Morphological Analysis of the *Cinara cupressi* complex: A Comparison of Specimens from Africa, Europe, and North America

by

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Part I

Material Examined

In addition to the material received from IIE, specimens were borrowed from museums throughout North America. Requests were sent to those where material was expected to be found, unfortunately some have not responded to date and some had no material of the *C. cupressi* complex. Many of the specimens received were not useful due to inadequate clearing prior to mounting, poor mounting techniques or a combination of both. As with most *Cinara* there is much misidentification in the collections here. Data has been taken on over 300 apterae, approximately 40 alatae 12 oviparae and about 30 specimens which were later determined not to be in the *cupressi* complex.

Data taken on specimens

Lengths of: body, hind tibia, rostrum, frons between eyes, antennal segments III, IV, V, VI base, VI pt, base and tip of ultimate rostral segment (sometimes referred to as rostral IV and V), siphuncular cone (measured parallel to body axis), longest setae on abdominal segment V, hind tibia and antennal segment III. Number of setae on: third rostral segment, ultimate rostral segment, antennal segment VI base and process terminalis, subgenital plate and abdominal segment VIII. Secondary sensoria on antennal segments III, IV and V were counted. The presence and approximate size of scleroites and sclerites on abdominal tergites IV - VII was noted.

Analyses

The data was analyzed in a number of ways. Initially the distribution of the values was examined using notched box plots and bivariate plots with 95% confidence ellipses. These provide a visual indication of the range of the measurement in question as well as an indication of the statistical difference between sets of data. Data were compared by host for each continent, by host for all collections, by continent with no regard for host, and as individual collections. Multivariate analysis techniques were used and characters were examined for size independence and relatedness using cluster analysis for data from host related groups. Alates were not available from most collections so analyses were confined to data from apterae. All statistical analyses were run using Systat ver. 5.0 for the Macintosh. The data set has been sent to Dr. R. Foottit in Ottawa who is more experienced than I in statistical analysis of such data sets. Any conclusions he may develop will be forwarded to you.

Details of Analyses

In all the analyses there have been no a priori categories imposed on the material other than host, distribution or collection. No decision was made as the material was examined that this collection was *cupressi*, *sabinae* or *canadensis* or for that matter some other distinguishable species. Specimens which clearly fell outside the *cupressi* complex were deleted from the data set.

Bivariate Plots

The overlap in ranges for virtually every character made these plots almost useless. When the default confidence level of 0.50 was used the ovals did not overlap. however, moving up to the 0.95 level in all instances the ellipses overlapped to such a degree that all potential separation is lost. The only thing that becomes clear is that the African material is always smaller than the North American material but the ranges always overlap.

Notched Box Plots

These plots are very useful analytical tools in that they provide a good visualization of the data distribution as well as an indication of significant differences between the medians (Figures 1-4). The center line is the median, the ends of the box divides the higher and lower values in half, and the range is indicated by the entire line. Asterisks and circles beyond the line indicate outlying values. The notches surrounding the medians provide a measure of the rough significance of differences between the values. Specifically, if the notches about two medians do not overlap, the medians are, roughly, significantly different at about a 95% confidence level.

Two sets of notched box plots are shown on Figures 1 and 2. In Figure 1 the aphids were sorted by distribution for each host. It is quite clear that specimens from *Juniperus* in North America are significantly larger than those from *Juniperus* in Europe. The aphids from *Cupressus* are somewhat larger in North America than in both Africa and Europe. Significant differences are in size of siphuncular cone and length of antennal segment IV. Collections from *Thuja* in North America which were examined did not fall into the *cupressi* complex so comparisons could not be made. The *Thuja* material could be separated by the longer setae, especially from specimens taken on *Juniperus* in North America.

In Figure 2 specimens are grouped by distribution and compared by host. The four characters displayed on this figure represent the general range of variation seen in other characters. The European material shows some interesting host related variability. What is interesting is the remarkable differences shown by the specimens collected on *Callitris*. The other category in the North American group is primarily two collections of both apterae and alatae made by Knowlton in his yard in Utah on *Pinus mugho*! These were sent to Quednau and Pepper who mounted the material and returned some of it. Both determined the aphids as *C. cupressi* and said the host was wrong but Knowlton most certainly could separate *Pinus mugho* from anything in the Cupressaceae. For a long time I included them in the analyses until I found that antennal segment VI base and pt were much shorter than in any of the *cupressi* group.

Discriminant Functions

These functions are often criticized because they impose an a priori categorization then determine a mathematical function that will separate the included entities. If there is other indication that the material can be divided into groups these functions are useful in that they provide a way to assign additional specimens to a group. Because one of the questions in this particular case is the origin of the African *Cinara* the data were assigned distributional codes on the basis of continent. The characters used were length of hind tibia, length of antennal segment IV and diameter of siphuncular cone. Using these three characters for the African and North American specimens from all hosts a discriminant function based on a randomly selected subset of half the specimens

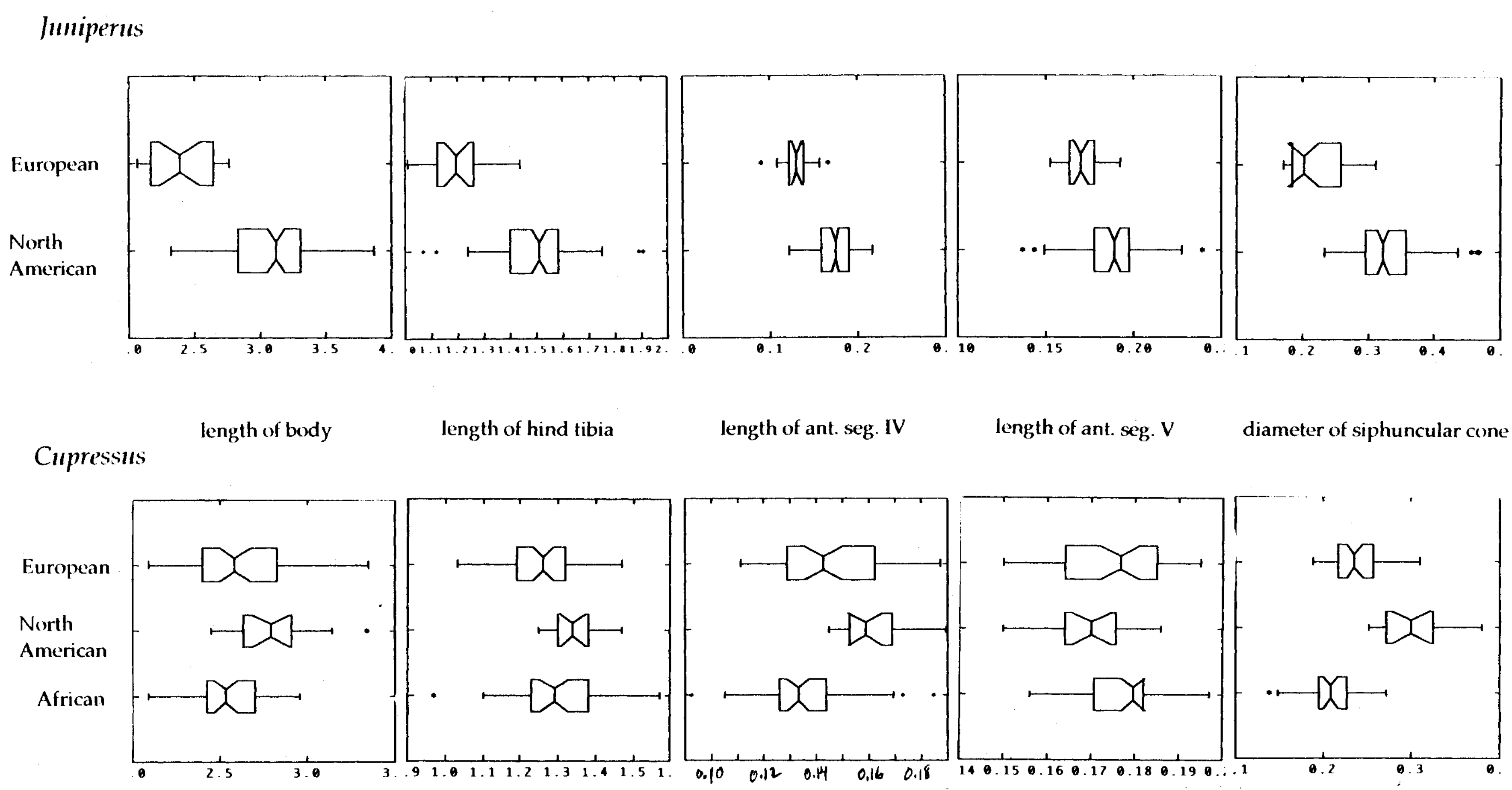

Figure 1. Notched box plots for five measurements taken from apterous viviparae of the *Cinara cupressi* complex. Specimens are sorted by host and compared by origin. Only two hosts *Juniperus* and *Cupressus* had specimens in this complex collected on them from more than one continent. All measurements are in mm.

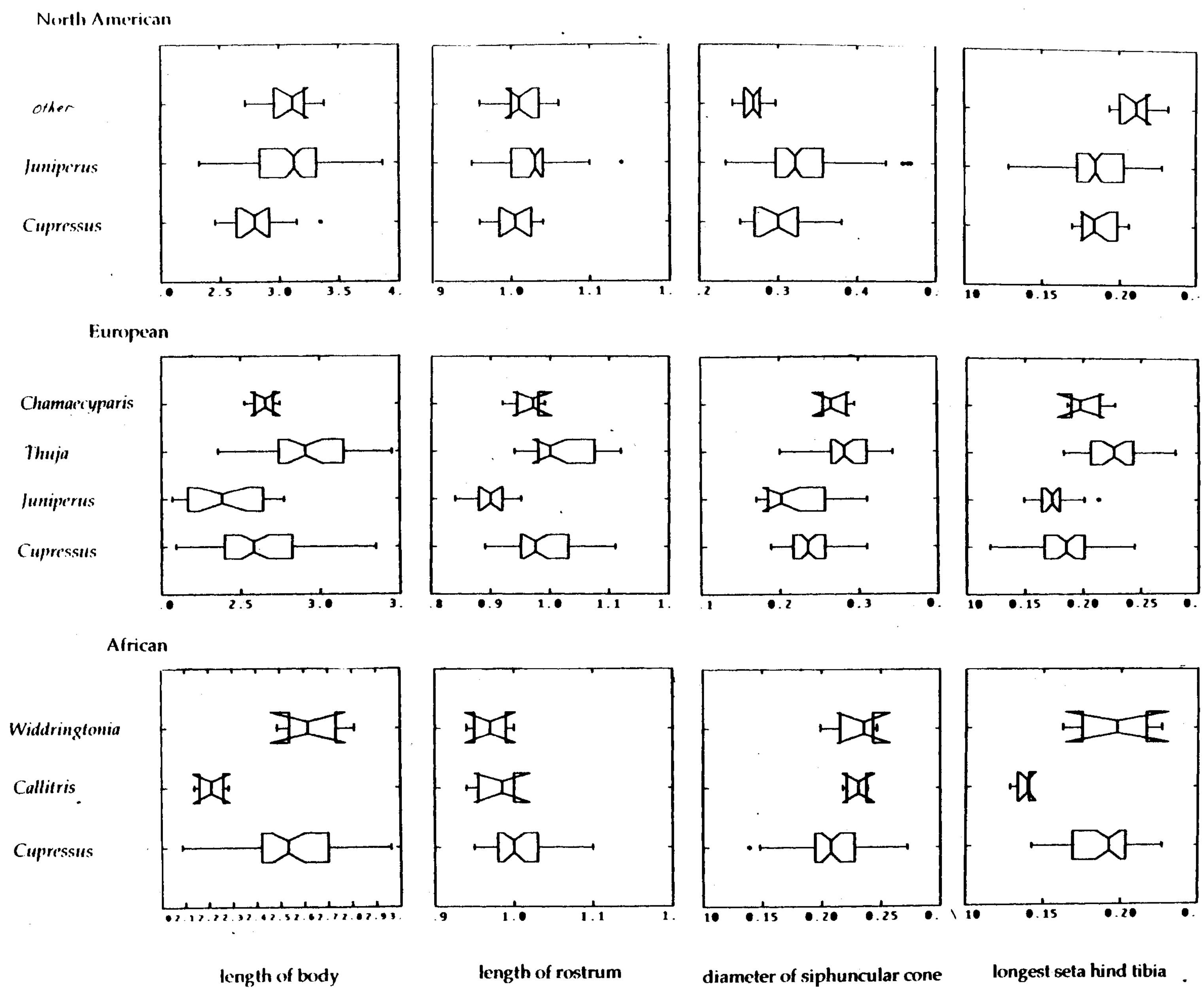

Figure 2. Notched box plots for four measurements taken from apterous viviparae of the *Cinara cupressi* complex. Specimens are sorted by distribution and compared by host. Samples from *Juniperus*, *Cupressus* and *Thuja* are represented by a reasonable number of specimens. Boxes where the notch extends past the range usually represent small samples, often only one collection. All measurements are in mm.

was calculated. This function was tested on the remaining specimens and correctly placed all of the African and 87% of the North American specimens.

Equivalent tests were run comparing African and European, and European and North American. Neither of the discriminant functions developed were nearly as powerful as the first, maximally correctly placing 70% of the specimens.

Discriminant functions calculated using all three distributional categories were similarly less powerful. Analysis by host and by distribution have produced discriminant functions of varying efficiency.

In all discriminant functions developed whether using the complete data set, or subsets containing data from two distributions misidentifications occurred between the African and European and the North American and European but rarely between African and North American specimens. In discriminant functions between host related groups misidentifications of specimens from *Cupressus* and *Juniperus* were more common than those from *Thuja*.

Principal Components

Sets of characters from all the specimens were submitted to principal components analyses. The results were inconclusive at best. Separation of the data into files where only two of the three geographic regions were represented produced somewhat better resolution but not strong enough to support any conclusions. The data set combining the North American and African provided scores which when plotted showed two overlapping but relatively distinct clusters.

No clear association between variables was noted, however, when factors are plotted the African material always forms a fairly tight association.

Part II

The above analyses were made without categorization into groups other than distributional and host. After spending too much time attempting to discover mathematically based groupings in the data I decided it was time to examine all the specimens and categorize them into subjective categories based on their appearance and partially on what was observed in prior analyses. All specimens were examined and placed into six groups. Groups 3 and 4 were made for comparative purposes only.

1. Specimens from *Cupressus* and *Juniperus* in Europe with paired sclerites on abdominal tergites I and II.
2. Specimens from Africa on *Cupressus, Callitris* and *Widdringtonia*.
3. Specimens from *Cupressus* in North America with paired sclerites on abdominal tergite 1.
4. Specimens from *Cupressus* in Europe with paired sclerites on abdominal tergite 1.
5. Specimens from *Thuja* in Europe.
6. Specimens from *Juniperus* in North America.

Group 1 was a surprise in that it was not until I went through the slides a second time that I realized that some of the European collections had paired sclerites on abdominal tergite II as well as I. The presence or absence of this character holds for all specimens in a collection. Paratypes of *cupressi*, *sabinae* and *canadensis* do not have sclerites on abdominal tergite II. Categories 2-6 have only large paired sclerites on the meso-and metathorax and a smaller pair of sclerites on abdominal tergite I. Collections in this group are from ENGLAND Berkshire, Sunninghill; Cornwall, Looe, Silwood (cultured on *Cupressus macrocarpa* and *Juniperus virginiana*); SPAIN Baiona (*Cupressus macrocarpa* and *Juniperus scopulorum* skyrocket); FRANCE Antibes. ITALY Stella, Duma, Nozzano, La Glabella, Ceriara. TURKEY Istanbul.

Group 2 seems to be very close morphologically. The specimens from South Africa are slightly larger and those taken from *Callitris* are smaller.

Group 3 and 4 were kept separate to compare material from *Cupressus* in North America and Europe. The type of *cupressi* is in group 4 but surprisingly after group 1 is separated there are very few specimens from *Cupressus* in Europe.

Group 5. In the first phase of analysis I was convinced that the specimens from *Thuja* constituted a separate group. They seemed to have distinctly longer setae than the European specimens from *Cupressus* and *Juniperus*. However, once Group 4 is removed from the European material the remaining specimens (including the paratypes of *cupressi*) are similar to those from *Thuja*.

Group 6 contains specimens from *Juniperus* in North America. These probably should remain under the names *sabinae* and *canadensis* for now. I think molecular analysis would probably support the separation of *canadensis* and *sabinae* but I cannot maintain this separation with morphological data.

I have prepared a series of notched box plots using these six groups (Figure 3). The arrangement is in increasing length of hind tibia (median). This closely reflects body size but the later is more varied depending on the quality of the slide. When arranged in this manner size independent characters become apparent. The surprise was group 1 which shows that the other characters support the distinctness of the sclerotic pattern. Groups 1 and 2 also are more often not significantly different from one another while some characters show clear and significant differences from the other four groups. Also very interesting is the complete accord of the length of the base of antennal segment VI over all groups.

Discriminant Function

The data for specimens in categories 2 and 6 above were used to develop a discriminant function. Characters used were the length of the hind tibia, length of antennal segment IV and diameter of siphuncular cone. The function correctly placed all African specimens but misidentified 9 of the specimens from *Juniperus* in N.A. The key below will separate these two categories without the use of this function.

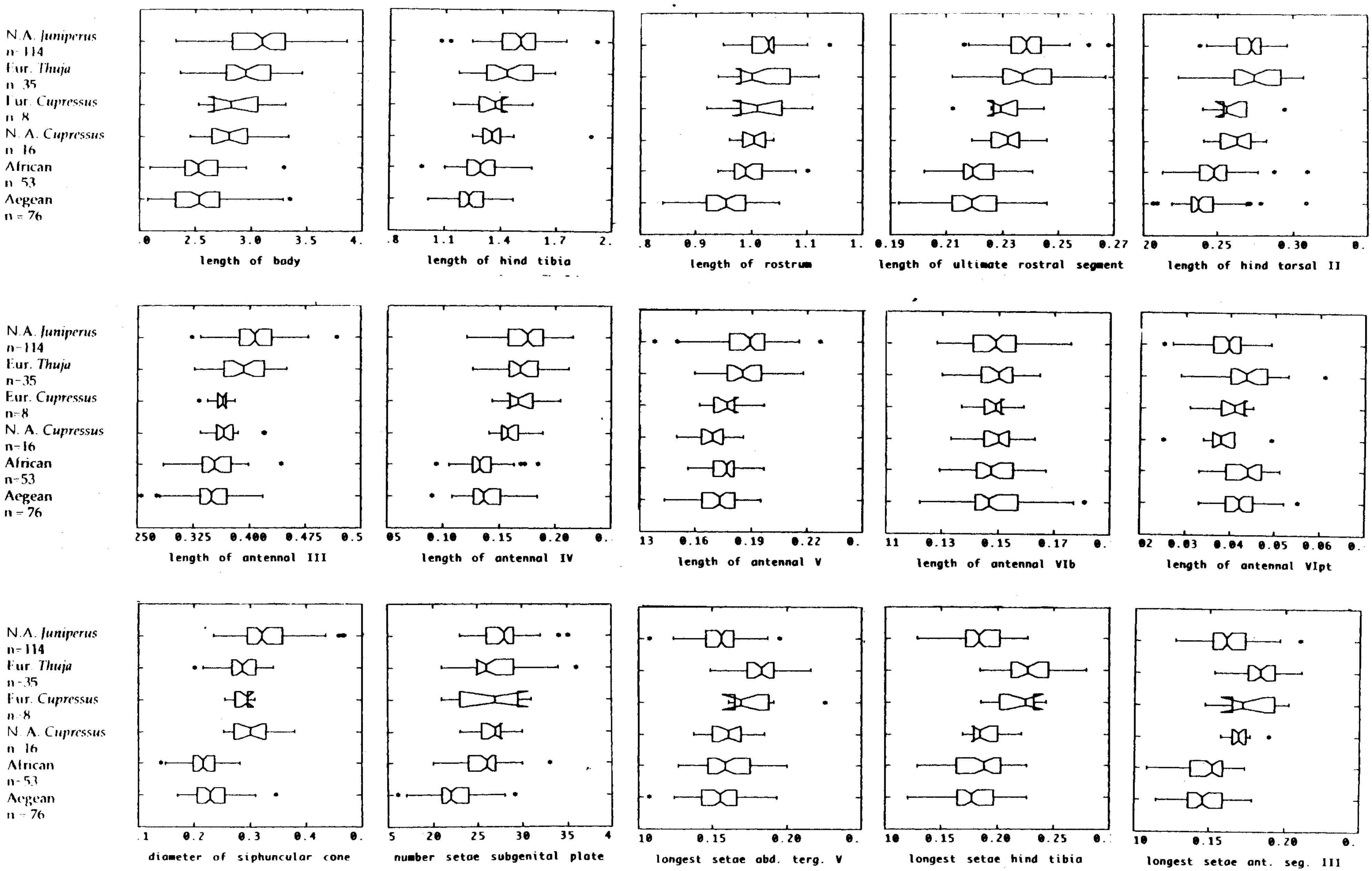

Figure 3. Notched box plots for fifteen characters taken from apterous viviparae of the *Cinara cupressi* complex. Specimens are sorted into subjective or host related categories. Arrangement of categories is by increasing median length of hind tibia. All measurements are in mm.

Observations and Conclusions

1. In general the aphids from Africa are smaller than those from Europe and North America. This may be host related since the aphids on *Cupressus* are also smaller than those from *Juniperus* and *Thuja* (however see below regarding clonal response on *Cupressus* transferred to *Juniperus*).

2. It seems quite likely that there is some host specificity in this group. None of the material I examined from *Thuja* in North America was in the *cupressi* group. That may be just a function of collecting but I doubt it. If we do not have *cupressi* on *Thuja* in this continent it follows that we do not have *cupressi* or the aphids from *Thuja* in Europe are not *cupressi*. Aphids from *Cupressus* were transferred successfully to *Juniperus* and apparently did well. I suspect that acceptance in a culture situation is not the same as in the field. The fact that it is possible to generate a discriminant function that will separate 80% of the specimens depending on their host also suggests some host specificity.

3. Unfortunately I cannot say that the source of the *Cinara* in Africa is either Europe on North America. I think it is quite possible that it is not represented by any of the collections I have examined. I believe that the introduction originated from *Cupressus* and not *Juniperus* or *Thuja*. Specimens from *Cupressus,* regardless of locality, always looked more like the African material than did those from other hosts. This "gestalt" is difficult to define! Until I discovered the sclerites on abd. tergite II I was convinced that the source of the African *Cinara* was southern Europe. This has shaken my conviction somewhat, however, the notched box plots demonstrate the size similarity between groups 1 and 2. There are also a couple of specimens in group 1 on which I could see only one sclerite on the second abdominal tergite (may be due to mount). One thing that was quite obvious during my examination of this material is that the African population, with possibly the exception of those from South Africa, is the result of a single introduction possibly a single clone.

4. Size differences between host related groups is probably genetic and not environmental. In the two sets where aphids from *Cupressus* were transferred to

Juniper the size has been reduced which is the opposite of what is seen when comparing field collected material from both hosts.

5. The North American material and some of the European show considerable more sclerotization on the dorsum of the body. This is particular evident in the presence of large scleroites on abdominal IV - VII. Most setae stand on a small scleroite only slightly larger than the setal base. Larger scleroites. which I have categorized as ≤ 5 times the base diameter of the seta which stands on them, are present in most of the African specimens on tergite VII, sometimes on VI, rarely on V and never on IV. In North American specimens from *Juniperus* there are larger scleroites, often > 10 times the base diameter of the seta on them, and these are always on VII and VI usually with smaller 5-10x, or ≤5x scleroites on V and IV.

6. Specimens from North America have considerably more setae. Because of time constraints and the difficulty of making accurate counts I did not attempt to count all the setae on tergite V but some samples I did count were in the 80+ range. This is quite outside the range given by Eastop for *cupressi* (30-50).

Taxonomic Fallout

1. I think that the *Cinara* from Africa are not *cupressi.* The aphids closest to them in size and appearance are those in group 1 (see 2 below). However, as noted above the low level of morphological variation of the African material suggests that the introduction was possibly clonal. In such a case the aphids could represent a portion of the gene pool of a species that would be far from the "average" for that species.

2. I believe the specimens in category number 1 above are also not *cupressi*. It may be one of the synonyms of *cupressi* My guess is that it is endemic on *Cupressus sempirvirens* and *Juniperus* spp. in the Aegean region. This has undoubtedly been introduced into England. It is clearly distinct enough to warrant species designation. Some of the material in this category is included in Eastops (1972) discussion of C. *cupressi* (Bacekoy, Istanbul, Turkey, 1966). I suspect that there may be more of these specimens in the material he cited which I have not seen. Binazzi in Redia (1978) pages 340-344 discusses this species (as

C. cupressi) and his drawing shows the sclerites on the first and second abdominal segment. My Italian is nonexistent but I think he considers Del Guercios species *tujae* to be this species. Whether it will live on *Thuja* or not is the question. Only one of his collections is from *Thuja*.

3. I suspect that *canadensis* and *sabinae* are geographically isolated populations which are in the process of speciating on different species of *Juniperus*. It may be impossible to separate them morphologically. What is interesting is to check host records of *Cinara* collected in these regions. *Cupressus* spp are widely planted ornamentals throughout North America. There are no records of *Cinara* from *Cupressus* in Palmer (1952) or in Bradleys (1961) unpublished manuscript on the *Cinara* of Canada or in Knowltons (1983) "Aphids of Utah" or in Forbes and Chan (1989) "Aphids of British Columbia". It seems unlikely that either of these species will feed on *Cupressus*. The only specimens I received on loan or have in my collection from *Cupressus* were collected in California or Mexico. It is also interesting to note that Palmer found *sabinae* only on *J. scopulorum* even though she collected on J. *osteosperma* (as *J. utahensis*) and Knowlton has records of *Cinara* spp. from *J. communis* and *J. osteosperma* but has *sabinae* only from *J. scopulorum*. This suggest a rather strong host specificity which makes it unlikely that these species would feed on *Cupressus.*

4. Since there are no native *Cupressus* in central and northern Europe, the aphids that Buckton described from *Cupressus* in all likelihood were introduced. The origin of these specimens could be North America and if so they would be from *Cupressus*.

5. There are also no native *Thuja* in Europe so the origin of the aphids from that species is also in question. As noted above it is possible that they could transfer from *Juniperus* which has native European species. I have not seen any aphids that would fall into the *cupressi* group from *Thuja* in North America. Either they are misidentified so were not sent to me or *sabinae / canadensis* will not feed on it.

Key

Key to apterae of the more or less recognizable entities of the *Cinara cupressi* complex.

1 (2) Abdominal tergite II with paired sclerites. On *Cupressus, Juniperus* and possibly *Thuja*. Native to the Aegean region.

Cinara tujae Del Guercio ?

2 (1) Abdominal tergite II without paired sclerites.

3 (4) Siphuncular cones usually less than 0.3 mm in diameter, antennal segment IV usually < 0.15mm, abdominal tergite VI and VII with scleroites usually < 5x diameter of the seta standing on them, abdominal tergites IV and V without scleroites. On *Cupressus, Widdringtonia* and *Callitris* in Africa. Origin unknown but most likely from *Cupressus.*

Cinara problematica n. sp.

4 (3) Siphuncular cones generally larger than 0.25 mm, antennal segment IV usually > 0.15. Often with scleroites on abdominal tergites IV and V > 5x the base of the seta on them and scleroites on abdominal tergites VI and VII often > 10x the seta on them.

5 (6) Ratio of hind tibia to longest setae on it usually > 6.8. Number of setae on abdominal segment V >60.

On *Juniperus virginiana*	*Cinara canadensis* (Hottes &Bradley)
On *Juniperus scopulorum,*	*Cinara sabinae (Gillette & Palmer)*
On *Cupressus* spp.	*Cinara ?*

6 (5) Ratio of hind tibia to longest setae on it usually < 6.8. Number of setae on abdominal segment V <50 (setal count from Eastop 1972). On *Cupressus* and *Thuja* in Europe.

Cinara cupressi Buckton

The last couplet above will separate approximately 75% of the specimens correctly. The ratio is different than that given by Eastop (1972) but I believe he

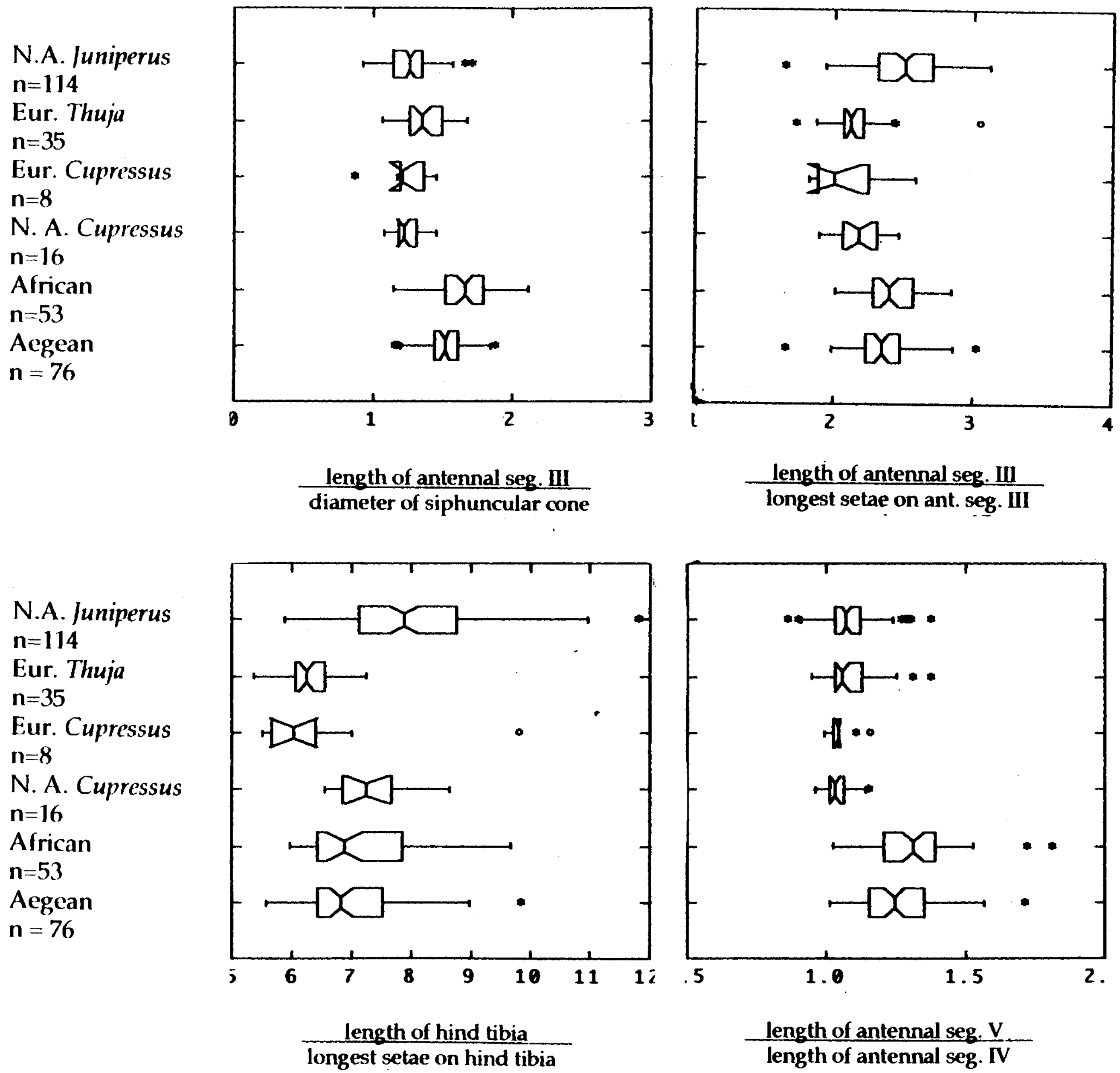

Figure 4. Notched box plots showing the values of four ratios. Categories are the same as shown on Figure 3.

included what I call the Aegean species in his ratio. I have included one more series of notched box plots which will explain the cutoff used in key couplet 5 (6) (Figure 4). I cannot find any other feature either quantitative or qualitative with which to separate these. I believe they are biologically distinct and that is the reason I have attempted to separate them in the key. For a while I believed that specimens from *Cupressus* in Europe and North America were the same. Now I am not so sure. If *C. cupressi* will feed on both *Thuja* and *Cupressus* in Europe than there is no doubt that it is biologically distinct from the North American species on *Cupressus*.

Recommendation for Biological Control Efforts

1. I am convinced that the African *Cinara* are originally from *Cupressus* and that they are clearly distinct from the aphids found on *Juniperus* in North America (*canadensis* and *sabinae*). Although there is discussion and research presently on the potential of parasitoids from closely related species, which *canadensis* and *sabinae* are, I think the best bet for a successful parasite would be to find one associated with a *Cinara* living on a *Cupressus*. Considering the source of the trees planted throughout Africa, logical regions for collecting are the southwestern US, Mexico and Guatemala or the Aegean region on *Cupressus sempervirens* and *Juniperus*. However *Cupressus* spp. are found all the way to Asia so there is a great deal of ground to cover.

2. There is an interesting collection determined as *C. cupressi* by R. C. Dickson taken on cypress in Ojai, California. The collection was made during an extended series of collecting trips made by the biological control group at Berkeley in the early 1960's with the object of collecting aphids and their parasites. There are no *Cupressus* spp. which occur naturally in Ojai. It is just south of the range of *Cupressus sargentii* so the cypress was most likely an ornamental. I am not sure how to find out if they reared parasites from this collection. Presumably this would be recorded somewhere at U. C. Berkeley. I made a phone call to Dr. Donald Dahlsten but have not yet received an answer.

3. Lack of parasitism seen in collections of *Cinara* from *Juniperus* is either a seasonal phenomenon or the control mechanism is due to a predator or predator complex. Collections from July should provide parasites. I am less certain about

collections made later in the season. It is also sometimes difficult to raise parasites from collected *Cinara*. Often parasitized *Cinara* leave the colony and move considerable distances prior to mummification which means they will not be collected. When I was studying this group in the Sierra Nevada I was unable to rear parasites from *Cinara curvipes*. The parasite larvae inevitably cut their way out of the aphids and died. Strange behavior.

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