10). However, the sex comparisons for the Haller's organ of *A. americanum* also include the wireframe diagrams to show the locations of variance in PC3 and PC4 (Figure 1.11).

Procrustes ANOVA Results

Overall I found that, regardless of sex, the Haller's organs of *I. scapularis*, *D. variabilis*, and *A. americanum* were all significantly different from each other (Table 1.1). Additionally, when comparing only males or only females of each species, my analyses reveals that the organ is also significantly different between species (Table 1.1). Intraspecific sex comparisons unveiled that *D. variabilis* shows high morphological variation between males and females, while *I. scapularis* and *A. americanum* do not (Table 1.1). Overall, my 8 landmark data analyses reflects the same results as found in the 12 landmark analyses (Table 1.2). The pairwise and symmetry morphometric analyses of the Haller's organ using both 12 landmarks and 8 landmarks, also supports my results from my initial 12 landmark 3-way comparisons (Table A.2-A.3).

CVA Results

With these analyses I found additional support that the Haller's organs of *I. scapularis*, *D. variabilis*, and *A. americanum* were all significantly different from each other (Table 1.3). However, the Procrustes distance revealed that the Haller's organ of *I. scapularis* differs the most morphologically. When comparing only males or only females, these ticks are still significantly different from each other (Table 1.4-1.5). Both my CVA and my DA comparing males and females of these species further confirm that only *D. variabilis* shows signs of sexual dimorphism (Table 1.6-1.7).

DISCUSSION

The ability to identify tick species is key to being able to narrow down the disease carrying potential of a particular tick. My morphometric analyses ANOVA results revealed that the Haller's organ of all three medically important tick species found in Illinois are morphologically different. My average approximate length and width data, although taken, proved to be difficult to draw conclusions from due to irregularity in overall Haller's organ morphology (Figure A.1). Although these data provide clear evidence that the Haller's organ varies morphologically among species, potential mechanisms as to why remain to be tested. Overall, the Haller's organ has been shown to have a common function among all ticks: host location (Sonenshine and Roe 2013). Therefore, one possible reason for differences in the morphology of these organs may be linked to how these ticks acquire hosts. *Amblyomma americanum* is considered to be aggressive when host seeking and will actively seek out hosts (Kollars et al. 2000). On the other hand, *D. variabilis* and *I. scapularis* are more likely to passively quest for their hosts (Bloemer 1988, Keirans et al 1996). It is possible that morphological differences observed in the Haller's organs of these species reflect an adaptation to increase the possibility of finding a preferred host. This is because with the exception of *D. variabilis,* which is more of a generalist species (Bloemer 1988, Dodds et al. 1969), these species are more commonly found on specific hosts in the adult life stage. Unlike the other species, *I. scapularis* uses the white tailed deer as their main host (Keirans et al. 1996). Another example is that adult lone star ticks are commonly found on wild turkeys; this appears to be a host unique to *A. americanum* (Kollars et al. 2000). Finally, differences in the Haller's organ morphology are not due to differences in the type of life cycle because all three tick species possess a three-host life cycle (Goddard et al 2008, Scoles 2004, Keirans et al. 1996).

A PCA acts as a method to redistribute total variance among data sets on orthogonal axes and cannot provide any definitive information about variance in overall shape (Elewa 2010). Thus, I cannot draw any conclusions from the PCAs about how the shapes are different between species, such as length, width, roundness, size, *etc*. Principal component analyses can be used to identify points of interest that may be

causing variances observed (Viscosi et al. 2011). However, my PCAs revealed that many landmarks are the cause of the variance and it is not possible to point out any single landmark without exhibiting a bias.

The ANOVA results revealed that differences in morphology between sexes was absent in *I. scapularis* and *A. americanum* but present for *D. variabilis*. Although both sexes find hosts and take blood meals, females gorge for a long period of time for adequate egg provisioning, while males take a small and short blood meal to prime their sperm for mating (Sonenshine and Roe 2013). In most cases, after feeding, the male locates a feeding female on the host and begins to mate with the female (Sonenshine and Roe 2013). Occasionally, male and female ticks in the genus *Ixodes* will mate off the host before either feeds (Sonenshine and Roe 2013). It is possible that the lack of sexual dimorphism is due to the fact that these differences in male and female behaviors are not governed through the use of the receptors in their Haller's organ. It is also possible that males and females of *D. variabilis* have varying sensory needs not found in other tick species, however, neither my data nor other existing work support or reject these hypotheses. I found that my CVA and DA analyses support my ANOVA results. The Haller's organs of *D. variabilis* and *A. americanum* are more similar to each other than *I. scapularis* which can be seen not only from the *P*-value, but the lower value for the Procrustes distance when compared to *I. scapularis.* This result was the same for both the only male and only female tick comparisons. One reason for this may be that *D. variabilis* and *A. americanum* might actually have a more similar host seeking behavior then that of *I. scapularis.* Although *D. variabilis* does quest for hosts, it has been found that this species occasionally aggressively seeks out hosts, a behavior also observed in *A. americanum* (Bloemer 1988, Kollars et al. 2000). It is possible that this Haller's organ morphology is linked to this aggressive host seeking behavior, which is seen in *A. americanum* (Bloemer 1988, Kollars et al. 2000). Additionally, my thin-plate spline graphs from each of these CVA and DA analyses exhibits the extremity of the morphological differences for each of my landmarks (Figures A.2-A.10). These graphs support the differences seen in my PCA wireframe graphs.

Although these data only support the conclusion that the Haller's organs of *I. scapularis*, *A. americanum*, and *D. variabilis* are morphologically different and that there

is sexual dimorphism in the Haller's organ of *D. variabilis*, this study contributes additional knowledge about the Haller's organ to other fields outside of morphometrics. It may be possible to utilize geometric morphometrics to analyze the Haller's organ as additional method to use to identify ticks to species, but further research is necessary.

In the future, larger scale studies should include more tick species to allow better comparisons of the morphologies of the Haller's organ to host seeking strategies (passive versus active) or host specificity (specialist versus generalist). It would also be interesting to conduct within genus comparisons in order to see if there is similarity in morphology among closely related species. Because I found evidence of sexual dimorphism in *D. variabilis*, it would also be useful to study the Haller's organ of nymphal and larval ticks of this species since these stages do not have any other easily observable sexual features. Adult females are more likely to spread disease due to their longer feeding times, therefore having a method for sexing ticks in earlier life stages and determining the sex ratios within populations, can possibly lead to better predictions of disease spread. The Haller's organ is important to ticks for locating hosts and any additional information about the organ, such as is described in this study, ultimately helps in designing better tick management strategies and tick borne disease control programs.

CHAPTER 2: IDENTIFICATION OF CHEMOPERCEPTION GENE EXPRESSION IN THE HALLER'S ORGAN OF *IXODES SCAPULARIS* (ACARI: IXODIDAE)

INTRODUCTION

Many ticks belonging to the family Ixodidae (hard ticks) are major disease vectors worldwide. Currently ticks are gaining even more importance in the medical and veterinary fields because of their involvement in the spread of emerging diseases (Parola et al. 2005, Sonenshine and Roe 2013, Ostfeld et al. 2015). Additionally, the incidence of known diseases, such as Lyme disease, is growing as well. The black-legged tick, *Ixodes scapularis,* is the main vector for Lyme disease. Both the disease and host ranges are expanding further across the United States as well as into Canada (Simon et al. 2014). Despite their importance to human and animal health, ticks still remain relatively understudied disease vectors.

The mechanisms of chemosensory in insects have been studied in some detail. However, this area has not been as intensely studied in other arthropods such as ticks. The study of chemoreceptors, such as odorant receptors (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) found in antennae and other chemosensory organs of insects has been the focus of genomic studies. These studies have been key in understanding how insects such as longhorn beetles detect pheromones (Mitchell et al. 2012) or how gall flies find host plants (Andersson et al. 2014). Additionally, major disease vectors such as *Anopheles gambiae* and *Aedes aegypti* are the focus of many studies concerning olfactory receptors, which overall has increased our understanding of how these mosquitoes locate their hosts (Carey et al. 2010, Bohbot et al. 2007).

In contrast to insects, ORs have not been annotated from non-insect arthropod genomes. The OR family evolved in insects, which explains their absence in annotated, non-insect arthropods such as the crustacean *Daphnia pulex* and the centipede *Strigamia maritima* (Peñalva-Arana et al. 2009, Chipman et al. 2014). GRs have been found to be related to ORs and many insect GRs are used to detect pheromones required for mating as well as carbon dioxide (Hallem et al. 2006). Unlike GRs, ionotropic receptors are not related to ORs. It has been argued that IRs are actually

related to another chemosensory family, which includes ionotropic glutamate receptors (iGluR) due to the homology of their molecular structures. Insect IRs have been found to detect various acids and amines (Rytz et al. 2013). Due to the recent sequencing of the *Ixodes scapularis* genome, it is now possible to study tick chemoreceptors at the genetic level, specifically IRs and GRs (Hill et al. 2005).

Ticks need to find an animal host in order to transmit diseases. They find their host by using a specialized sensory organ, found on the tarsi of the forelegs, called the Haller's organ. Although not all functions of the Haller's organ are understood, the organ contains sensilla involved in mechanosensation and olfaction. The Haller's organ is responsible for recognizing chemicals such as carbon dioxide, ammonia, and hydrogen sulfide, as well as other common host odorants (Sonenshine and Roe 2013). Additionally, a study on *Amblyomma hebraeum* (South African bont tick) concluded that this organ plays a factor in sensing tick pheromones as well (Rechav et al. 1977).

In this project, I have identified the gustatory receptors and ionotropic receptors potentially expressed in the Haller's organ of *Ixodes scapularis* by sequencing RNA libraries constructed from adult male and female forelegs and hindlegs, assembling the reads into transcriptomes, and comparing relative gene expression levels. I expected to see that some *I. scapularis* GRs and IRs would be foreleg specific due to the fact that the Haller's organ is solely found in the forelegs of ticks. Additionally, I wanted to determine if any of the tick receptors were sex specific or if any receptors had orthologs to described insect GRs or IRs. My GR phylogeny suggests an absence of *I. scapularis* GRs related to described insect fructose receptors, sugar receptors, and carbon dioxide receptors. The Burrows-Wheeler Alignment (BWA) analysis indicates there may be a few GRs directly involved with chemoperception in the Haller's organ. Additionally, I found that there might be GRs that are more highly expressed in the female Haller's organ, compared to males. The IR phylogeny presents evidence of two conserved IRs, Ir25a and Ir93a, which have been identified in multiple arthropods, as well as another 14 identified in the Ixodes genome project (H. M. Robertson, manuscript submitted), and another 76 intron-less and divergent IR genes. My data supports expression of IsIr25a and IsIr93a solely in the *I. scapularis* forelegs, indicating that these IRs are likely to be present in the Haller's organ. There was no indication of any sex specificity in the IRs.

MATERIALS AND METHODS

Tick Collection and Processing

Ticks were collected from three locations: Allerton Park (Piatt County, Illinois), Danada (DuPage County, Illinois) and Waterfall Glen County Forest Preserve, (DuPage County, Illinois). All three locations contain oak forest woodland and are recognized as sites with *I. scapularis* (Rydzewski 2011, “Ticks,” 2014). Tick collection took place from April-May 2014, during peak adult activity. Ticks were collected alongside existing trails using dragging and flagging methods (Rydzewski 2011). After every 2-3 meters, the drag cloth was inspected, and any *Ixodes spp.* were collected and kept alive in vials.

After collection, ticks were brought back to the lab and identified to species (Keirans et al. 1989). Over the course of the collection period, more than two hundred *I. scapularis* ticks were collected: 101 males and 112 females. All ticks identified as *I. scapularis* were then individually placed on a chill plate. Using sterilized forceps, forelegs and hindlegs of male and female ticks were removed and placed in separate RNase-free ©Eppendorf tubes embedded in pelleted dry-ice. Tick carcasses were also placed into tubes based on sex, and then all six tubes were stored in a -80˚C freezer for further processing.

RNA Isolation and Gels

The tubes containing legs were very briefly centrifuged, and the contents were placed in separate 1mL RNase-free glass tissue grinders containing 500µL Trizol (Invitrogen). The tissues were ground, and another 500µL Trizol were added to the tissue grinders. The leg tissues were ground further and then centrifuged over a Qiashredder column (Qiagen) for 1 minute at 16000Xg at room temperature. The column flow-through was transferred to a 1.5mL tube. Next, 200µL chloroform and 1µL linear polyacrylamide (LPA, 10mg/mL) were added to each tube. The tubes were shaken for 15 seconds, incubated at room temperature for 3 minutes and centrifuged for 10 minutes at 12000Xg at 4˚C. The aqueous layer from each tube was transferred to a

new tube, and then 650µL isopropanol was added to the tubes. The tubes were inverted, incubated for 10 minutes at room temperature and centrifuged for 10 minutes at 12000Xg at 4˚C. The supernatant was decanted from the pellets, and then 500µL of 80% ethanol was added to each tube followed by brief mixing. The samples were then centrifuged for 5 minutes at 7500Xg at 4˚C. The ethanol was decanted and the pellets were air-dried. The pellets were then resuspended in 25µL of RNase-free water and stored overnight in the -20˚C freezer.

All RNA samples were quantified using a Qubit fluorometer (Life Technologies). This was done using the Qubit RNA Broad Range Assay Kit following the kit instructions. Either 100ng or 250ng of total RNA from the four leg samples were visualized using ethidium bromide on a 1.0% agarose gel in TBE Buffer, which ran at 94V for 1 hour.

Sequencing and IR and GR Identification

The University of Illinois at Urbana-Champaign W.M. Keck Center for Comparative and Functional Genomics prepared the RNAseq libraries from total RNA sheared to an average fragment size of 250nt using the TruSeq Stranded RNAseq Sample Prep kit from Illumina. The 4 libraries were individually barcoded and quantitated using qPCR before pooling and sequencing both ends with the TruSeq SBS Sequencing Kit version 3 on a single lane of a HiSeq2500 instrument. The data was processed with Casava 1.8.2 (Illumina) before conversion into FASTQ files.

The FASTQ files were evaluated with FASTQC: (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to determine the degree of 5' trimming needed to remove biased nucleotides. Reads were trimmed at the 5’ end and the 3’ end to remove low quality bases (-t 20) with the FASTX-Toolkit software (http://hannonlab.cshl.edu/fastx_toolkit/).

Trimmed reads from all 4 leg samples were assembled together with SOAPdenovo-Trans-127mer v1.02 (http://soap.genomics.org.cn/SOAPdenovo-Trans.html) using default parameters with the exception of K=49. Scaffolding was allowed using available mate-pairs. Sub-assemblies were also generated for each

specific tissue. Trinity (Release 2014-04-13) (http://trinityrnaseq.github.io/), was also used to generate a second transcriptome assembly of all 4 tissue types with default parameters, except minimum reported contig length=100. Each algorithm has it own strengths and weaknesses, and their output can be compared to check transcript completeness.

H.M. Robertson (University of Illinois at Urbana-Champaign) initially built GR gene models from the available *Ixodes scapularis* genome (Vectorbase, http://www.vectorbase.org, *Ixodes scapularis*), and some IR models were provided by Richard Benton (University of Lausanne; pers. comm.) Transcripts from both transcriptome assemblies were aligned against the models when available to validate, improve, or correct the models, and additional models were added for the IRs.

Alignment and Phylogenetic Trees

The IR and GR amino acid sequences were aligned with CLUSTALX v2.0 (Larkin et al. 2007) using its default settings. The alignments were trimmed using TRIMAL v1.4 (Capella-Guiterrez et al. 2009), which only retained positions present in more than 80% of the sequences. For phylogenetic analyses I used PHYML v3.0 (Guindon et al. 2010) with its default settings to determine maximum likelihood and FIGTREE v1.4 (http://tree.bio.ed.ac.uk/software/figtree/) to build trees. The resulting IR and GR trees were then color coded and further labeled in Adobe Illustrator.

BWA analysis

To determine relative levels of gene expression, trimmed RNAseq reads from each leg library were separately aligned against the IR and GR cDNA models using the Burrows-Wheeler Aligner (BWA) (Li *et al.* 2009a) using default "aln/samse" settings in single-end mode. Samtools (Li *et al.* 2009b) converted the BWA output into a bam file that was then sorted, indexed, and summarized in a tab-delimited format for comparison. BWA analysis results were compared to raw GR reads in order to insure that the expression levels I obtained correlated with my raw data.

RESULTS

Sequencing and IR and GR Identification

The raw FASTQ files yielded approximately 52 million paired reads from the female forelegs library, 53 million paired reads from the female hindlegs library, 66 million paired reads from the male forelegs library, and 52 million paired reads from the male hindlegs library.

H.M. Robertson (University of Illinois at Urbana-Champaign) built 62 GRs models from the *Ixodes scapularis* genome. With the four tick RNAseq libraries combined, SOAPdenovo-Trans assembled 10 of the GR models as transcripts. Trinity assembled 35 GR models as transcripts.

Of the 15 distinct IRs provided by R. Benton (University of Lausanne; pers. comm.), the SOAPdenovo-Trans and Trinity transcriptomes assemblies confirmed 14 of these IR models. SOAPdenovo-Trans assembled 8 transcipts and Trinity assembled 14 transcripts. Additionally, I built 76 intron-less IR gene models from the existing *Ixodes scapularis* genome, none of which were present in the Benton lab set.

Alignment and Phylogenetic Trees

During the alignment process in CLUSTALX v2.0 (Larkin et al. 2007), all amino acid sequences for both GRs and IR aligned well except for IsIr279. IsIr279 was excluded from my IR phylogenetic tree due to inconsistencies during alignment (it may not be a real IR).

Based on phylogentic analysis, none of the *I. scapularis* GRs are related to the known carbon dioxide, sugar, or fructose receptors found in *Drosphila melanogaster*, *Apis mellifera*, and *Tribolium castaneum*. Of the 72 total IsGrs mapped in the tree (Figure 2.1), there are 49 IsGrs grouped together in an IsGr specific expansion. There are 11 IsGrs that appear to be sister groups to multiple GRs belonging to the predatory mite, *Metaseiulus occidentalis*.

All of the intron-less IsIrs are grouped in an IsIr specific expansion (Figure 2.2). Seven of the remaining IsIrs were found to be grouped in an IsIr specific expansion as a sister group to multiple *M. occidentalis* IRs. Ir25a and Ir93a, are conserved among all four groups, *D. melanogaster* (DmIr25a and DmIr93a), *Zootermopsis nevadensis* (ZnIr25a and ZnIr93a)*, M. occidentalis* (MoIr25a and MoIr108), and *I. scapularis* (IsIr25a and IsIr93a). There is no evidence for an *I. scapularis* IR equivalent to Ir8a, which is found in *D. melanogaster* and *Z. nevadensis* (Rytz et al. 2013).

BWA analysis

After running the Burrows-Wheeler Aligner analysis, I can conclude that there are 8 IsGrs of interest. Overall, the number of BWA read alignments are quite low. However, IsGr1FIX, IsGr21FIX, IsGr26, IsGr32FIXb, and IsGr35 can be considered of interest because there are read alignments only in the tick forelegs libraries (Figure 2.4-2.5). Another IsGr of interest is IsGr9 because there are read alignments only in the female forelegs. Two IsGrs, IsGr11FIX and IsGr15, are also striking because they have read alignments specific to the female tick libraries.

There are 2 IRs, IsIr25a and IsIr93a, which appear to be expressed in only the forelegs of both male and female ticks (Figure 2.6). The rest of the IRs have very few BWA read matches (hits) (Figure 2.7-2.9). Of the IRs in these tables only two IRs are considered of interest: IsIr271 and IsIr276. Even though the BWA number of reads was low for IsIr271 and IsIr276, the reads in the foreleg libraries of both sexes are higher than the hindleg libraries. None of the IRs showed any signs of sex specific expression.

DISCUSSION

Phylogenetic Trees

The GR phylogenetic tree presented no evidence of any IsGrs having obvious orthologs to any described carbon dioxide GRs of insects. I expected to find some evidence of a carbon dioxide receptor due to the fact that ticks use the Haller's organ to detect carbon dioxide (Sonenshine and Roe 2013). Although the tree does not indicate a receptor or group of receptors linked to carbon dioxide detection, it is probable that non-insect arthropods have their own set of GRs to detect carbon dioxide. Like ticks, mosquitoes are hematophagus, and there is evidence that mosquitoes express Gr21a and Gr63a, the gustatory receptors linked with carbon dioxide reception, in their maxillary palps (Jones et al., 2007). I found that IsGrs have no common orthologs to the most conserved sugar or fructose GRs of insects. If ticks are capable of sensing sugar and fructose, then it is possible that they have their own set of GRs for these odorants as well.

The IR phylogenetic tree indicates that Ir25a and Ir93a are conserved across the four groups I included in the tree: *D. melanogaster*, *Zootermopsis nevadensis, M. occidentalis,* and *I. scapularis*. There is evidence that Ir25a may be an ancestral IR since it has been found to be conserved across many protostomes such as in *Lottia gigantea* and *Capitella capitata* both of which are organisms outside of the phylum Arthropoda. (Rytz et al. 2013). The function of neither Ir25a nor Ir93 has not yet been identified (Rytz et al. 2013). In insects, there is evidence that Ir8a detects acids as well as other odors (Rytz et al. 2013), however my tree does not support any IsIr to be related to Ir8a. My findings cannot conclude that ticks cannot sense acids. In fact, it has been shown that the tick *Rhipicephalus microplus (*the cattle tick, formerly *Boophilus microplus*) can sense heptanoic acid (Osterkamp et al. 1999). In the case of both GRs and IRs, further investigation is required in order to identify tick equivalents to these well studied GRs and IRs of insects. Once identified, they can be placed on a phylogenetic tree and compared for relatedness to the described insect GRs and IRs.

BWA Analysis

The GRs and IRs of interest I describe here are currently the only set of tick receptors with evidence suggesting that they are either foreleg- or sex-specific receptors. Although I am not able to make definitive conclusions about the gene expression of these receptors in these ticks, I have outlined receptors in need of further investigation.

The GRs of interest, IsGr1FIX, IsGr9, IsGr11FIX, IsGr15, IsGr21FIX, IsGr26, IsGr32FIXb, and IsGr35, fall into three major categories: foreleg-specific, female-specific, and female foreleg-specific. I expected to find some GR's unique to the tick forelegs because the Haller's organ, the main chemosensory structure of ticks, is found on the forelegs only (Sonenshine and Roe 2013). It is possible that the GRs expressed in both male and female forelegs, IsGr1FIX, IsGr21FIX, IsGr26, IsGr32FIXb, and IsGr35 are involved in the Haller's organ functions in host and mate-finding (Rechav et al. 1977, Sonenshine and Roe 2013).

I also found that some of these GRs may be sex-specific, e.g. IsGr9, IsGr11FIX, and IsGr15. Since only *I. scapularis* males are solely responsible for finding a mate (Sonenshine and Roe 2013) I expected some IsGrs to be male specific because the Haller's organ has been found to aid in mate finding (Rechav et al. 1977). In contrary to my expectations, IsGrs appear to be female-specific, not male specific. This finding contradicts what has been have found in some insects. For instance, a previous study comparing GRs of male and female gall-inducing flies found no sex-specific differences in GR expression (Andersson et al. 2014). Although there has not been any research comparing specifically GR expression of hematophagus insects, a comparison of ORs, another type of chemosensory receptor, in *Anopheles gambiae* revealed ORs that were highly expressed in females and not in males (Iatrou et al. 2008). In ticks, unlike mosquitoes, males are required to both find a host and take a small blood meal, which stimulates the production of sperm. These vital needs, finding a host and taking a blood meal, are shared traits between both sexes (Oliver 1989, Sonenshine and Roe 2013). Therefore, it is possible that female-specific IsGrs in ticks are involved in the process of

a female finding a suitable area to lay her clutch. After feeding, female *I. scapularis* will fall of her host and crawl through vegetation and lay her eggs in an area of high humidity and appropriate temperature (Harris 1959, Peavey et al. 1996). This provides some explanation for the low expression levels. The female tick tissues used in this study were from unfed females and laying a clutch only occurs after a blood meal. Therefore, these unfed ticks would not need to highly express any GRs related to locating an ideal spot for a clutch. The conflicting results from published work concerning sex specific chemoreceptors in insects (Iatrou et al. 2008, Andersson et al. 2014), along with varying sensory needs of male and female ticks, allow further consideration of IsGr9, IsGr11FIX, and IsGr15 as receptors of interest. Ideally, with further research it will be possible to confirm or reject sex specificity of these GRs and discover why these differences exist. It is also important to note that the low number of IsGr transcripts assembled compared to total GRs modeled from the genome may be due to the narrow focus on tick fore- and hindleg gene expression. Other GRs might be expressed in the tick palps and chelicerae, which are also involved in tick chemoreception (Waladde 1982, Sonenshine and Roe 2013).

I found three IsIrs of interest (IsIr271, IsIr25a, and IsIr93a) since they appear to be foreleg specific. Although IsIr271 had a low number BWA read matches, it may just be expressed at low levels. Because of the high read matches, it is very likely that IsIr25a and IsIr93a are in fact expressed IRs in the forelegs of *Ixodes scapularis*. Because of the leg specificity of these IRs, IsIr25a and IsIr93 are likely to be directly related to the Haller's organ function. Unfortunately, because the functions of Ir25a and Ir93a remain uncharacterized I cannot make any definitive conclusions about how these IRs are utilized (Rytz et al. 2013). Some studies have shown that in *D. melanogaster*, Ir25a acts as a co-receptor to many other IRs such as Ir75d and Ir41a. Both Ir75d and Ir41a have been found in the mosquitoes *Aedes aegypti* and *Anopheles gambiae* (Rytz et al. 2013). This could mean that Ir25a may have some involvement in controlling other IRs related to host finding. It was not surprising that there was no evidence of sex-specific IRs because previous studies on insects have found few sex specific IRs (Andersson et al. 2014). It is also likely that my total number of IRs found to be expressed were lower than the number of IRs found in the *Ixodes scapularis* genome.

This is probably due to the fact that I only was looking at IR expression in the fore- and hindlegs, not all tissues.

My study has barely scratched the surface of chemoperception by the Haller's organ of *Ixodes scapularis* and chemoperception of this tick as a whole. Future studies would include running a Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) of these four tissues: female forelegs, female hindlegs, male forelegs, and male hindlegs. Focusing only on my IRs and GRs of interest mentioned above, qRT-PCR can be used to help support my arguments concerning RNA expression of the IsGrs and IsIRs (Heid et al. 1996). Once the receptors expressed are known, ligand identification using frog oocyte assays would follow (Mitchell et al. 2012). These processes would lead to a better understanding of the Haller's organ as a whole because it would be possible to identify which IRs and GRs are expressed as well as what these receptors can sense.

This study, along with additional techniques outlined above, should be repeated under a number of different conditions, which could also further knowledge about *I. scapularis* chemoperception. Additional comparisons would be mated versus unmated ticks and fed versus unfed female ticks. Looking at IR and GR expression in other life stages of *I. scapularis* may also be of interest. Different life stages of *I. scapularis* utilize different hosts, so IR and GR expression may vary in nymphal or larval ticks to those found in adults (Sonenshine and Roe 2013). Comparing IR and GR expression in the palps and Haller's organ is another possibly interesting research direction. Ticks utilize their palps (located neat their mouthparts) to find feeding spots and to identify suitable females (Sonenshine and Roe 2013). There is evidence that the tick palps express GRs and ticks utilize their palps to find feeding spots on hosts as well as to identify mates (Sonenshine and Roe 2013). When more tick genomes become available, it would be interesting to compare IR and GR expression of 1-host ticks such as *Rhipicephalus microplus* and 3-host ticks such as *Ixodes ricinus* (Osterkamp et al. 1999, Sonenshine and Roe 2013). These two tick species' questing responses to different volatiles vary significantly (Osterkamp et al. 1999). With a deeper understanding of the IR and GR expression of *I. scapularis*, monitoring and trapping methods of these ticks can be improved, leading to better control of the ticks and the diseases they spread.

FIGURES AND TABLES

Table 1.1 – ANOVA results using 12 Landmarks

Samples compared – LM=12	Shape, f-value	df	Shape, p-value
All Three Tick Species	52.42	40	<0.0001*
All Male Ticks	30.94	40	<0.0001*
All Female Ticks	34.42	40	<0.0001*
I. scapularis Males versus Females	0.60	20	0.9156
A. americanum Males versus Females	1.29	20	0.1796
D. variabilis Males versus Females	9.99	20	<0.0001*

Table contains the results of my initial comparisons

* Signifies a p-value denoting significant differences

Table 1.2 – ANOVA results using 8 Landmarks

Samples compared – LM=8	Shape, f-value	df	Shape, p-value
All Three Tick Species	27.95	24	<0.0001*
All Male Ticks	14.62	24	<0.0001*
All Female Ticks	27.39	24	<0.0001*
I. scapularis Males versus Females	0.81	12	0.6355
A. americanum Males versus Females	1.76	12	0.0562
D. variabilis Males versus Females	13.40	12	<0.0001*

Table contains the results of my initial comparisons.

* Signifies a p-value denoting significant differences

Table 1.3 – CVA: All Ticks

	D. variabilis	***A. americanum***
A. americanum	0.1367	
	P= <0.0001*	
I. scapularis	0.3637	0.3839
	P= <0.0001*	*P*= <0.0001*

Top value is Procrustes distance; bottom value is *P* value

* Signifies a p-value denoting significant differences

Table 1.4 – CVA: Male Ticks

	D. variabilis	***A. americanum***
A. americanum	0.1148	
	P= <0.0001*	
I. scapularis	0.3822	0.3754
	P= <0.0001*	*P*= <0.0001*

Top value is Procrustes distance; bottom value is *P* value

* Signifies a p-value denoting significant differences

Table 1.5 – CVA: Female Ticks

	D. variabilis	***A. americanum***
A. americanum	0.2197	
	P= <0.0001*	
I. scapularis	0.3747	0.3935
	P= <0.0001*	*P*= <0.0001*

Top value is Procrustes distance; bottom value is *P* value

* Signifies a p-value denoting significant differences

Table 1.6 – CVA: Male versus Female Ticks

	***A. americanum* Female**	***D. variabilis* Female**	***I. scapularis* Female**	***A. americanum* Male**	***D. variabilis* Male**
D. variabilis	0.2190				
Female	*P*=<0.0001*				
I. scapularis	0.3931	0.3742			
Female	*P*=<0.0001*	*P*=<0.0001*			
A. americanum	0.0611	0.2053	0.3840		
Male	*P*=0.2212	*P*=<0.0001*	*P*=<0.0001*		
D. variabilis	0.0916	0.1828	0.3849	0.1146	
Male	*P*=0.0029*	*P*=<0.0001*	*P*=<0.0001*	*P*=<0.0001*	
I. scapularis	0.3896	0.3605	0.0465	0.3762	0.3829
Male	*P*=<0.0001*	*P*=<0.0001*	*P*=0.6333	*P*=<0.0001*	*P*=<0.0001*

Top value is Procrustes distance; bottom value is *P* value

* Signifies a p-value denoting significant differences

Table 1.7 – DA: Male versus Female Ticks

	D. variabilis	***A. americanum***	***I. scapularis***
Procrustes distances	0.1846	0.0620	0.0481
***P*-value**	*P*= <0.0001*	*P*= 0.2090	*P*= 0.6510
T^2 value	T^2=<0.0001	T^2=0.0830	T^2=0.4210

* Signifies a p-value denoting significant differences

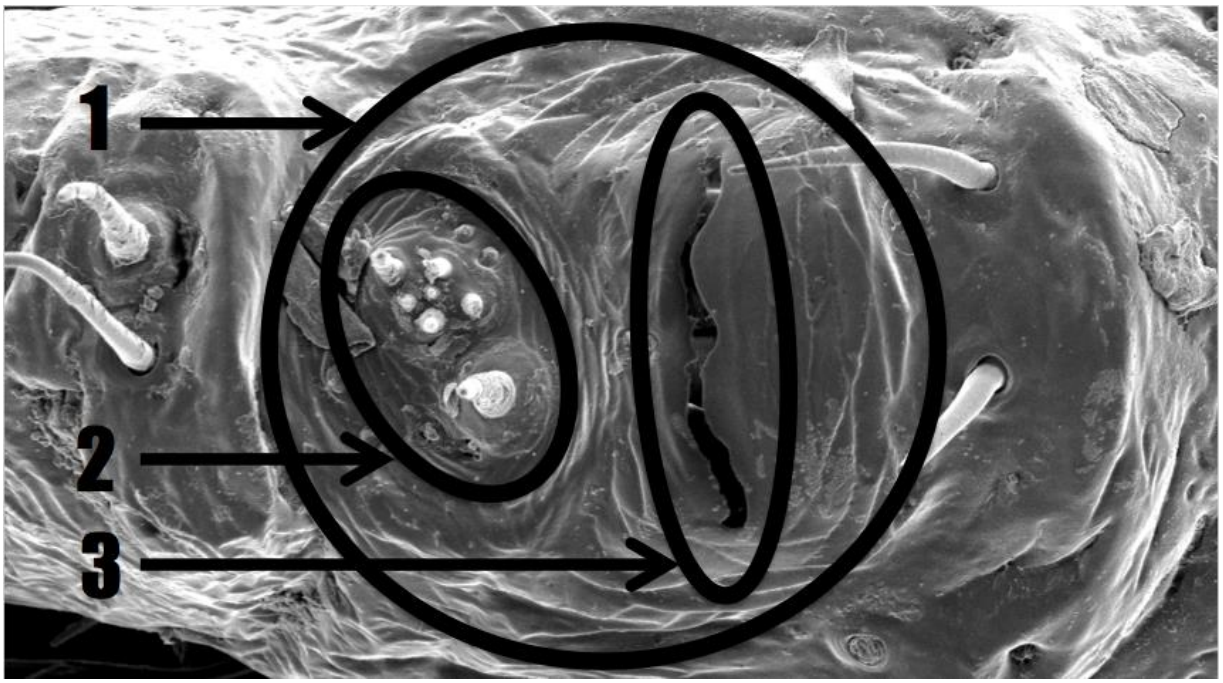

Figure 1.1 – The Haller's organ of *Amblyomma americanum*. 1 outlines the whole pit, 2 outlines the anterior pit containing setae, and 3 outlines the capsule aperture.

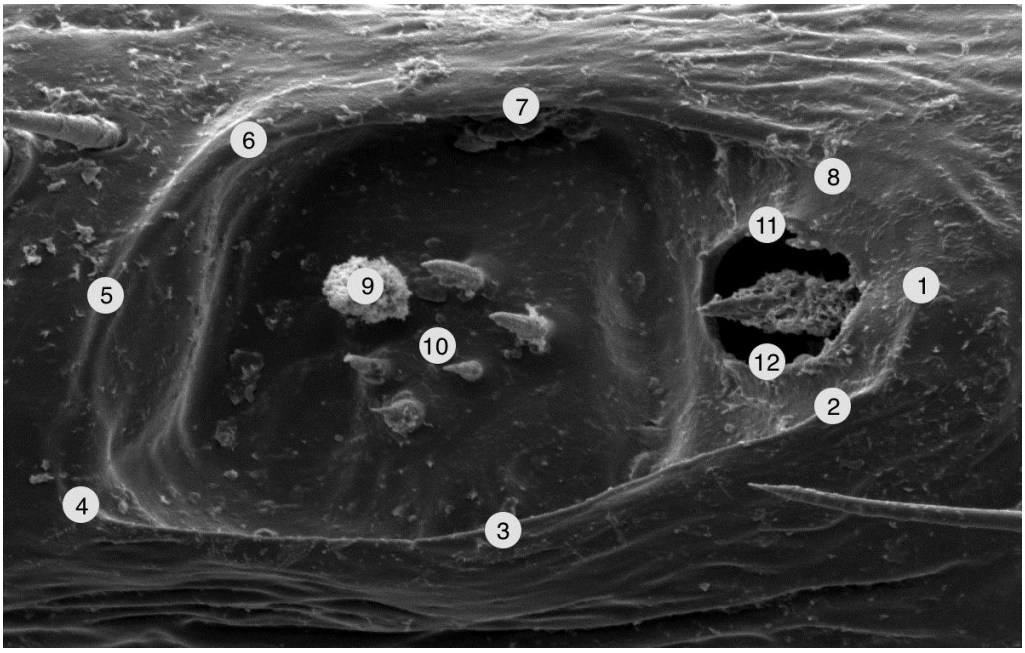

Figure 1.2 – Image of the landmarks placed on the *Ixodes scapularis* Haller's organ

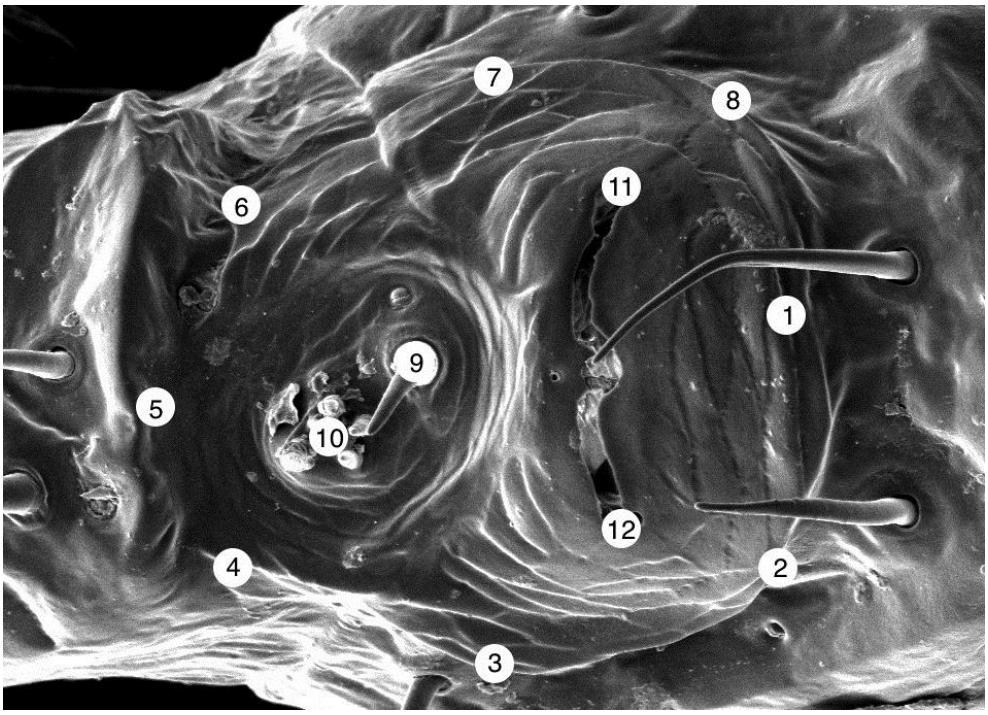

Figure 1.3 – Image of the landmarks placed on the *Amblyomma americanum* Haller's organ

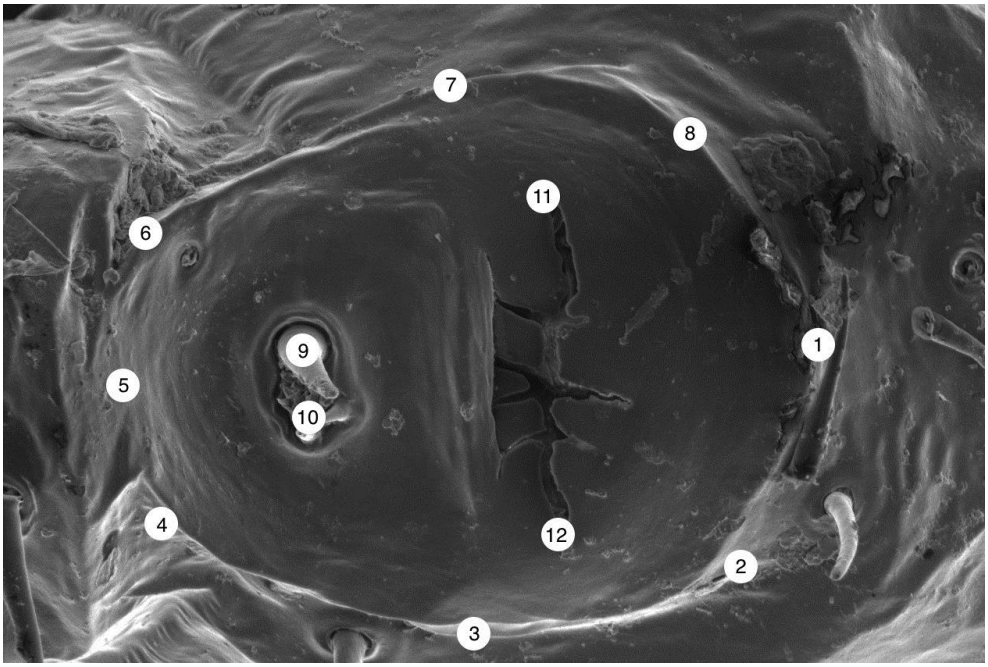

Figure 1.4 – Image of the landmarks placed on the *Dermacentor variabilis* Haller's organ

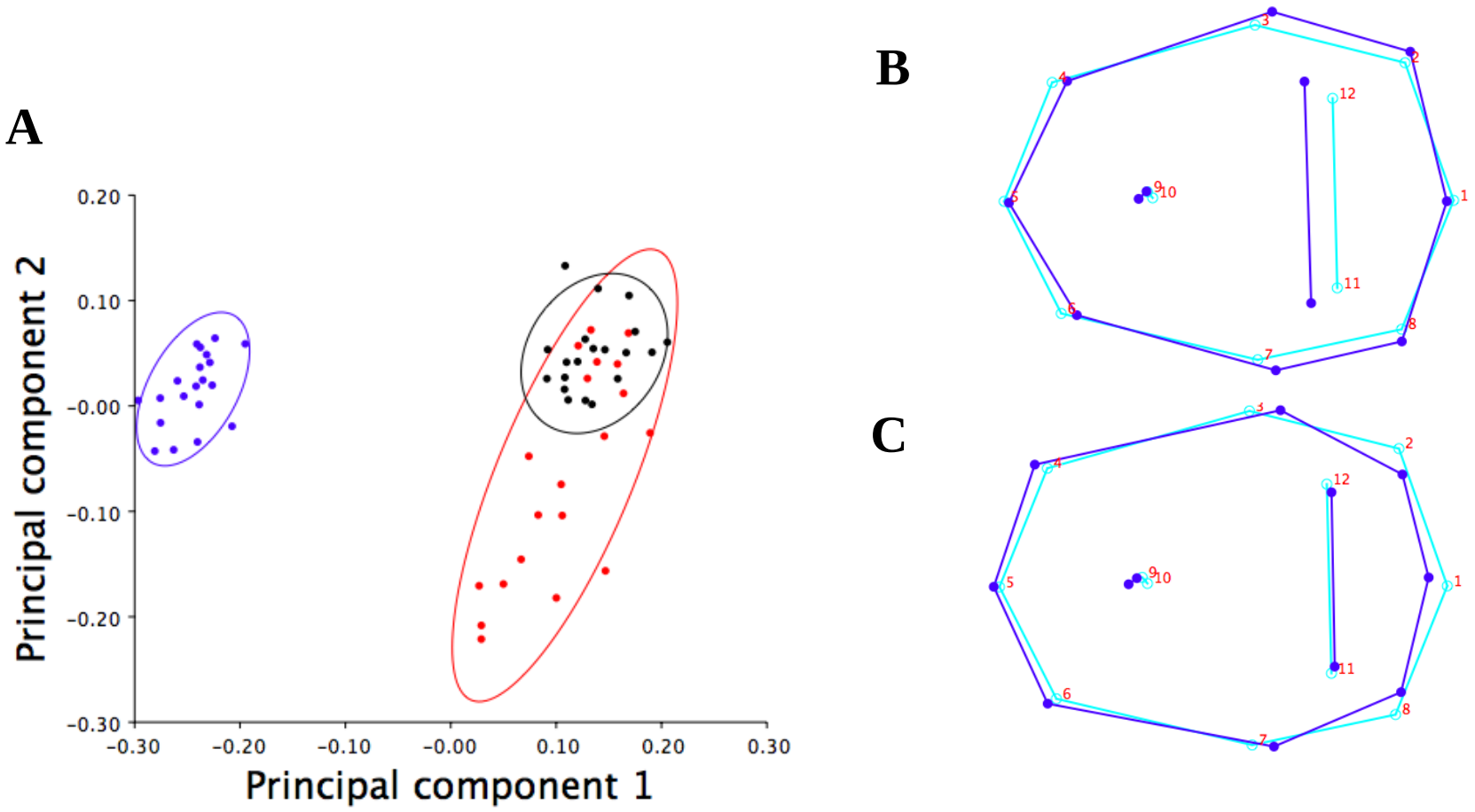

Figure 1.5 – All ticks principal component analysis: **A**: scatter of the geometric landmark data from only males of all three tick species: *D. variabilis* (black), *A. americanum* (red), and *I. scapularis* (blue), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

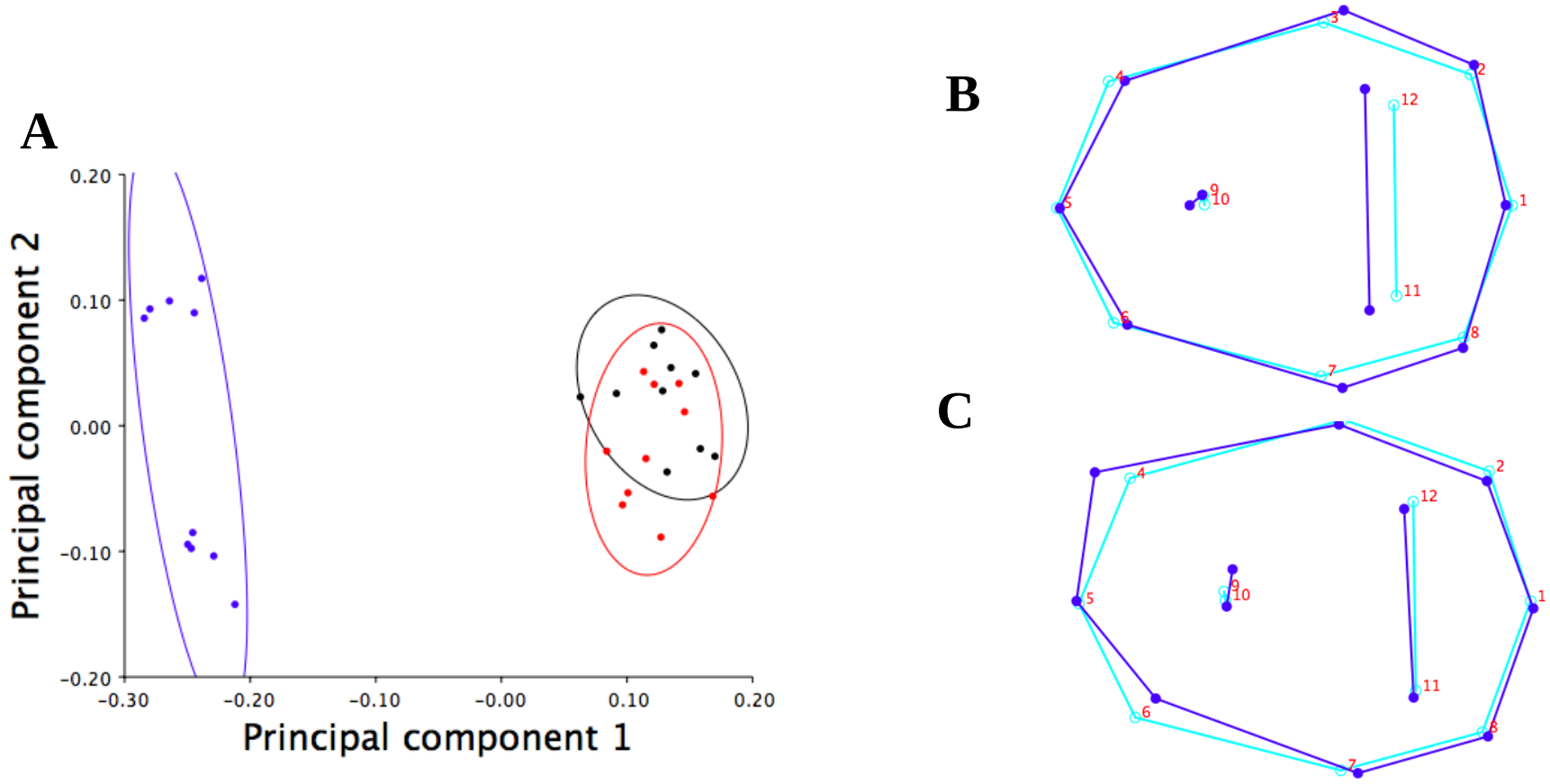

Figure 1.6 – All male ticks principal component analysis: **A**: scatter of the geometric landmark data from only males of all three tick species: *D. variabilis* (black), *A. americanum* (red), and *I. scapularis* (blue), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

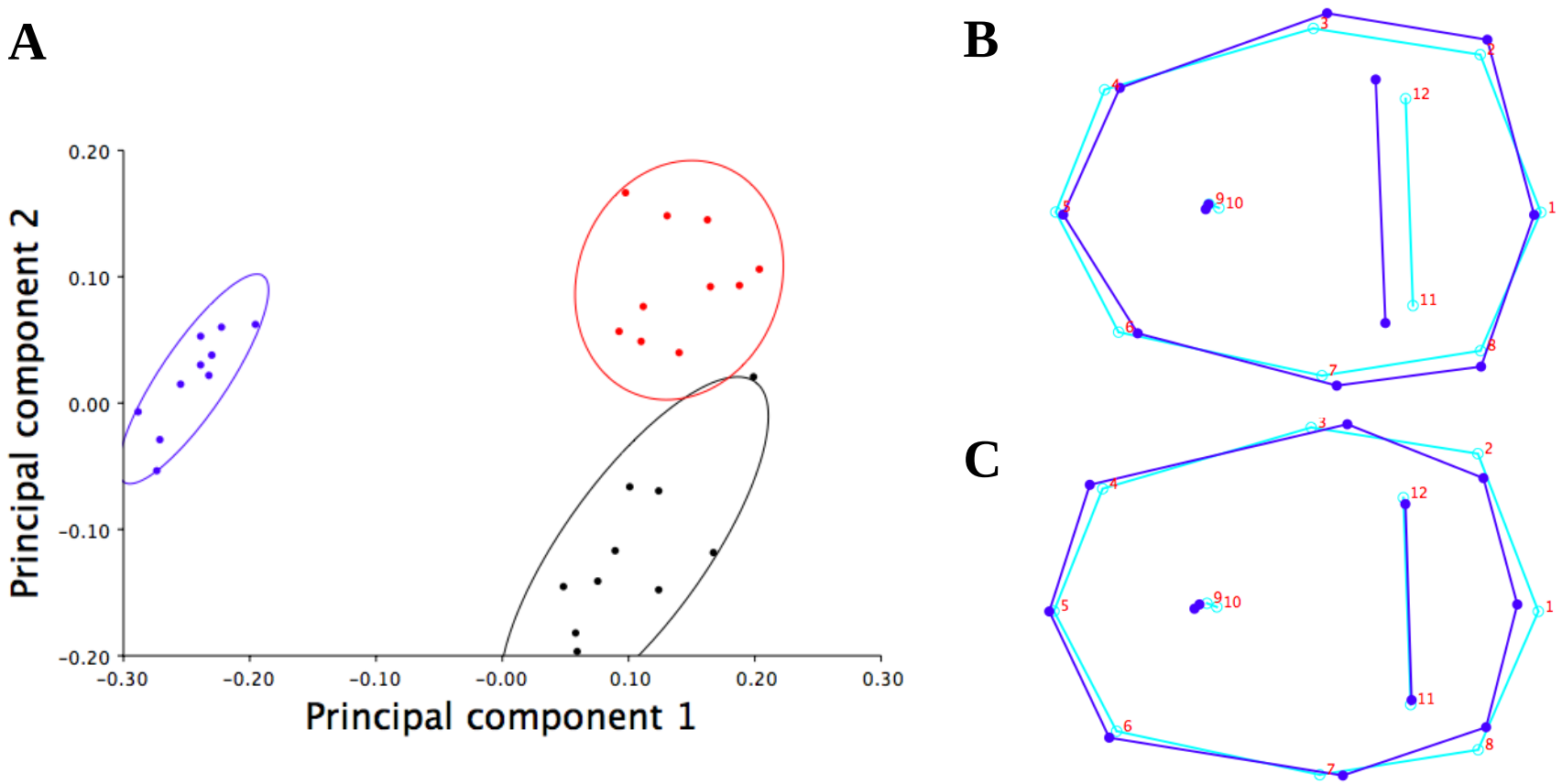

Figure 1.7 – All Female Ticks Principal component analysis: **A**: scatter of the geometric landmark data from only females of all three tick species: *D. variabilis* (black), *A. americanum* (red), and *I. scapularis* (blue), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

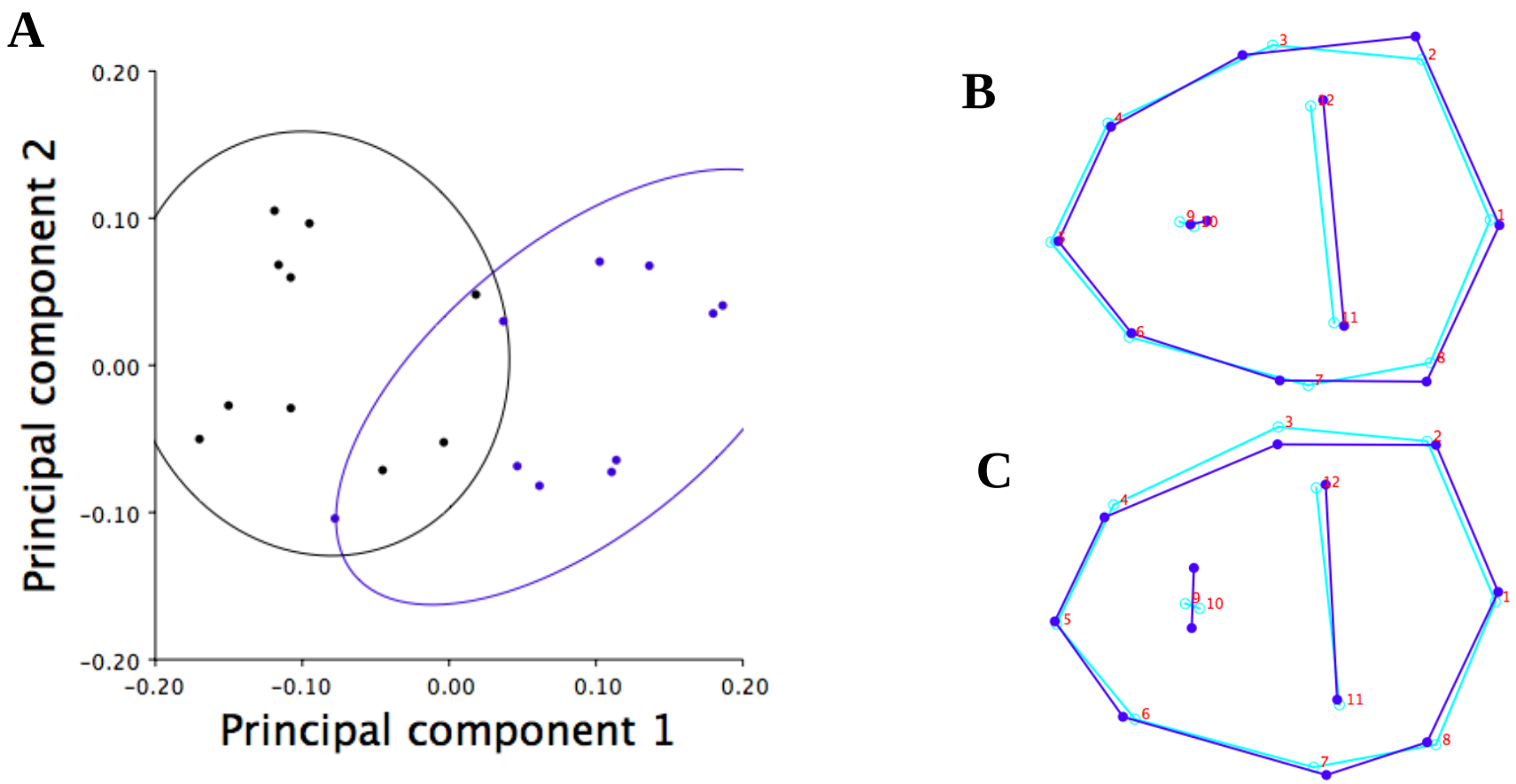

Figure 1.8 – *D. variabilis* sexual dimorphism principal component analysis:: **A**: scatter of the geometric landmark data , female (blue) and male (black), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

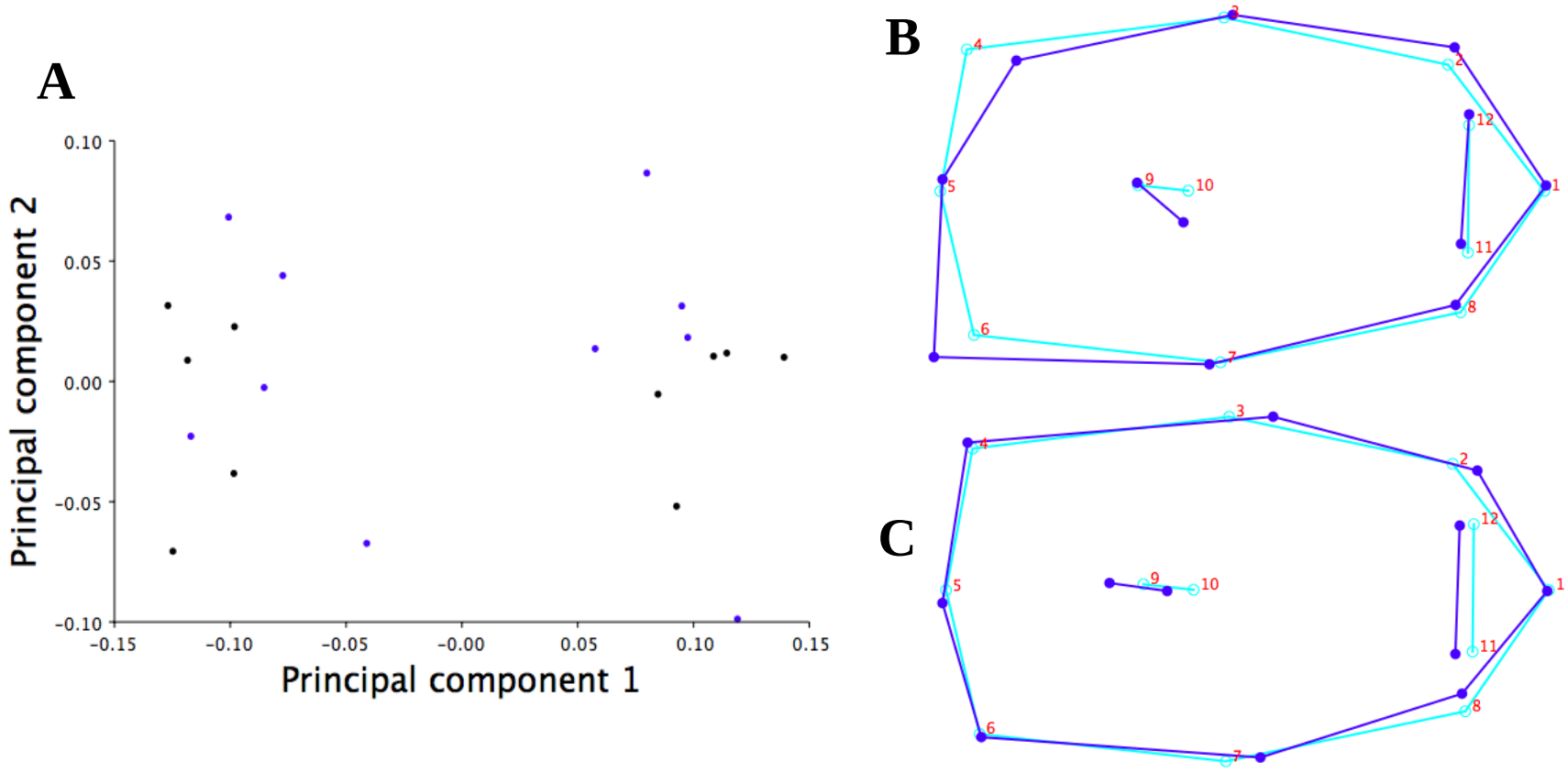

Figure 1.9 – *I. scapularis* sexual dimorphism principal component analysis:: **A**: scatter of the geometric landmark data , female (blue) and male (black), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

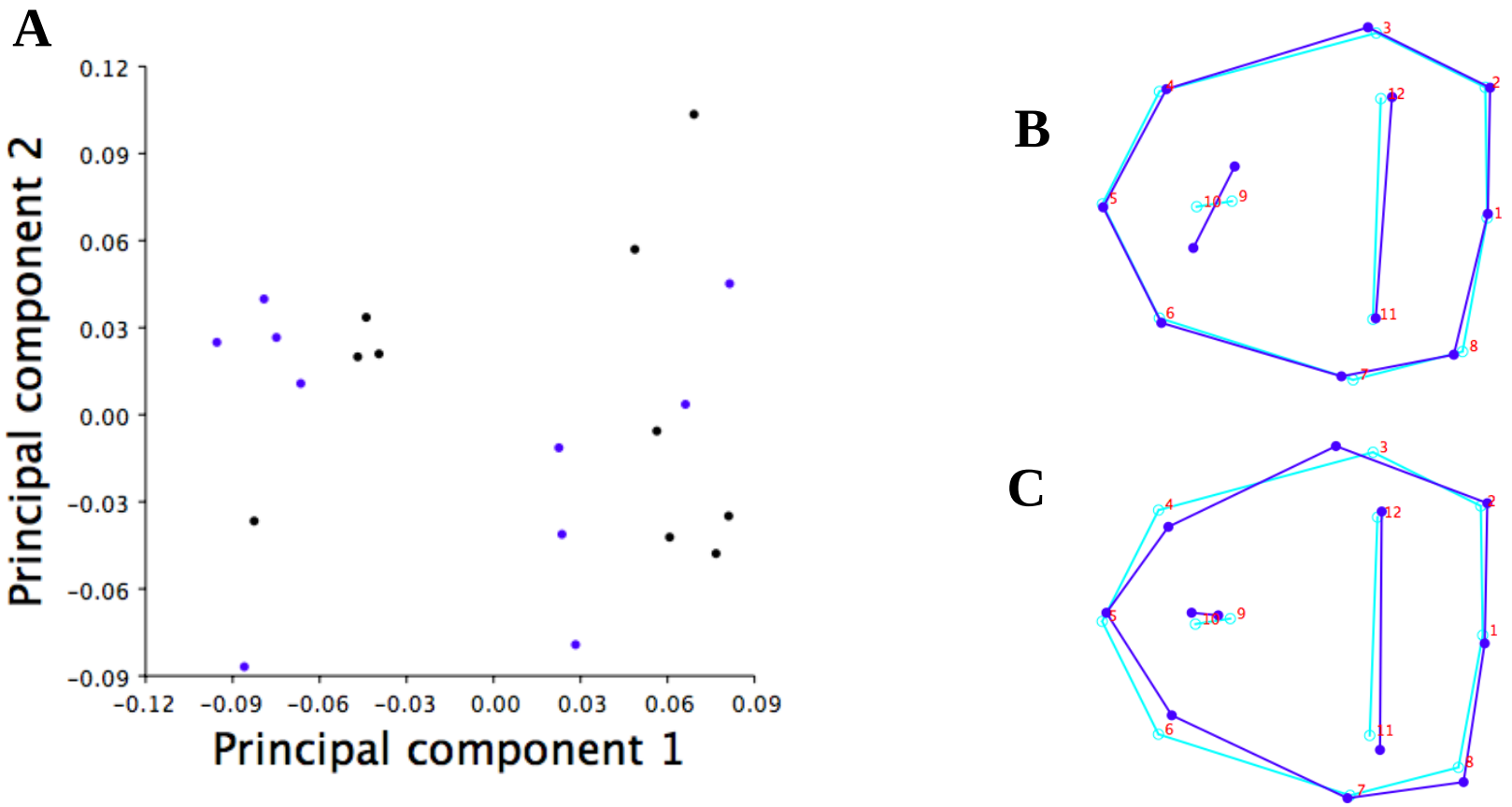

Figure 1.10 – *A. americanum* sexual dimorphism principal component analysis:: **A**: scatter of the geometric landmark data , female (blue) and male (black), **B**: Wireframe connecting landmarks of PC1 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC2 (blue) over average shape of the sample (cyan)

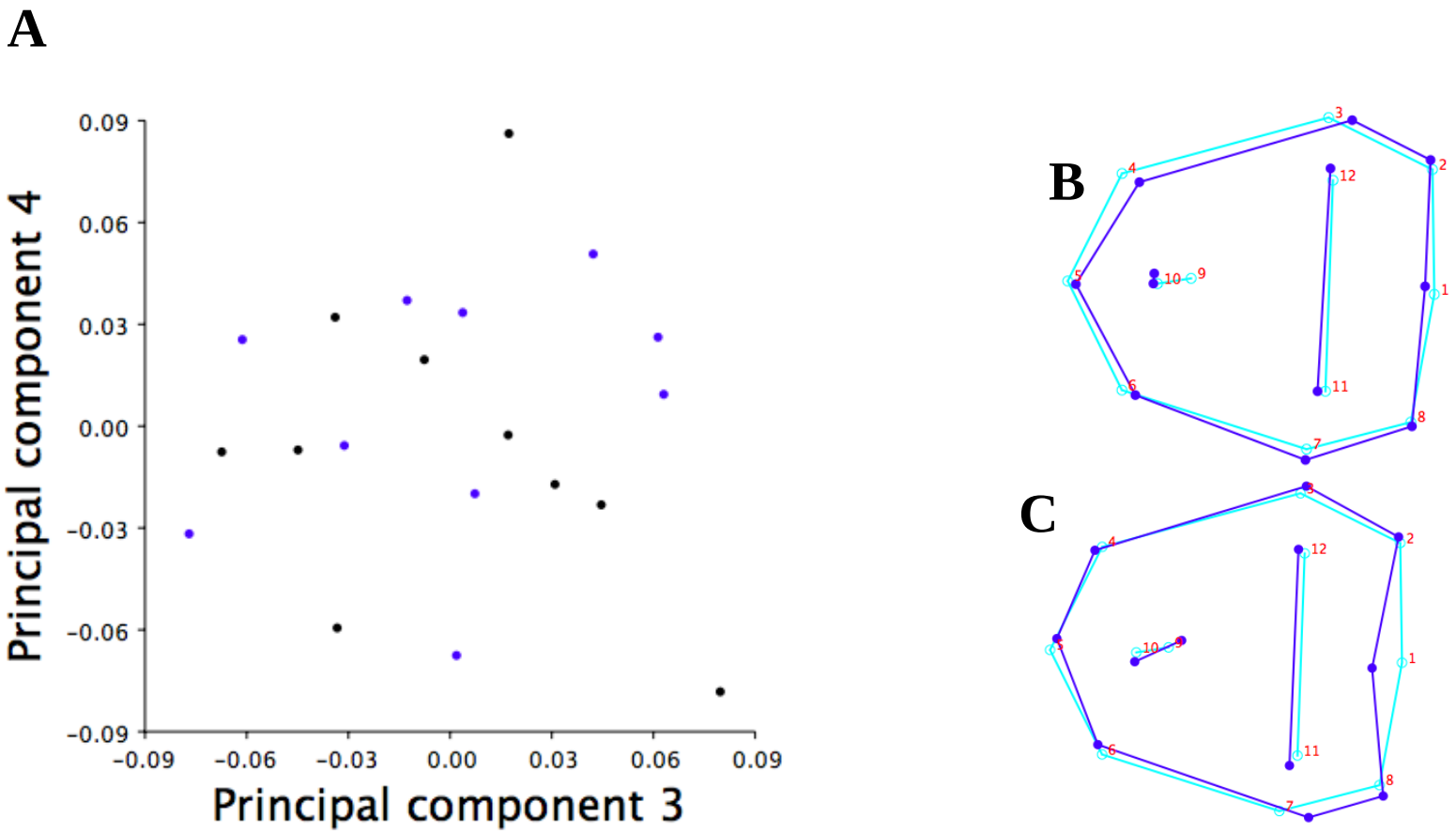

Figure 1.11 – *A. americanum* sexual dimorphism principal component analysis: **A**: scatter of the geometric landmark data for examination of possible in: female (blue) and male (black), **B**: Wireframe connecting landmarks of PC3 (blue) over average shape of the sample (cyan), **C**: Wireframe connecting landmarks of PC4 (blue) over average shape of the sample (cyan)

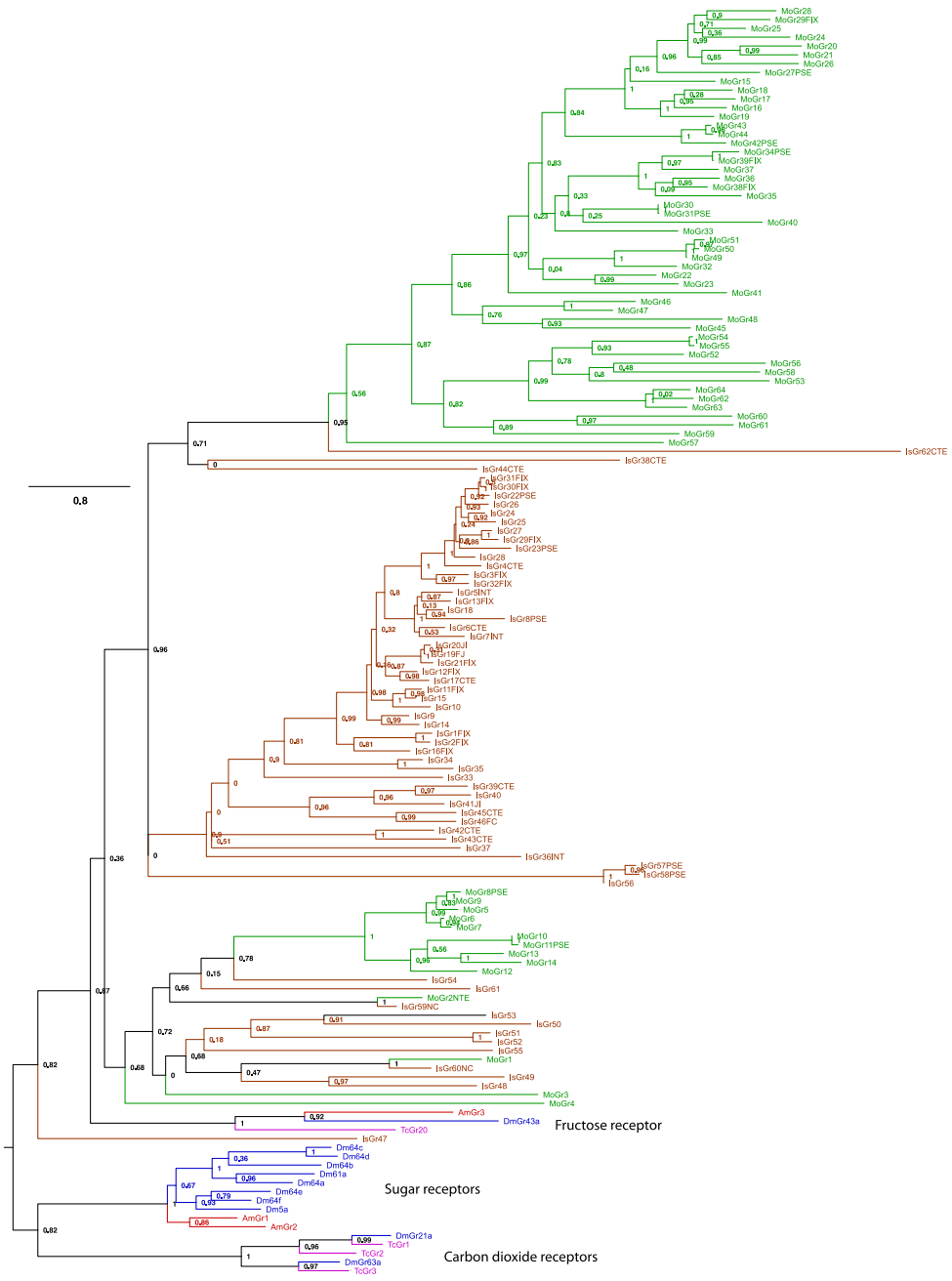

Figure 2.1 – GR sequences are of *Drosphila melanogaster* (blue), *Apis melifera* (red), *Tribolium castaneum* (pink), *Metaseiulus occidentalis* (green), and *Ixodes scapularis* (brown). *Ixodes scapularis* has no GRs related to existing carbon dioxide, sugar, nor fructose receptors. There are some (49) *I. scapularis* GRs that are grouped in an IsGr specific expansion as well as multiple (11) GRs with mixed relation to various *Metaseiulus occidentalis* GRs.

Figure 2.2 – IR sequences are of *Drosphila melanogaster* (blue), *Zootermopsis nevadensis* (purple), *Metaseiulus occidentalis* (green), and *Ixodes scapularis* (brown). The intron-less *I. scapularis* IRs are grouped in an IsIR specific expansion. Of the remaining *I. scapularis* IRs, 7 are grouped in an IsIr specific expansion as a sister group to many *Metaseiulus occidentalis* IRs. Ir93a and Ir25a are conserved among all four groups

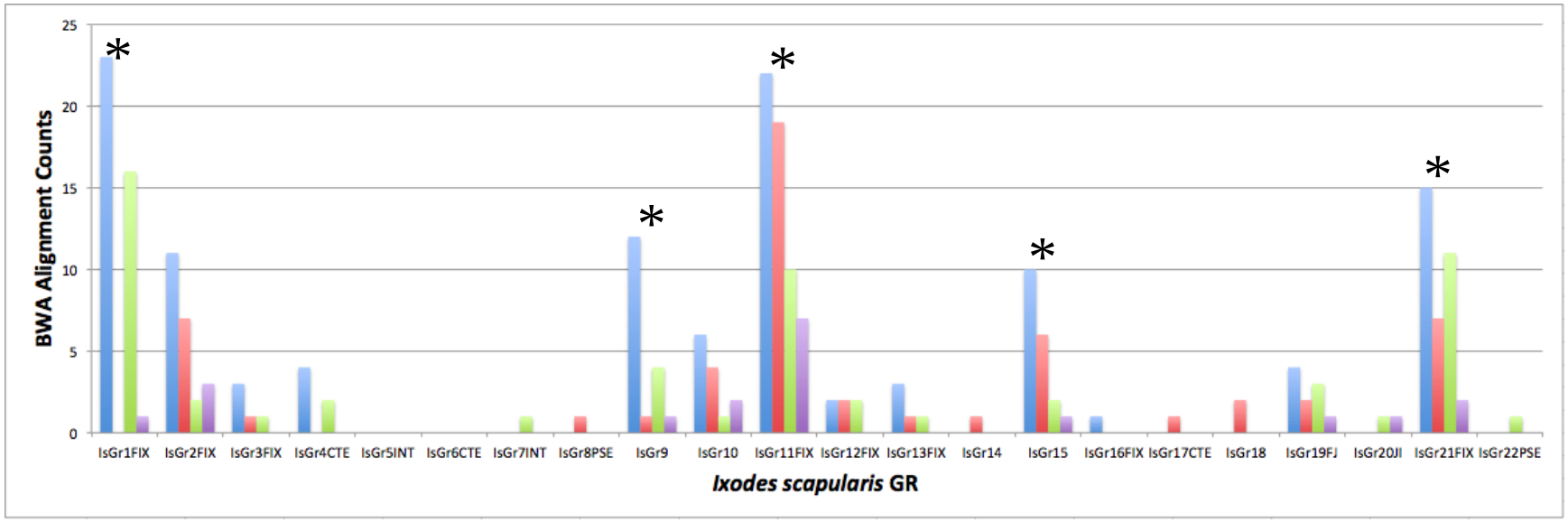

Figure 2.3 – IsGr1Fix to IsGr22PSE; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple). * Receptor of interest

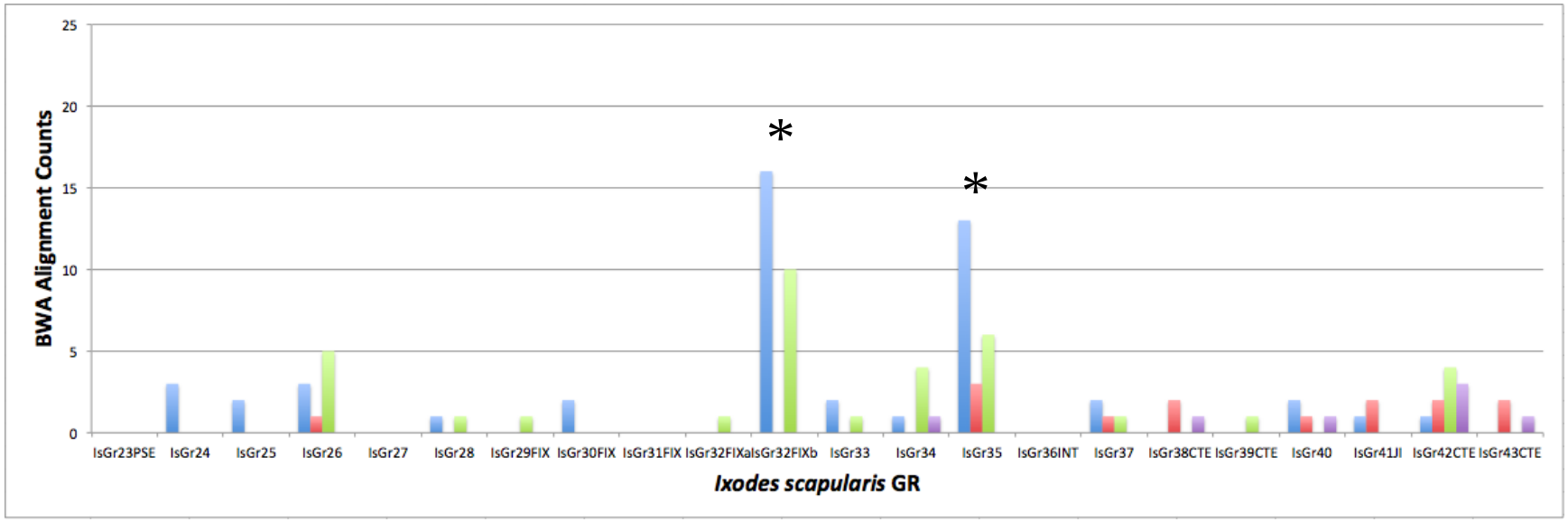

Figure 2.4 – IsGr23PSE to IsGr43CTE; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple). * Receptor of interest

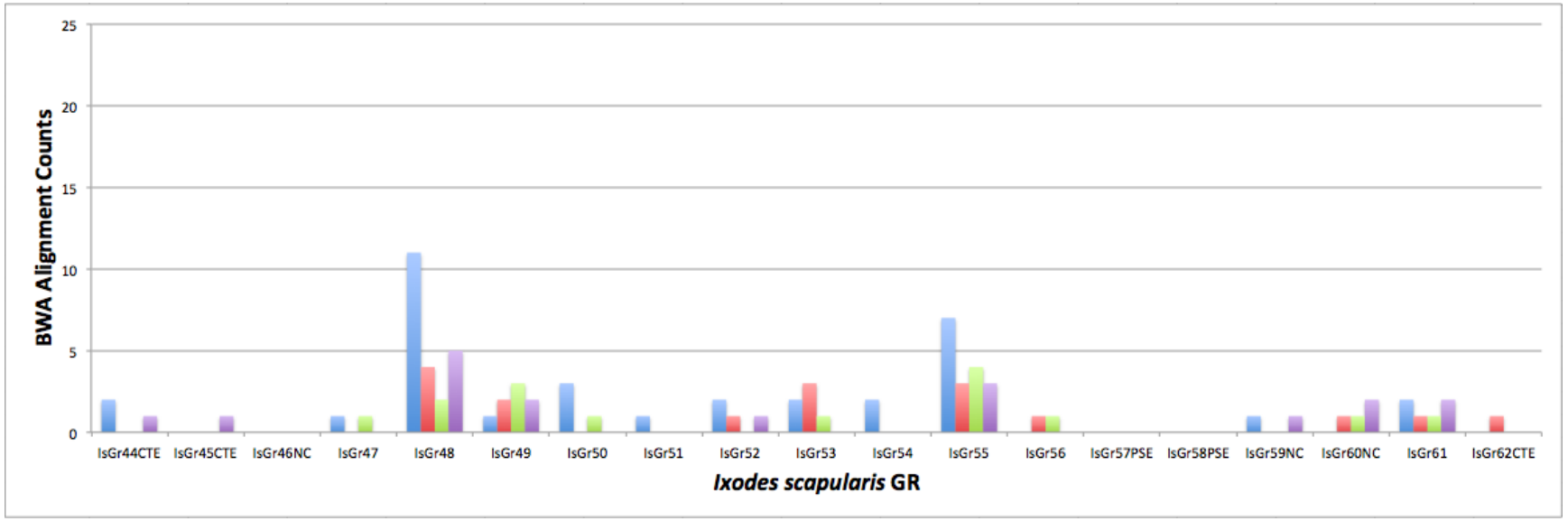

Figure 2.5 – IsGr44CTE to IsGr62CTE; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple).

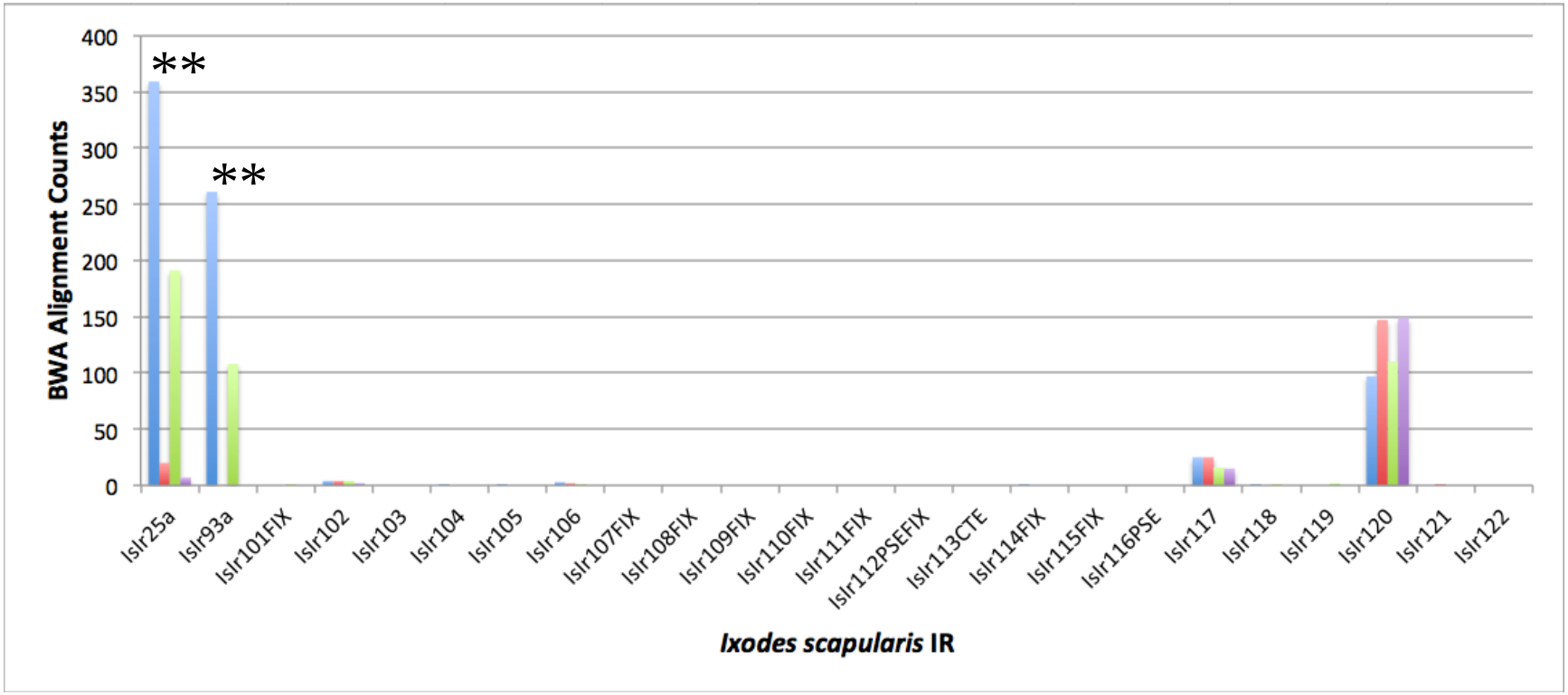

Figure 2.6 – IsIr25a to IsIr122; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple). ** Receptor with high BWA read alignments

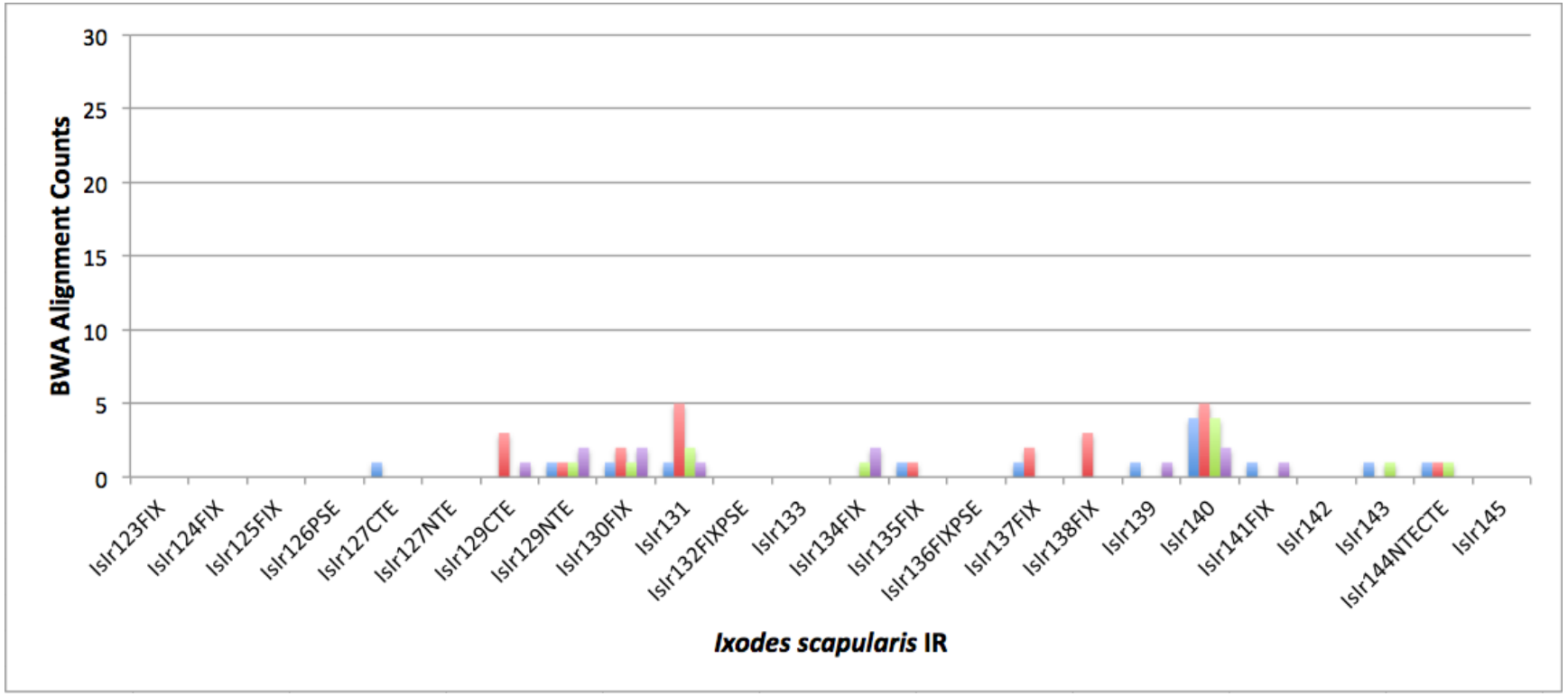

Figure 2.7 – IsIr123 to IsIr145; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple).

(Note the change in y-axis values from previous table; these values will remain the same in following two tables)

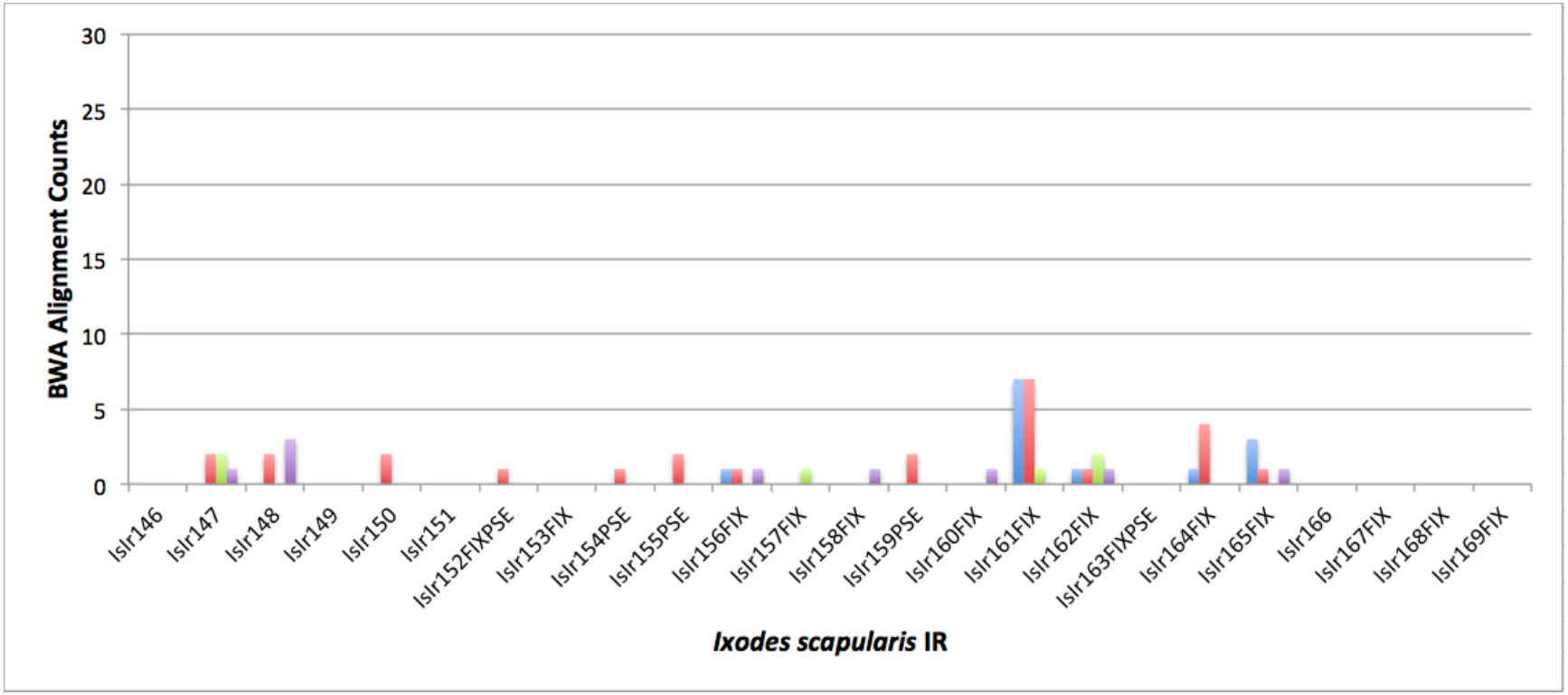

Figure 2.8 – IsIr146 to IsIr169FIX; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple).

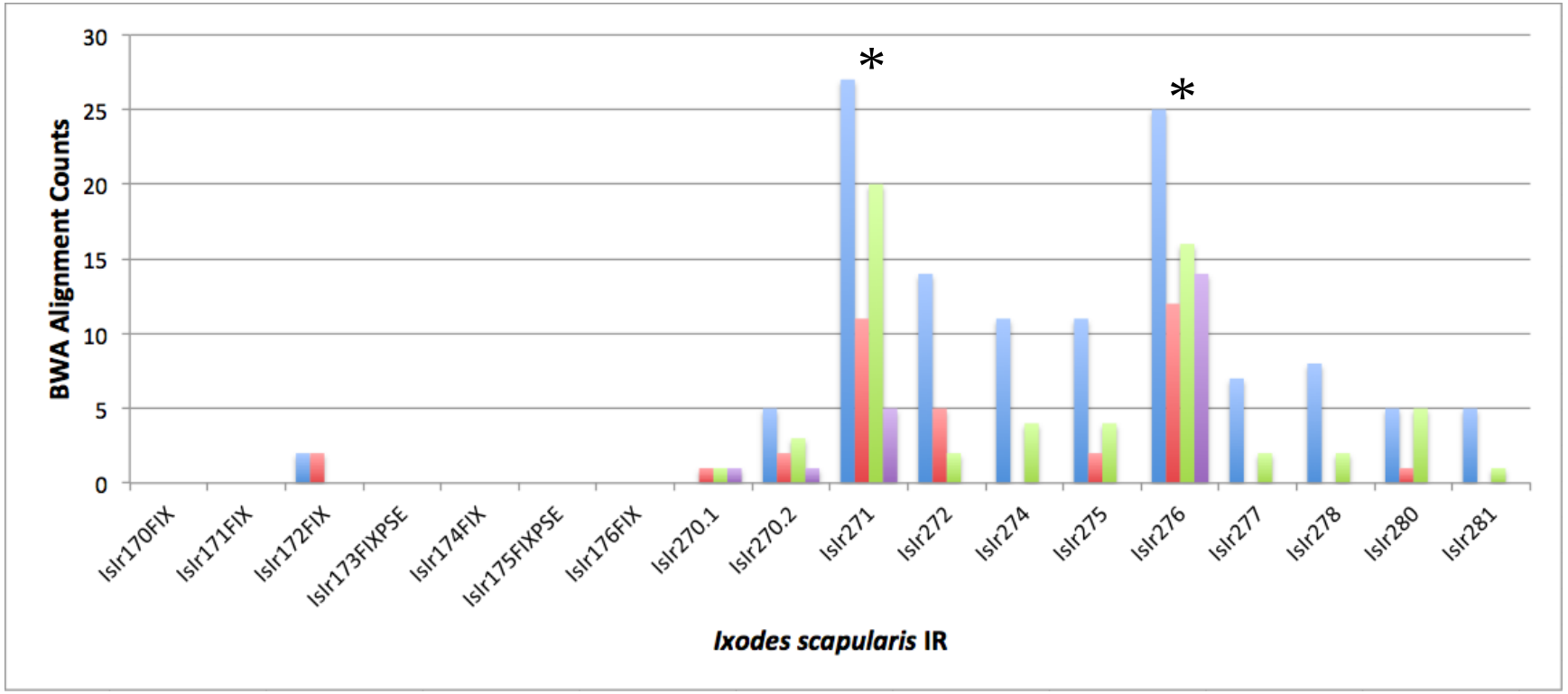

Figure 2.9 – IsIr170FIX to IsIr281; Female forelegs (blue), female hindlegs (red), male forelegs (green) and male hindlegs (purple). * Receptor of interes

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APPENDIX

Table A.1 – Haller's organ measurements

Tick Sample	Average Approximate Length (μm)	Average Approximate Width (μm)	Average Capsule Length (μm)	Average number of setae
Female *I. scapularis*	60.06	36.08	13.58	7
Male *I. scapularis*	59.15	36.82	13.56	6
All *I. scapularis*	59.61	36.45	13.57	6.5
Female *A. americanum*	95.74	89.02	62.24	6.8
Male *A. americanum*	89.37	88.3	58.53	6.9
All *A. americanum*	92.56	88.66	60.38	6.85
Female *D. variabilis*	107.89	94	63.42	5.9
Male *D. variabilis*	103.87	84.23	56.64	5.7
All *D. variabilis*	105.88	89.12	60.03	5.8

Table A.2 – ANOVA results using 12 Landmarks

Samples compared – LM=12	Shape, f-value	df	Shape, p-value
All *D. variabilis* versus all *A. americanum*	9.45	20	<0.0001*
All *I. scapularis* versus all *A. americanum*	92.16	20	<0.0001*
All *I. scapularis* versus all *D. variabilis*	63.38	20	<0.0001*
All M *D. variabilis* versus all M *A. americanum*	4.43	20	<0.0001*
All F *D. variabilis* versus all F *A. americanum*	14.66	20	<0.0001*
All M *I. scapularis* versus all M *A. americanum*	42.69	20	<0.0001*
All F *I. scapularis* versus all F *A. americanum*	49.63	20	<0.0001*
All M *I. scapularis* versus all M *D. variabilis*	43.44	20	<0.0001*
All F *I. scapularis* versus all F *D. variabilis*	39.72	20	<0.0001*
All Three Ticks Left Leg only	44.24	40	<0.0001*
All Three Ticks Right Leg only	36.75	40	<0.0001*
All Male Ticks Left Leg	26.54	40	<0.0001*
All Male Ticks Right Leg	29.27	40	<0.0001*
All Female Ticks Left Leg	26.81	40	<0.0001*
All Female Ticks Right Leg	28.48	40	<0.0001*
Males versus Females *Ixodes* Left Leg	1.25	20	0.2231
Males versus Females *Ixodes* Right Leg	1.06	20	0.3983
Males versus Females *Amblyomma* Left Leg	0.96	20	0.5121
Males versus Females *Amblyomma* Right Leg	1.14	20	0.3188
Males versus Females *Dermacentor* Left Leg	3.83	20	<0.0001*
Males versus Females *Dermacentor* Right Leg	12.72	20	<0.0001*

Top section contains the results of my pairwise comparisons. The bottom section contains the results of my left or right side only comparisons.

* Signifies a p-value denoting significant differences

Table A.3 – ANOVA results using 8 Landmarks

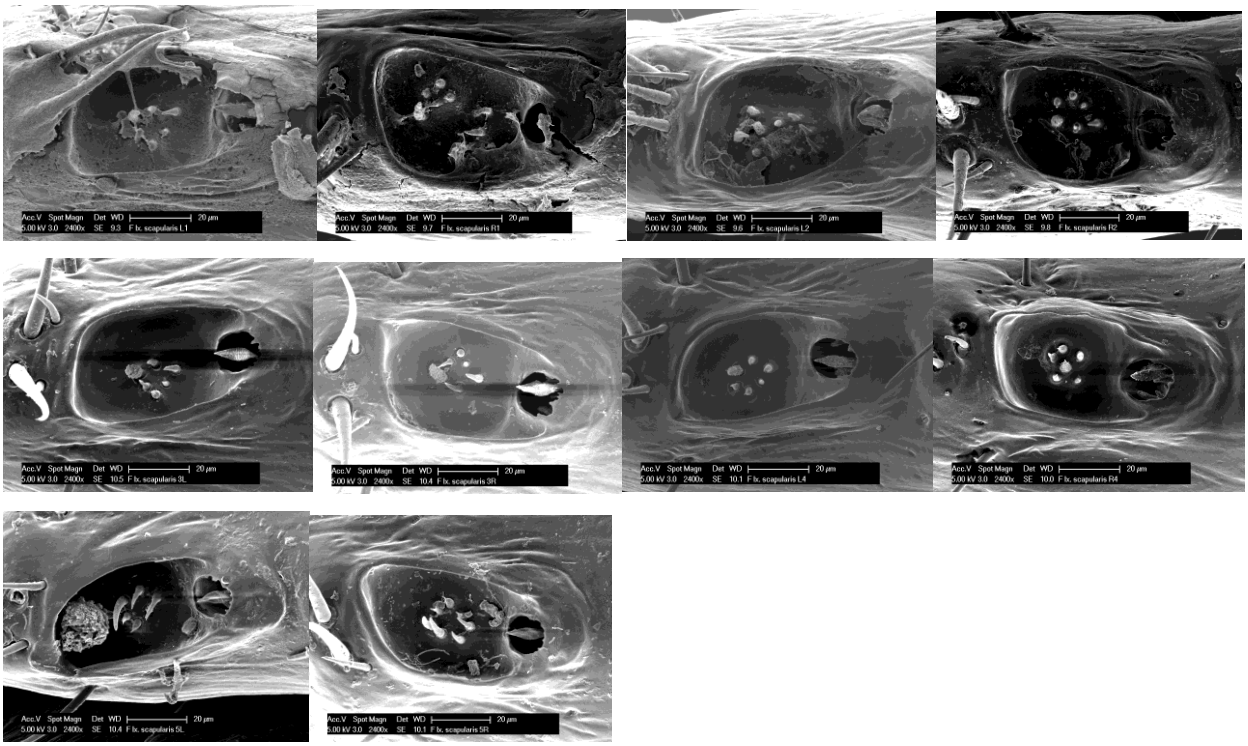
Samples compared – LM=8	Shape, f-value	df	Shape, p-value
All *D. variabilis* versus all *A. americanum*	11.13	12	<0.0001*
All *I. scapularis* versus all *A. americanum*	47.98	12	<0.0001*
All *I. scapularis* versus all *D. variabilis*	26.66	12	<0.0001*
All M *D. variabilis* versus all M *A. americanum*	3.33	12	0.0002*
All F *D. variabilis* versus all F *A. americanum*	25.68	12	<0.0001*
All M *I. scapularis* versus all M *A. americanum*	21.44	12	<0.0001*
All F *I. scapularis* versus all F *A. americanum*	28.20	12	<0.0001*
All M *I. scapularis* versus all M *D. variabilis*	15.73	12	<0.0001*
All F *I. scapularis* versus all F *D. variabilis*	27.98	12	<0.0001*
All Three Ticks Left Leg only	21.43	24	<0.0001*
All Three Ticks Right Leg only	20.93	24	<0.0001*
All Male Ticks Left Leg	12.13	24	<0.0001*
All Male Ticks Right Leg	16.43	24	<0.0001*
All Female Ticks Left Leg	17.24	24	<0.0001*
All Female Ticks Right Leg	22.52	24	<0.0001*
Males versus Females *Ixodes* Left Leg	1.85	12	0.0579
Males versus Females *Ixodes* Right Leg	1.46	12	0.1517
Males versus Females *Amblyomma* Left Leg	0.86	12	0.593
Males versus Females *Amblyomma* Right Leg	1.06	12	0.4026
Males versus Females *Dermacentor* Left Leg	5.00	12	<0.0001*
Males versus Females *Dermacentor* Right Leg	14.84	12	<0.0001*

Top section contains the results of my pairwise comparisons. The bottom section contains the results of my left or right side only comparisons.

* Signifies a p-value denoting significant differences

Figure A.1 – ESEM Images

Ixodes scapularis Females



Ixodes scapularis Males

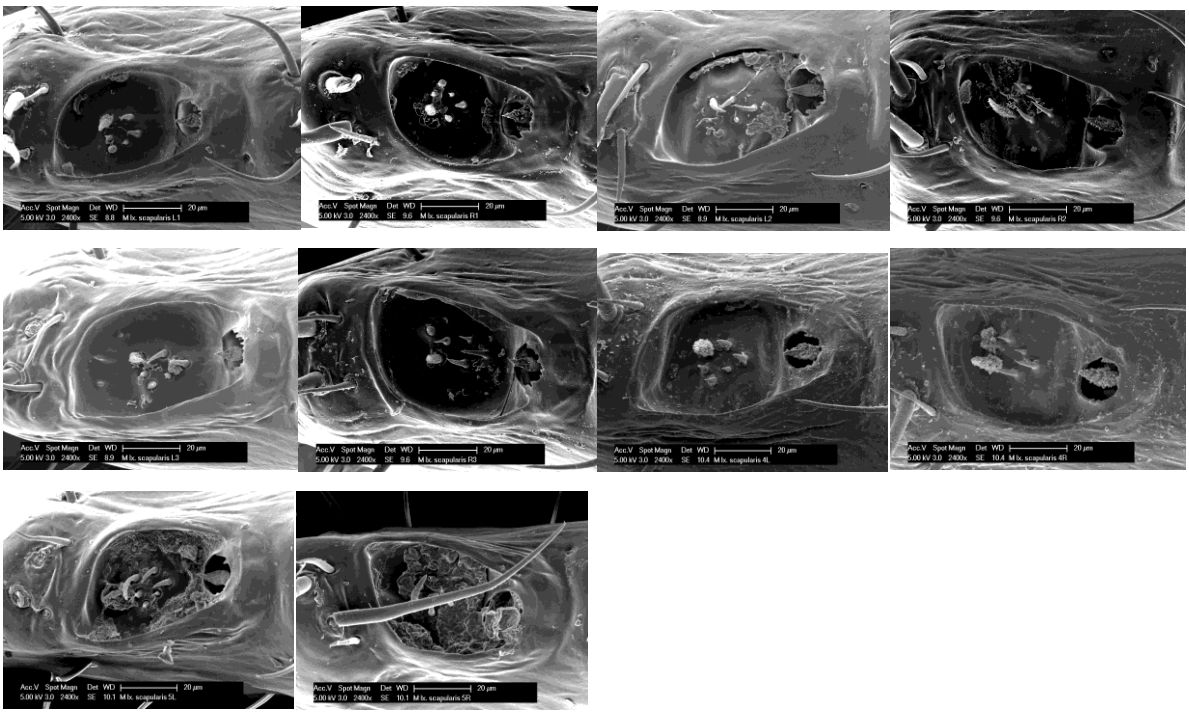
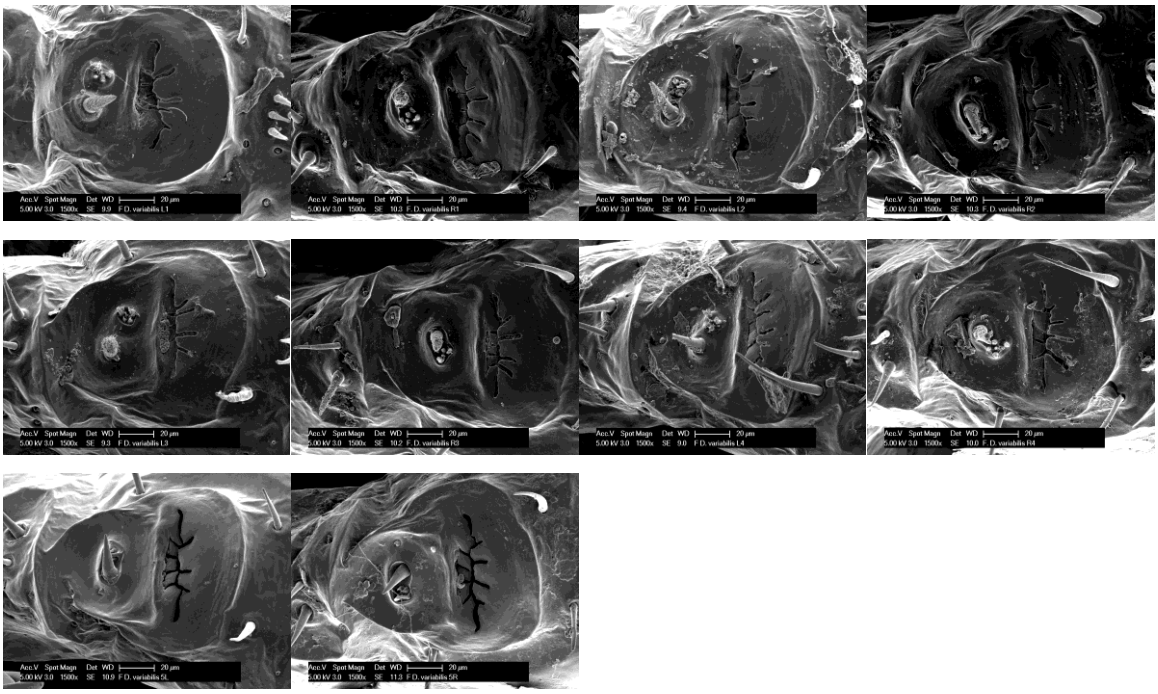


Figure A.1 (cont.)

Dermacentor variabilis Females



Dermacentor variabilis Males

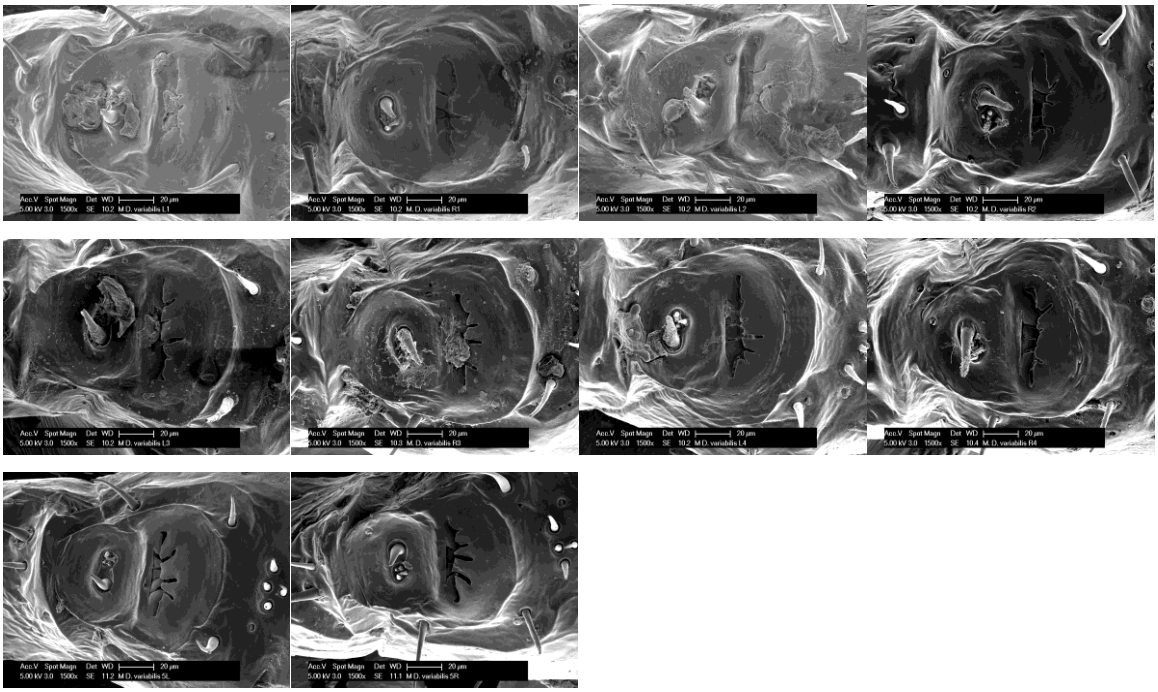
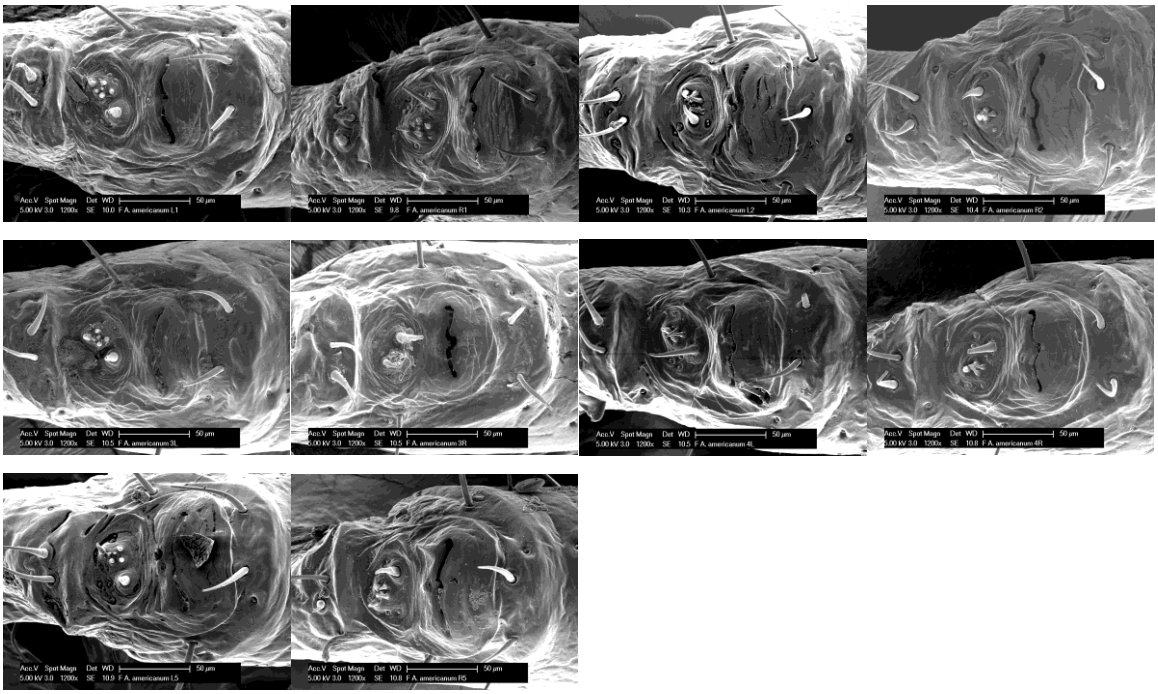
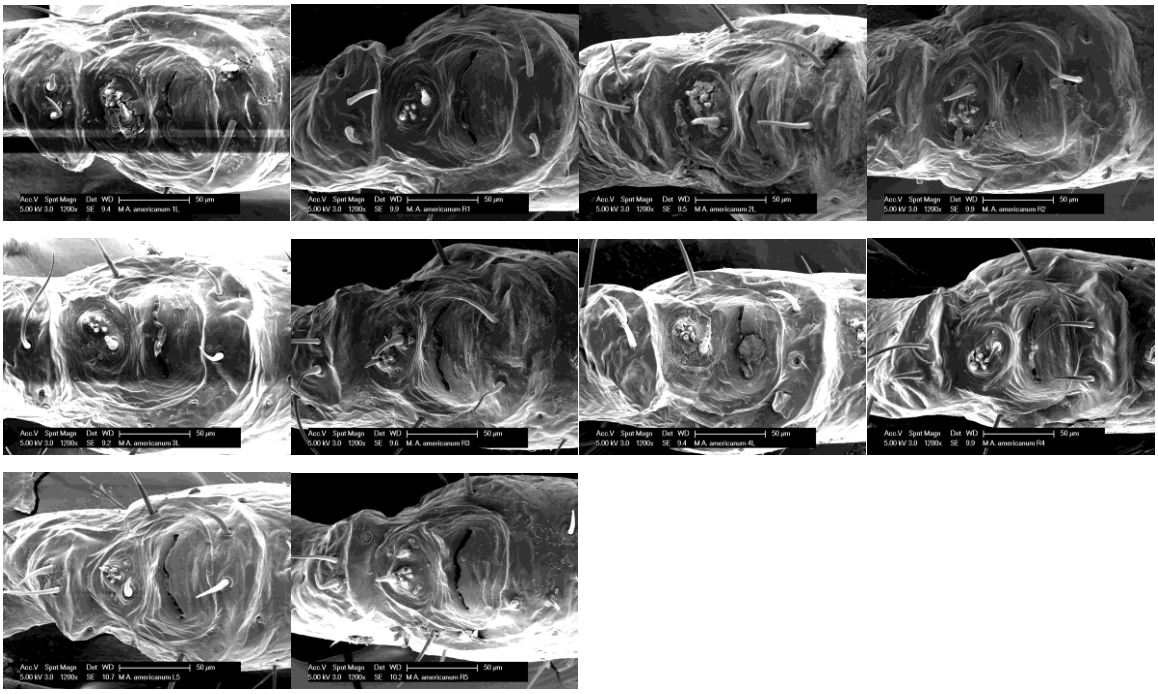


Figure A.1 (cont.)

Amblyomma americanum Females



Amblyomma americanum Males



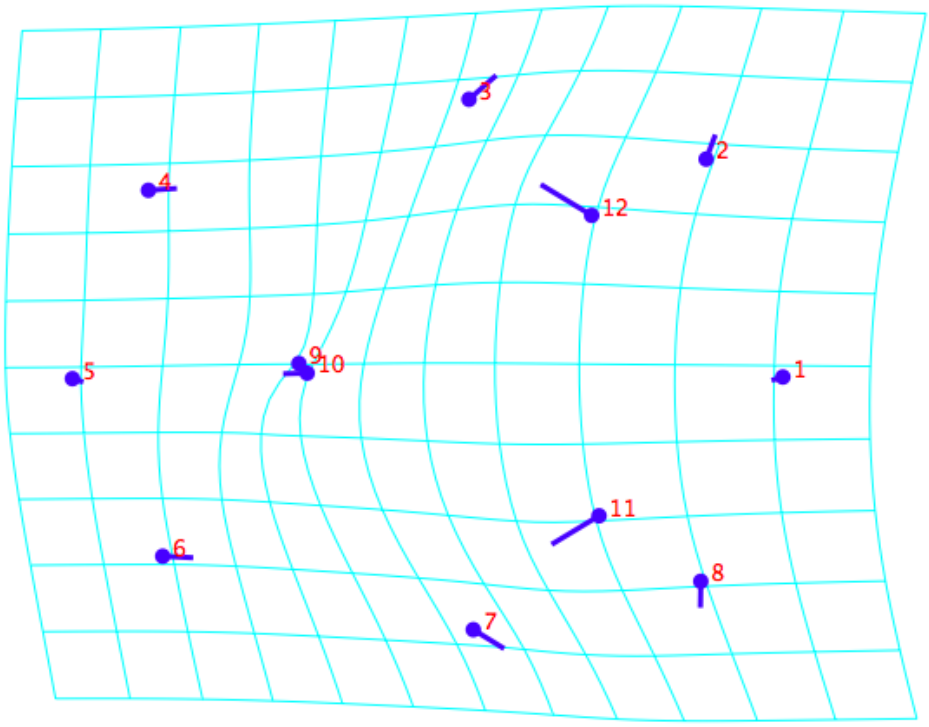


Figure A.2 – CVA thin-plate spline graph – CV1 for all ticks

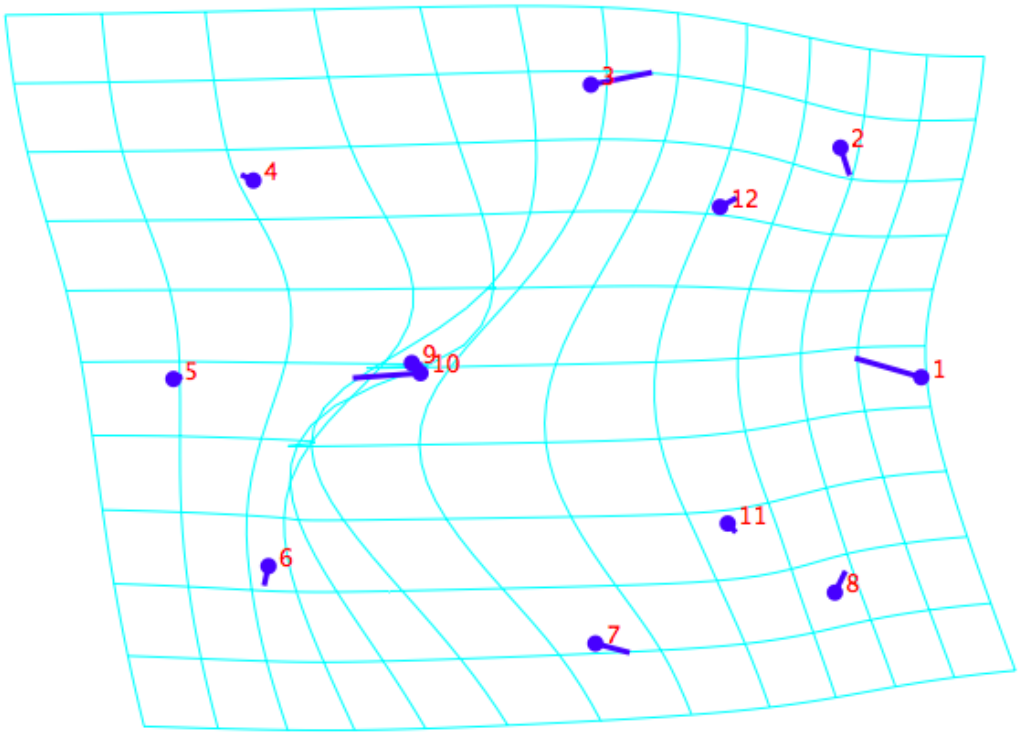


Figure A.3 – CVA thin-plate spline graph – CV2 for all ticks

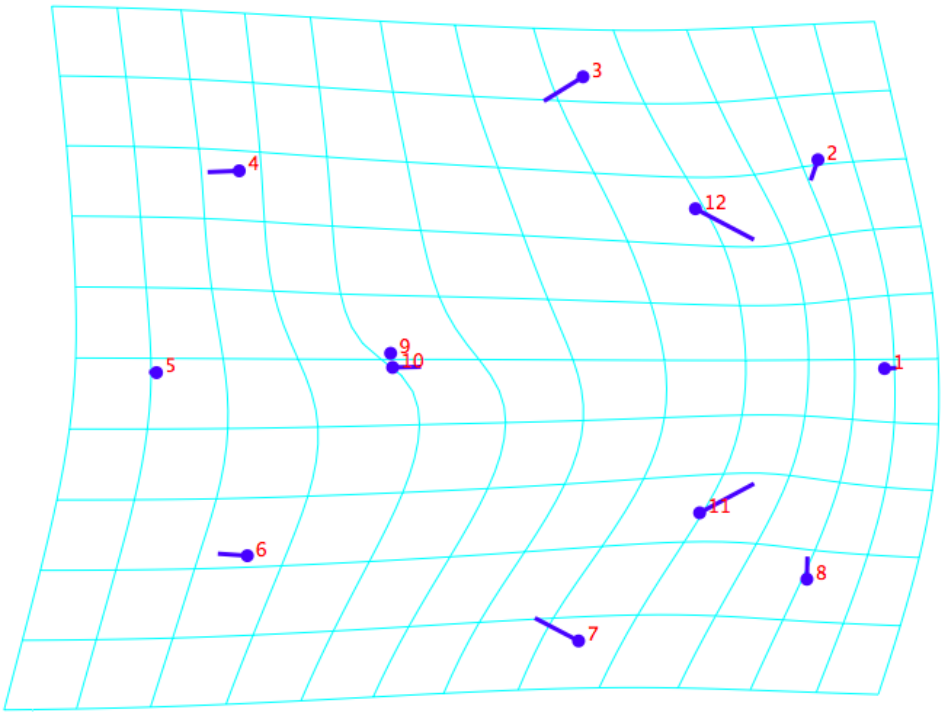


Figure A.4 – CVA thin-plate spline graph – CV1 for all male ticks

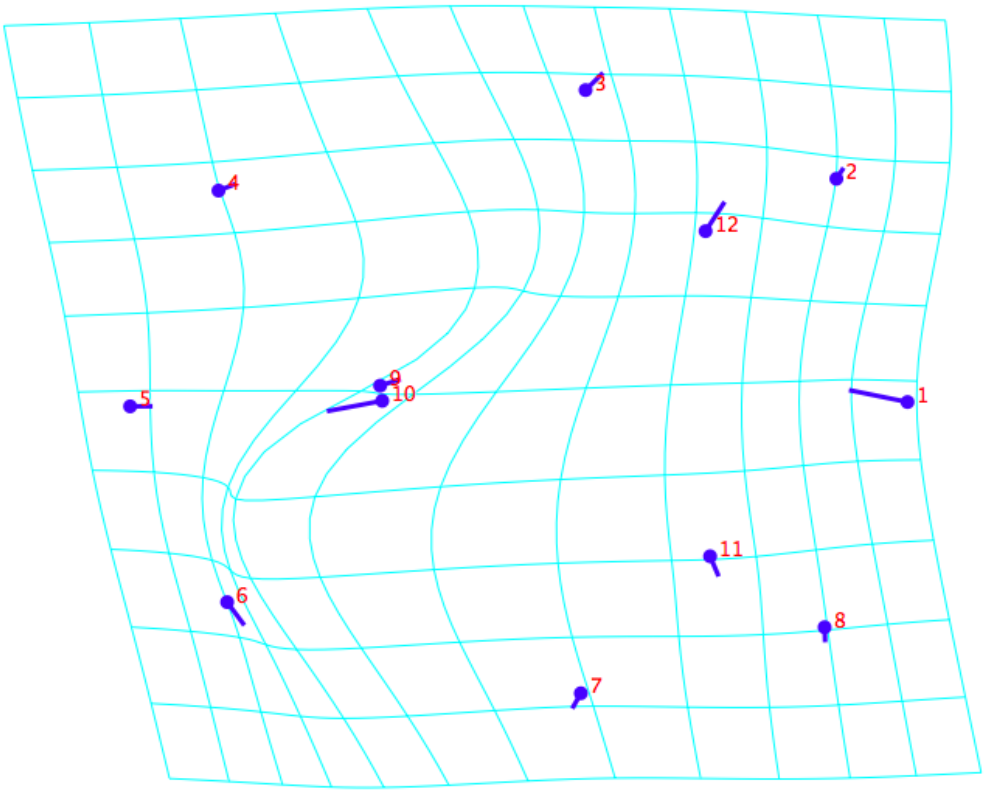


Figure A.5 – CVA thin-plate spline graph – CV2 for all male ticks

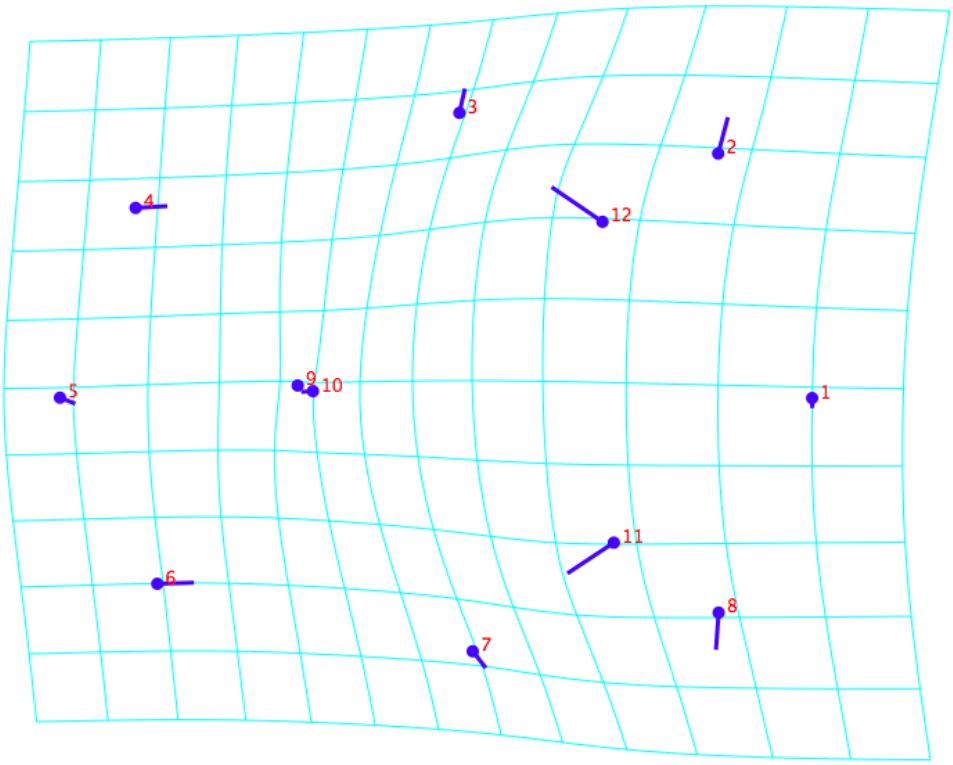


Figure A.6 – CVA thin-plate spline graph – CV1 for all female ticks

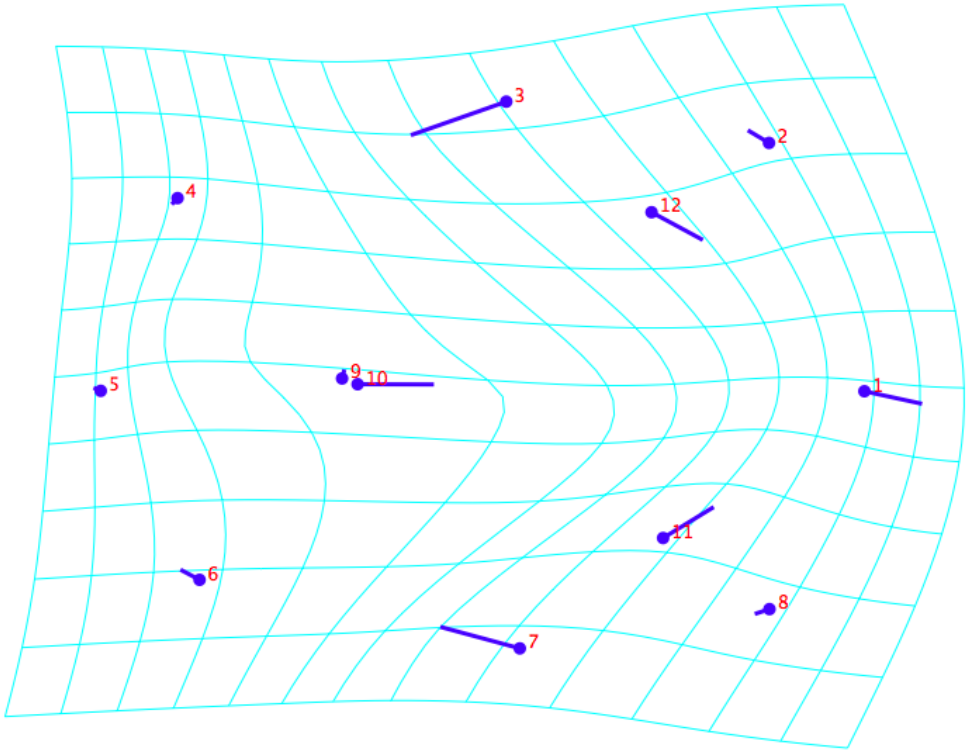


Figure A.7 – CVA thin-plate spline graph – CV2 for all female ticks

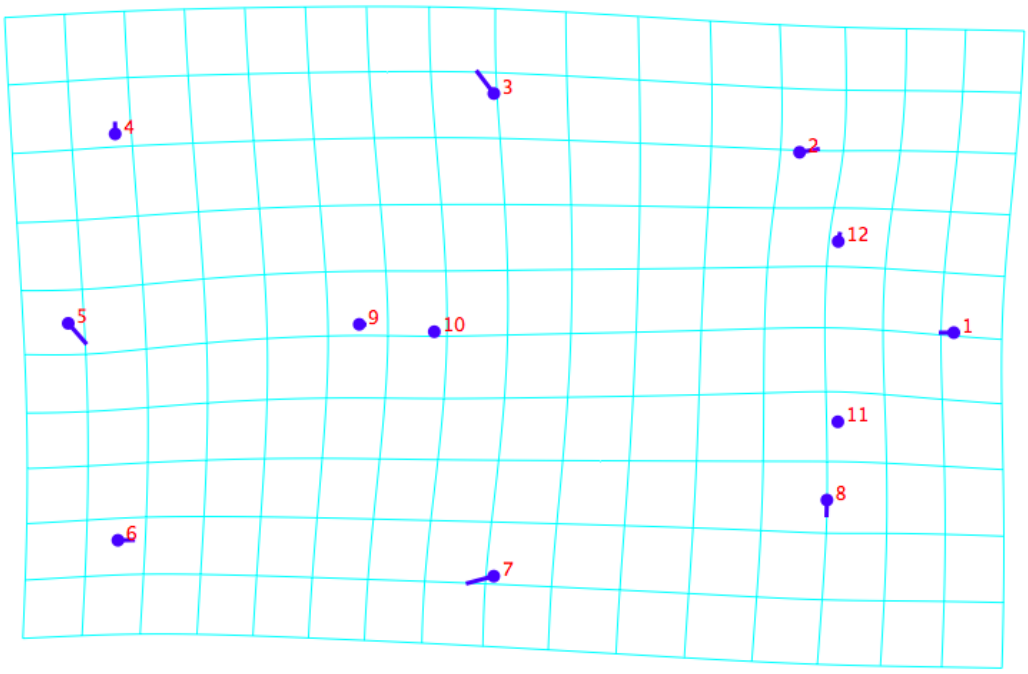


Figure A.8 – DA thin-plate spline graph for all *Ixodes scapularis*

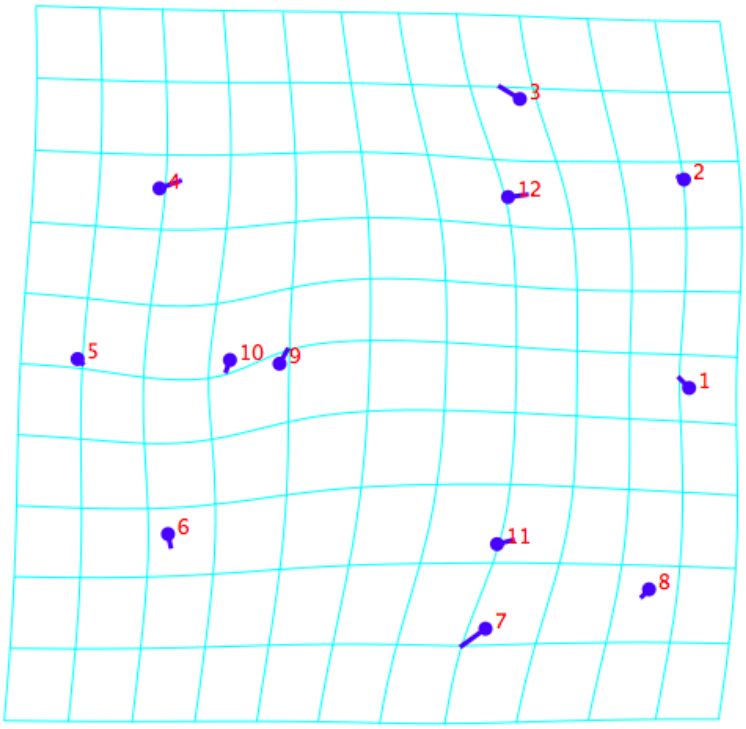


Figure A.9 – DA thin-plate spline graph for all *Amblyomma americanum*

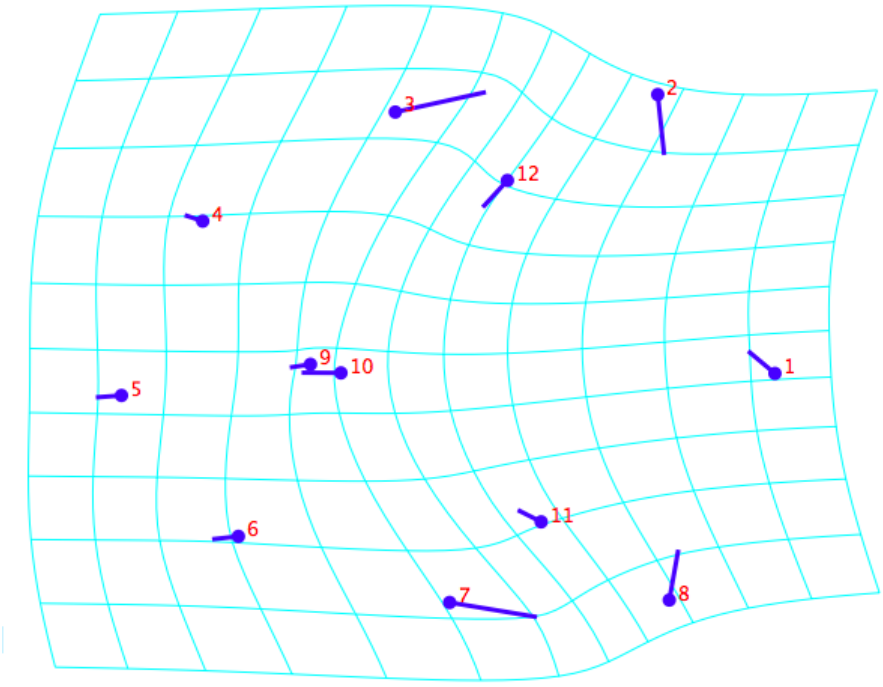


Figure A.10 – DA thin-plate spline graph for all *Dermacentor variabilis*