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Homology as a parsimony problem: a dynamic homology approach for morphological data

Martín J. Ramírez*

Museo Argentino de Ciencias Naturales—CONICET. Av. Angel Gallardo 470, C1405DJR Buenos Aires, Argentina

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Abstract

The primary data used to reconstruct phylogenies comes organized in the conceptual grid of homology correspondences, and the construction of this theory-rich grid depends in part on knowledge of relationships. This situation is not satisfactory as a conceptual system, because the evidence is not clearly delimited from the results. I explore the testing of alternative hypotheses of morphological correspondences in a quantitative cladistic context. The varying homology assessments implied by classical criteria of homology (topological equivalence, or position and connections; composition of structures, or commonality in details of construction) can be expressed as regular characters in a cladistic analysis. Doing so provides adequate transformation costs for changes in schemas of correspondences. Correspondences imply evolutionary transformations, and multiple schemas of correspondences can be compared according to the evolutionary transformations that they imply. The method is used to test the correspondences in sclerites of the male copulatory organs of spiders of the subfamily Amaurobioidinae (Arachnida, Araneae, Anyphaenidae). The correspondences of three sclerites are tested, in a data set of 93 species having one, two or three sclerites, using a simultaneous analysis of all the morphological characters. Most parsimonious trees are identified together with the correspondences they imply. Once the correspondences are integrated in the phylogenetic analysis, it is easy to evaluate the robustness of trees or decay in optimality after changes in anatomical interpretations. A Bremer support for anatomical interpretations is proposed, calculated as the increase in tree length when the specific interpretation is not used.
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Background and context

Homology, the defining theme of comparative biology, is perhaps the most elusive and recurrent issue in phylogenetics, and a permanent source of discomfort among systematists. How we came to this situation is nowadays clear: the primary data used to reconstruct phylogenies comes organized in the conceptual grid of homology correspondences, and the construction of this theory-rich grid depends in part on knowledge of relationships. In practical terms, this intermingling of "evidence" and "results" may be more or less influential on the trees and correspondences for particular phylogenetic analyses, but it is still a problem for the conceptual system. In this paper I explore the testing of alternative hypotheses of morphological correspondences in a quantitative cladistic context.

The correspondences

De Pinna (1991) distinguished two independent steps for the determination of homology. In a first step, the correspondences are decided by recourse to comparative biology; these correspondences are "primary homology" statements. In a second step, the characters derived from those correspondences are mapped (optimized) onto optimal phylogenetic trees. Corresponding states whose origins can be traced to common ancestors become "secondary homology" statements. The optimization procedure does not involve any alteration of the correspondences that were previously determined as primary homologies.

*Corresponding author:
E-mail address: ramirez@macn.gov.ar

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Primary homology correspondences are built following some guidelines known as "homology criteria" summarized in two main ideas (for a recent review see Rutishauser and Moline, 2005): (1) topological relations (position and connections), and (2) composition of structures (commonality in details of construction). A third criterion, of linkage by intermediate forms or the continuum criterion, will be discussed separately below. Of those, the topological criterion is usually considered as the most important one (but see Richter, 2005).

The application of these criteria results in a list of conditions that may partially conflict with each other (e.g., position versus composition), or even within the same criterion (e.g., connections with vascular and nervous system suggest different correspondences). Hence these criteria are better seen as guidelines or "indicator hypotheses": "Topology and connectivity are indicator hypotheses that indicate in a defensible manner in which direction to 'look for' potential homology, because topology and connectivity may, at least to some degree, be entrenched in the generative mechanisms of ontogeny." (Rieppel, 2005, p. 25).

There are no essential, logically necessary reasons by which topology and connectivity may be better indicators than others: it just happened that life on Earth is structured and evolves in such a way that parts of organisms usually preserve more their topological relations and connections through evolution than say, function, color, texture, or atomic composition. The relative importance of topological versus compositional information also depends on the biology of the specific cases, e.g., possibility and extent of recombination between chromosomes, localized or distributed cell types, single versus homonomous structures, etc.

If one considers that homologs themselves often exhibit hierarchical structure (i.e., homologous body parts carry homologous organs, which have structural details that are homologs themselves), the two homology criteria often intermingle depending on the level of analysis. In many cases "composition" or "specific quality" can be rephrased as topological correspondences of the small parts that compose larger structures. Ontogenetic information can also be included within these two criteria considering time as a further dimension (de Pinna, 1996; Schulmeister and Wheeler, 2004), thus considering change through developmental time as part of the morphological structure: a structure *is* its development, as much as organisms *are* developmental processes (Weston, 2000, p. 125; Sattler in Vergara-Silva, 2003, p. 263). As a short summary of the traditional procedures for the establishment of homology relations we can say that the comparative biologist examines a range of plausible homology relations, and tries to come up with the schema that maximizes the correspondences in topological relations of structures, and of the details of their constituent parts (reviewed in Collazo, 2000; Rieppel and Kearney, 2002; Richter, 2005). This process is often helped by feedback from previous classifications and preliminary results, involving cycles of reciprocal illumination (see below). Given that the criteria are not clearly delimited, I will not emphasize in the classifying of observations as pertaining to this or that criterion, or comparing the performance of criteria.

Similarity and transformations

Another way of expressing commonality in topology and composition is to use the more general idea of "similarity" (e.g., Rieppel and Kearney, 2002). The relation between "similarity" and homology promoted a hot debate in recent years (Kluge, 1999, 2003; Grant and Kluge, 2004; Cracraft, 2005; De Laet, 2005; Richter, 2005; Rieppel, 2005, 2006; Ghiselin, 2006, p. 92; Kluge and Grant, 2006). A basic criticism is that there are many, often contradictory aspects that could contribute to the similarity between structures ("similarity lies in the eye of the beholder") (e.g., Hawkins, 2000). A more elaborate criticism notes that a parsimony analysis attempts to minimize events of evolutionary transformations, which are not directly related to similarity measures: some evolutionary events introduce more "dissimilarity" than others. Single evolutionary events can be very drastic, involving many homologous structures at the same time; examples of those are deletions or insertions of fragments of DNA (De Laet, 2005). De Laet observed that in data sets with inapplicable data, minimizing transformations and maximizing homology are not equivalent procedures. His approach accounts for the finding of Maddison (1993) who demonstrated that in the presence of inapplicable characters, current parsimony algorithms can produce optimizations and counts of transformations that are internally inconsistent, i.e., counting transformations in structures that were absent. As there are currently no exact algorithms to implement the ideas of De Laet (2005), the analysis presented here uses regular parsimony, minimizing transformations. Some safeguards against the problem of inapplicables are discussed below.

As a synthesis of both points of view, it should be noted that it is not possible to obtain similarity values without reference to specific transformation events. Take for example the sequences ACTACGGATC and CTAGGCATCA: they are very similar or very different if inversions are considered as plausible events or not, respectively. This issue is also applicable to morphology. For example, the flowers of *Arabidopsis* and *Tulipa* are radically different when interpreted under a classic developmental model, but the discovery of a simple homeotic change in the expression of regulatory genes was enough to explain their differences (Theißen, 2005, Fig. 2).

Learning from molecular sequence data

In the analysis of molecular sequences of different length, homology and parsimony are tightly associated from the beginning, as the alignment is accomplished by minimizing edit events that are evolutionary transformations (insertions, deletions, transitions, transversions). Early treatments of the alignment problem were explicit in that alignments are tree-specific and that base-to-base correspondences should be represented over trees, with hypothetical ancestral sequences at the nodes (Sankoff, 1975; Sankoff et al., 1976). This one-step procedure of direct optimization (Wheeler, 1996) is computationally complex for real data sets (much more so at that time of such early discussions) and thus the alignment of sequences as a step prior to tree search was seen as a reasonable approximation (reviewed in Wheeler, 2001). This two-step schema is the same as the traditional way of defining correspondences and characters in morphology and became naturalized as the conceptual framework for sequence analysis (e.g., Simmons and Ochoterena, 2000; Simmons, 2004) instead of a pragmatic approximation. Nowadays it is computationally possible to obtain reasonable heuristic solutions for base-to-base correspondences and phylogenies at the same time (Wheeler, 1996; Wheeler et al., 2006). In this context hypotheses of correspondence are part of phylogenetic hypotheses, and are subject to the same optimality criteria as the trees, namely the minimization of evolutionary transformation events. This dynamic homology approach to sequence data offers a solution to the homology problem at the level of individual nucleotides: correspondences are not primary data, but results of the phylogenetic analysis. Under this paradigm, the distinction of primary and secondary homology dissolves (Grant and Kluge, 2004, p. 27).

Morphological homology as a parsimony problem

Although a method of dynamic homology for morphology was never proposed in detail, the issue of considering homology as a parsimony problem is already implied in current discussions. Welten et al. (2005) discuss the homology of fingers in the avian hand, and conclude that "[o]n the basis of current data, no one model of digit homology is more *parsimonious* than others" (p. 26, emphasis added). Rieppel (1996) proposed that in order to decide on alternative hypotheses of correspondences one should build data sets under each of the alternatives, and select the one that produces the more congruent results. Rieppel's approach is close in spirit to the ideas proposed here, although in his implementation he used the consistency index as a congruence measure to select among hypotheses, and eliminated the autapomorphies from the alternative data sets (p. 1397). Svensson (2004, p. 419) proposed "treating a certain phylogenetic tree based on, for example, a large molecular data set, as background knowledge. On this tree the changes in gene expression, demanded by different organ homology hypotheses, could be optimized and the most parsimonious hypotheses identified." De Laet (2005) mentioned that morphology could in principle be treated using similar algorithms for dynamic homology as with molecules, and Robillard et al. (2006) recently produced an analysis of discretized cricket songs using algorithms for direct optimization of molecular sequences.

The literature specific to the problem of morphological homology provides many examples amenable of a dynamic homology interpretation. As in molecules, the most paradigmatic cases involve the homology of serial components when they appear in different numbers. Classic examples are individual digits in tetrapods with less than five digits (Alberch and Gale, 1985; Oster et al., 1988; Feduccia, 1999; Wagner and Gauthier, 1999; Vargas and Fallon, 2005a,b; Welten et al., 2005), of identity of vertebrae along the body axis (Burke et al., 1995), and of appendages in arthropods (Edgecombe et al., 2000). Similar examples involve modular structures in plants (Sattler and Rutishauer, 1990; Svensson, 2004), or non-repetitive structures for which comparative anatomy or development produce mixed reports (see examples in Rieppel and Kearney, 2002; Theißen, 2005). Here I will try to implement a generalization of dynamic homology for morphological characters. Although most of the literature on homology and "homology criteria" refers either to morphology or molecules, the same discussion and arguments are, however, applicable with little alteration to other fields of comparative biology, as are behavior, developmental architecture, or metabolic pathways.

The taxa to be compared

The arguments for proposal, justification and discussion of homology (i.e., why some correspondences are preferred over others) are part of the standard discourse of comparative biology. While the problem of which structures in different organisms are to be compared has been well worked, the issue of which *organisms* are to be compared, however, has not received similar attention. Most commonly, argumentations about correspondences are presented as pair-wise comparisons between two, or a few representative species, for which detailed anatomical studies are available, or are produced for that purpose. Most real problems, however, involve multiple rather than two or a few species, and in this case pair-wise comparisons (or comparisons involving just a few representatives) may suggest different correspondences according to the species that are being compared. If we choose the schema of correspondences

favored by the majority of the comparisons, then there is the problem of non-independence: some of the pair-wise comparisons may come from closely related species. Morphologists typically order comparisons in series of gradual similarity, using the criterion of intermediate morphologies.

Intermediate morphologies

Recourse to intermediates to argue correspondences is an interesting elaboration, because the procedure is explicit in analyzing multiple (three or more) correspondences in a series, instead of only pairs. It is implicit in the reasoning that the series of comparisons should be compatible with the phylogenetic tree. This is so because the intermediate morphology should be a plausible intermediate in phylogeny, or at least bring some information about evolutionary intermediates. If the "intermediate" is known to be a distant taxon, and its morphology is a convergent elaboration, then intermediacy is not informative about ancestry, and cannot be invoked as a justification for homology. The issue of intermediacy illuminates a line of tension between the idea of homology as correspondences, figured as lines from one terminal to another terminal, and the idea of homology as common ancestry, depicted as lines along branches of a tree, spanning all hypothetical ancestors from one terminal to another ("homology lines", Wheeler, 2003, Fig. 2); those hypothetical ancestors are in general different from any of the terminals.

It is possible to envision the criterion of intermediacy in a way that becomes identical to the dynamic approach proposed here. That occurs when the intermediates are hypothetical ancestors on a tree, and are allowed to have combinations of characters not observed in the terminals. The comparisons are then arranged in a tree-like structure (instead of a linear series) and the tree is the optimal tree according to all available characters. In these circumstances, the correspondences that minimize the global sum of transformations over all characters are the optimal correspondences. Of course, we usually do not know the globally optimal tree, because it depends on the correspondences themselves, and that is why the correspondences and the trees must be evaluated simultaneously (see below).

Homology shifts

The core of the formulation of homology as a parsimony problem is realizing that homology correspondences, whatever they are, imply evolutionary transformations (Fig. 1), and that schemas of correspondences can be compared according to the amount of evolutionary change that they imply. If traditionally we defined homology correspondences according to

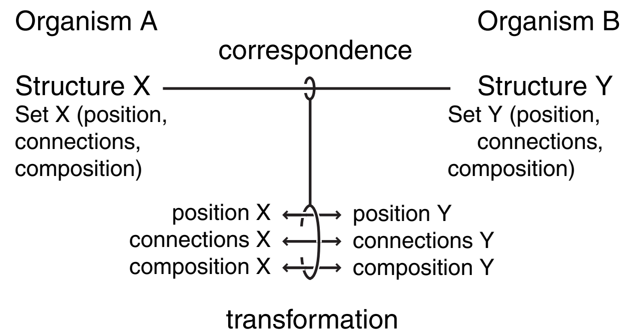


Fig. 1. Homology correspondences imply evolutionary transformations somewhere in the tree branches connecting organisms A and B.

positional and compositional characteristics that remain constant across homologs, considering multiple alternative correspondences means that these characteristics may differ in some of the homologs: somewhere in the phylogenetic tree, these differences imply evolutionary transformations. Hypotheses of homology between parts of organisms in different positions will imply more transformations than if they were in the same position, and so on.

The idea is illustrated with the hypothetical example of Fig. 2. In this example, Species 2 has two lobes, while Species 1 has only one lobe; the correspondence of lobes between species is unclear (Fig. 2A). Suppose we can summarize the positional (topological) information of the lobes as sets P and Q. We can also summarize the compositional information (internal details) as sets X and Y. The unique lobe in Species 1 shares all the positional information as the first lobe Species 2 (set P), but also shares all its compositional information with the proximal lobe in Species 2 (set Y). That is, position and composition conflict with each other when it comes to decide on correspondences. We will evaluate two correspondence schemas, where the unique lobe in Species 1 is homologous to (a) the distal lobe in Species 2 (Fig. 2C), or alternatively (b) to the proximal (Fig. 2D). As both species are part of the same phylogenetic tree, we trace homology lines and transformations through intermediate ancestors. For schema (a), rooting arbitrarily on Species 2, the correspondences imply at least one transformation from composition X to composition Y, and the loss of the proximal lobe (Fig. 2C). For schema (b), the correspondences imply at least one transformation from position Q to position P, and the loss of the distal lobe (Fig. 2D). (Rooting in Species 1 produces a symmetrical reconstruction, with gains instead of losses.) In order to decide which of the two schemas is more parsimonious, we need to assign costs of transformation between Q and P, X and Y, and of gains and losses of lobes. For doing so, we can simply represent the possible transformations as characters (Fig. 2E) expressing gains or losses, changes in position,

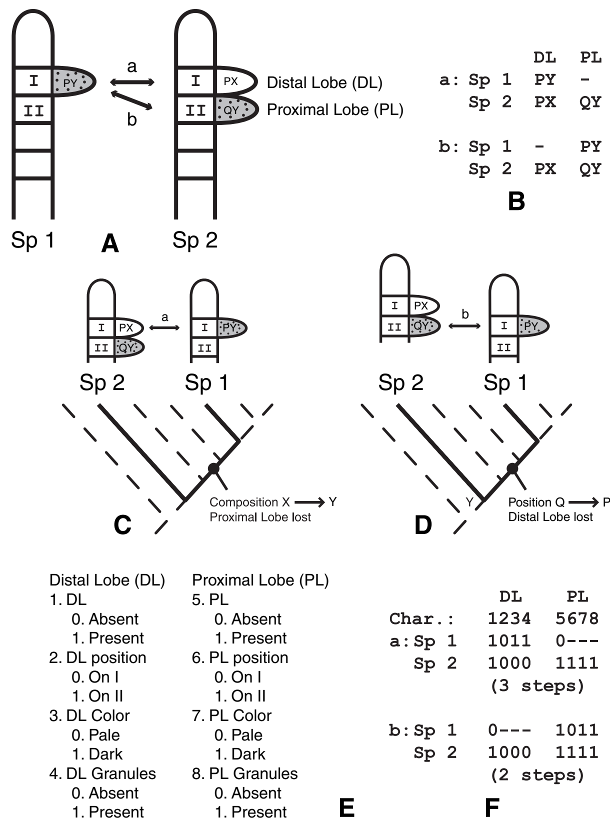


Fig. 2. (A) Hypothetical example of ambiguous homology, with two alternative correspondences: the lobe in Species 1 corresponds to the distal lobe in Species 2 (a), or to the proximal (b). The sets of positional (P, Q) and compositional (X, Y) information are in conflict. (B) Correspondences (a) and (b) represented as alignments of positional and compositional sets. (C) Species 1 and 2 are part of the same phylogenetic tree (dashed lines for the rest of the tree). Correspondence (a) implies an evolutionary transformation in composition (X–Y), and the loss of the proximal lobe. (D) Correspondence (b) implies an evolutionary transformation in position (P–Q), and the loss of the distal lobe. (E) Representation of the presence, positional, and compositional data as characters. (F) Correspondences (a) and (b) represented as alignments of presence, positional, and compositional characters; alignment (b) is one step shorter than (a).

or composition (color and granules, in this example). For the sake of the example, we will consider all these transformations as plausible, and of equal costs (we will discuss the issue of costs later). If there were additional information relevant to the homology of the lobes (e.g., further compositional details, connections to other organs, developmental patterns), they would be expressed as additional characters. The two alternative correspondences can be scored as two data sets (Fig. 2F). In this example, with these characters alone, schema (b) is more parsimonious, because it saves two steps in compositional characters, at the expense of one step in a positional character.

In Fig. 2(F) the two homology schemas (a) and (b) are represented as two alternative alignments. The unique lobe of Species 1 can be aligned with either lobe of Species 2 because both lobes are scored for the same conditions (characters 2–4 are equivalent to 6–8). The following sections will address in more detail the two main ideas behind this procedure:

1 It is possible to obtain proper transformation costs for homology shifts by representing as characters all the positional and compositional elements relevant to decide on homology correspondences.

2 When the potential homologs are scored for the same conditions, they are comparable, thus the alternative correspondences can be evaluated in terms of the evolutionary transformations that they imply.

Transformation costs for homology shifts

Homology correspondences are determined such that homologous structures in different organisms have similar position and connections in relation to other homologous structures, and share some structural details. This commonality can be traced to common ancestors, which are hypothesized to have similar positional and compositional conformation. In cases where both position and connections underwent evolutionary transformations, the resulting morphologies present a pattern that suggests conflicting correspondences. We can trace hypothetical correspondences, and place hypothetical conformations in ancestors, and count the evolutionary transformations that those reconstructions imply. That is, we can consider the arguments to defend correspondences, including the results of the application of "homology criteria", as regular characters, as in the example above of Fig. 2. Doing so will produce a direct relation between the justification of correspondences and the transformation costs that these correspondences imply (i.e., costs for each positional or compositional transformation). A shift in position may imply, for example, a homeotic transformation; a shift in compositional structure implies changes in compositional characters (the most common characters in cladistic analyses). The more controversial the correspondences, the more required evolutionary transformations, and the less anatomical configurations that can be traced as conserved in a common ancestor.

The characters involved in alternative homology correspondences should relate to biologically plausible transformation events. If we knew from other sources that a shift in position is too drastic to be invoked as a biological event, then we will not propose it as a possibility in the first place, and will consider the correspondences as settled. Equivalently, we can assign a very high (or infinite) cost to a transformation implying a change in position, thus making certain correspondences suboptimal on any tree. During the last

few decades, the comparative and experimental study of regulation of development discovered mechanisms that make plausible certain evolutionary transformations that would otherwise be considered highly speculative (e.g., Galis et al., 2005; Vargas and Fallon, 2005b; Wagner, 2005; Welten et al., 2005).

Justification of correspondences as characters

Expressing the application of ''homology criteria'' as characters is less problematic that it may appear. Perhaps this is more easily exemplified in developmental studies, as ontogenetic origin is one of the favorite sources of arguments for homology:

''Processes, or assemblies of processes into pathways or cassettes, are themselves characters. This idea has not penetrated discussions of homology in the evolutionary literature, perhaps because evolutionary biology and systematics have traditionally focused on adult structures. It is, however, essential for evolutionary analyses of development: many of the key characters of embryos are processes. Moreover, many embryonic structures are themselves transient (i.e., time dependent). Thus if we seek homologies in embryos, they should be homologies of processes rather than of structures. This is not a new conclusion, at least among those who have sought to integrate developmental and evolutionary biology.'' (Gilbert and Bolker, 2001, p. 5).

I found that even in classical morphology based on adults, the ''homology criteria'' are mostly embedded in the phylogenetic characters. Using as an example the case presented below of sclerites in the male copulatory organs of anyphaenid spiders, some of the characters could easily pass as if they were justifications of correspondences: a sclerite is fused or separate from some adjacent structure, or it is widely separated by a membranous area (connections); it arises from this or that region of the copulatory bulb (position); it bears a deep canal, or bears regularly disposed denticles (composition). Without knowing the details of each case, it is impossible to know which of these may qualify as justifications of correspondences, and which are regular characters; it seems that it would be only a matter of constancy or variation, respectively. If all the conductors had regularly disposed denticles, I may have invoked it as an indicator of correspondence, a compositional detail that supported the identification of a conductor as such. It turned out that only two genera had these denticles, hence I expressed it as a character, a synapomorphy of *Tomopisthes* plus *Araiya*. The conductors may also be firmly fused to some distal area of the tegulum. If all the species had fused conductors, I may have used this condition to argue that conductors are just an outgrowth of the tegulum. Because many species have the conductor separated from the tegulum, including all the basal clades, I considered the conductor as a separate sclerite, and the fusion as a character. In Ramírez (2003) I used the implantation of the conductors as a criterion to decide if they are primary or secondary conductors. In this contribution, I will test if the conductors are primary or secondary, and for doing this I will use the implantation as a character (see also Hübner, 2006, p. 391).

Comparable vectors for alternative homology correspondences

Expressing the justification of correspondences as characters provides adequate transformation costs for alternative homology schemas. This is easily done when the structures involved in alternative correspondences are represented by fully compatible character vectors. In other words, the structures, as represented by their characters, are made comparable. Doing so requires that the characters are expressed in a different way from a traditional cladistic analysis. A traditional character has the form ''Part X of homologous region A: condition 0, condition 1''. For dynamic homology, there are as many copies of the character as there are potential homolog areas. For example, for two regions A and B, the form is ''Part X of homologous region A or B: condition 0, condition 1'', and the character is used for both regions (for example, the characters 3 and 7 in Fig. 2E). This task is not simple, because comparable structures in different regions often receive different names, some characters involve connections with structures that are adjacent to region A but not B, some regions are very complex and provide many characters, while others are simpler, and so on. The real example used here is illustrative of the complexities involved (see characters in Appendix 2). Redefining the characters so that they are applicable to several regions at the same time was one of the more intellectually demanding tasks of this project, and required the reexamination of virtually all the terminals in the data set.

Absences and inapplicables

At this point we have the structures that are to be tested for homology correspondences represented as comparable character state vectors. In order to analyze these data with parsimony algorithms using fixed homology, each of these vectors is preceded by one character for the presence or absence of the structure in question; when a structure is absent, all its subsidiary characters are inapplicable. This strategy may produce paradoxical optimizations or counts of steps under fixed (Maddison, 1993), or dynamic homology (De Laet, 2005). The use of inapplicable cells is an approximate solution to the problem, and must be controlled for

```
a 000000000000    a 1000000000000 0------------ 0------------ 0------------ 0------------
b 111000010001    b 0------------ 1111000010001 0------------ 0------------ 0------------
c 111111000101    c 0------------ 0------------ 1111111000101 0------------ 0------------
d 111111111000    d 0------------ 0------------ 0------------ 1111111111000 0------------
e 111111111110    e 0------------ 0------------ 0------------ 0------------ 1111111111110

A                 B
```

Fig. 3. (A) Hypothetical data set of 12 characters for a morphological structure (shortest tree 15 steps, three homoplasious steps). (B) The same data set, reinterpreted as if there were five non-homologous structures appearing independently; each segment is preceded by a character 0: absent, 1: present (five steps, no homoplasy).

trivial solutions (Fig. 3). The pragmatic solution used here is to limit the maximum number of kinds of structures.

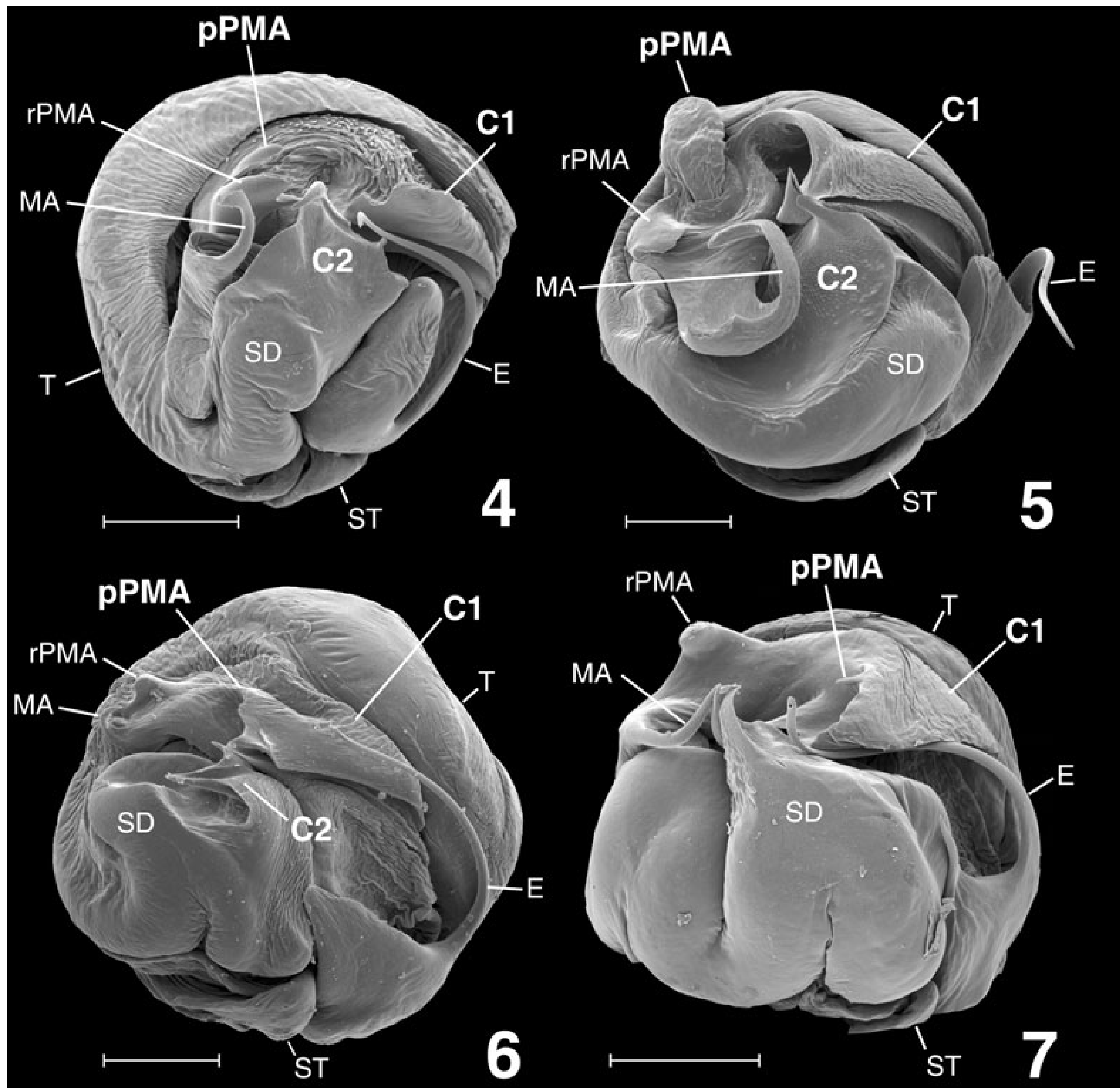
Specifying permitted positional changes within local homology problems

Before incorporating the alternative alignments into the tree optimization procedure, it is necessary to specify the permitted positional rearrangements within each local homology problem. A local homology problem is defined here as the set of body regions that participate in the specific correspondences under test. For example, in the problem of homology of fingers of the bird hands, the local problem is restricted to the five digits of the forelimbs; there may be other homology problems in the same data set (e.g., identity of vertebrae), but those problems do not interact with each other except by occurring on a common phylogenetic tree. The permitted rearrangements of correspondences will be specific for each local problem. Serially ordered structures will typically limit changes to one dimension, as in nucleotide sequences, avoiding inversions or more radical reshuffling. For example, if the first digit of a frog is aligned with the second of a lizard, then the frog's second digit cannot be aligned with the lizard's first. Other possible examples of one-dimension systems are vertebrae, teeth and series of ontogenetic stages. More complex systems may involve specific rearrangements in more dimensions, when considering local problems in surface, volume and ontogeny. However, because alternative homologies demand a very intense study of comparative anatomy, it is unlikely that this method will be applied to very complex systems. The study case presented below is linearly ordered.

The study case

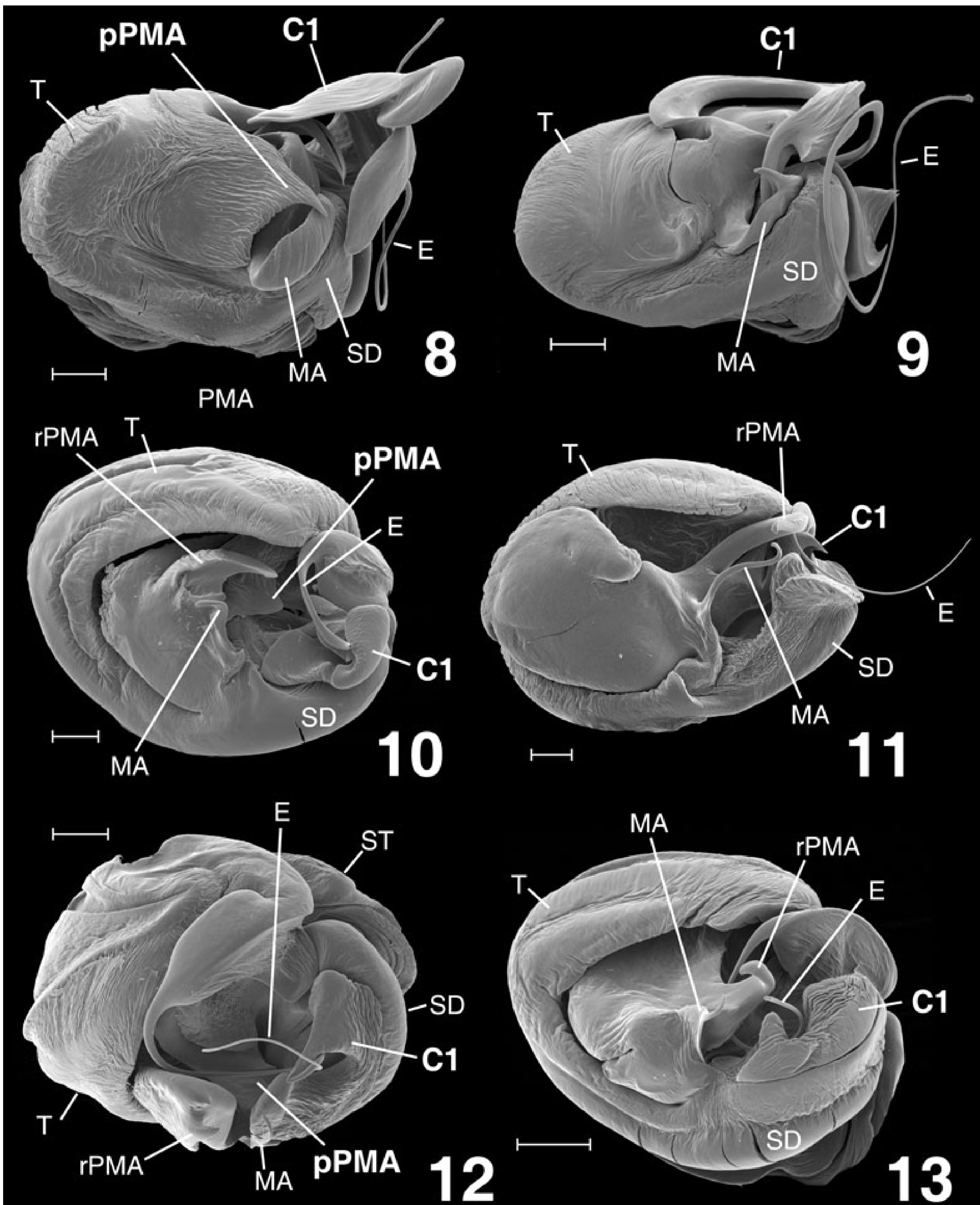
I will apply the methodology proposed here to a real cladistic analysis of anyphaenid spiders of the subfamily Amaurobioidinae (Ramírez, 2003), as updated in Ramírez et al. (2004). Those studies comprised representatives of all the 22 genera currently included in the subfamily, and were based in 93 species scored for 200 characters. About 25% of the characters come from the male copulatory organ, a very complex structure almost universally used for species identification (Figs 4–13). This remarkable organ is located on the tarsus of the male palp, and is not connected with the testis: after reaching maturity, the male deposits a drop of sperm in a small web made for that purpose, and fills the copulatory organs. Mating involves the intromission of parts of the copulatory organ in the female genitalia. In derived spiders, the movements of the copulatory organ are hydraulic, caused by expanding membranes (see Huber, 2004). Most characters from the copulatory organ come from the hard, sclerotized regions called sclerites. Some apical sclerites are generically denominated "conductors", without strong expectations of homology (Coddington, 1990). There may be a "primary" and a "secondary" conductor on the same copulatory organ, clearly identifiable within restricted groups (e.g., genera, tribes), but the correspondences between the "conductors" of more distantly related groups is contentious. In derived spiders there are no nerves or muscles inside the copulatory organ that might help elucidate correspondences (Huber, 2004), and the "conductors" are rather simple sclerites without specific glands or further structural details. The copulatory organ appears fully developed with the maturity moult; its ontogeny has been laboriously studied for a few species through histological sections (reviewed in Coddington, 1990). Details on the ontogeny of a conductor were only reported in one of the studies (for *Latrodectus*; Bhatnagar and Rampel, 1962), in which the conductor and the median apophysis originate from the dorsal lobe of the claw fundament; *Latrodectus* has only one conductor (Agnarsson, 2004). The homology correspondences that will be tested here involve three sclerites of the male copulatory organs: the primary conductor (C1), the secondary conductor (C2), and a prolateral cusp of paramedian apophysis (pPMA). These three sclerites were represented by 23 subordinate characters in Ramírez (2003).

The major groups inside Amaurobioidinae are the tribes Amaurobioidini (Figs 4–7), Gayennini (Figs 10–13) and the genus *Josa* (Figs 8 and 9), which is the sister group of Gayennini (Ramírez, 2003). Within each of these groups, the conformation of the copulatory bulb is sufficiently conserved for reliable hypotheses of correspondences for the apical sclerites, even when



Figs 4–7. Left copulatory bulbs of Amaurobioidini. 4. *Gamakia hirsuta* Ramírez. 5. *Coptoprepes flavopilosus* Simon. 6. *Ferrieria echinata* Simon. 7. *Aysenoides colecole* Ramírez. (C1 = primary conductor; C2 = secondary conductor; E = embolus; MA = median apophysis; pPMA = prolateral cusp of paramedian apophysis; rPMA = retrolateral cusp of the paramedian apophysis; SD = sperm duct on distal tegulum; ST = subtegulum; T = tegulum.) Scale bars = 0.1 mm.

some sclerites are missing in certain species. Between major groups, however, the correspondences are unclear. In Ramírez (2003) I made a thorough morphological examination of many representatives using an scanning electron microscope, expansions and dissections of the copulatory organs, and many cycles of preliminary runs, character mapping, and reexamination of specimens. At the end I adopted one schema of homologies that seemed to reflect better the comparative studies, especially the positional criterion, admitting that the election was to some extent arbitrary. Under an alternative schema of correspondences (Ramírez, 2003, p. 50), the C1 as identified in Gayennini could be homologous to the pPMA as identified in Amaurobioidini, while the C2 of Gayennini might correspond with the C1 of Amaurobioidini. The case presented here is aimed to test the hypothesis of correspondences of these three apical sclerites. In this data set, four of the representative



Figs 8–13. Left copulatory bulbs of *Josa* and Gayennini, according to the most parsimonious interpretation found here. 8. *Josa nigrifrons* (Simon). 9. *Josa calilegua* Ramírez. 10. *Tomopisthes varius* Simon. 11. *Oxysoma punctatum* Nicolet. 12. *Gayenna americana* Nicolet. 13. *Sanogasta x-signata* (Keyserling). (C1 = primary conductor; E = embolus; MA = median apophysis; pPMA = prolateral cusp of paramedian apophysis; rPMA = retrolateral cusp of the paramedian apophysis; SD = sperm duct on distal tegulum; ST = subtegulum; T = tegulum.) Scale bars = 0.1 mm.

Table 1
Pair-wise similarity scores between species having three and two terminal sclerites in the male copulatory organ, expressed as number of shared character states. Scores are computed for each of three alternative schemas of homology correspondences. Maximizing similarity favors different homology schemas for different pair-wise comparisons (asterisks)

Terminals with three sclerites	Terminals with two sclerites	Similarity score	Alignment type
Ferrieria echinata	*Gayenna americana*	34	C2, C1
		36	C2, pPMA
		39	C1, pPMA*
	Philisca puconensis	34	C2, C1
		37	C2, pPMA
		38	C1, pPMA*
	Araiya pallida	34	C2, C1
		36	C2, pPMA*
		35	C1, pPMA
	Josa riveti	31	C2, C1
		35	C2, pPMA
		39	C1, pPMA*
Gamakia hirsuta	*Gayenna americana*	34	C2, C1
		36	C2, pPMA
		39	C1, pPMA*
	Philisca puconensis	32	C2, C1
		37	C2, pPMA
		38	C1, pPMA*
	Araiya pallida	34	C2, C1
		36	C2, pPMA*
		35	C1, pPMA
	Josa riveti	31	C2, C1
		35	C2, pPMA
		39	C1, pPMA*
Coptoprepes flavopilusus	*Gayenna americana*	37	C2, C1
		39	C2, pPMA*
		39	C1, pPMA*
	Philisca puconensis	37	C2, C1
		40	C2, pPMA*
		38	C1, pPMA
	Araiya pallida	35	C2, C1
		37	C2, pPMA*
		35	C1, pPMA
	Josa riveti	34	C2, C1
		38	C2, pPMA
		39	C1, pPMA*
Coptoprepes nahuelbuta	*Gayenna americana*	35	C2, C1
		38	C2, pPMA*
		38	C1, pPMA*
	Philisca puconensis	35	C2, C1
		38	C2, pPMA*
		37	C1, pPMA
	Araiya pallida	34	C2, C1
		37	C2, pPMA*
		34	C1, pPMA
	Josa riveti	33	C2, C1
		37	C2, pPMA
		38	C1, pPMA*

species have the complete complement of three sclerites, 45 species have two sclerites, 37 have one, and three outgroup representatives lack the three sclerites altogether (see the additional material in http://www.cladistics.org/journal/data for further details).

The problem of pair-wise comparisons discussed above is exemplified here using the data from this real example. The values in Table 1 are similarity scores between pairs of terminals, considering three different schemas of homology correspondences. The scores were calculated following the methodology explained here, using both positional and compositional data, but are values of similarity (number of shared character states) rather than transformations. In this exercise, the male copulatory organs of three species of the tribe Gayennini, plus one species of *Josa*, are all compared with four members of the tribe Amaurobioidini. If we attempt to select the correspondences that maximize similarity scores between terminals, then different pairs of species suggest different correspondences. For example, comparing *Ferrieria echinata* with *Gayenna americana* suggests that the "conductor" of Gayennini is a primary conductor, but comparing *Ferrieria echinata* with *Araiya pallida* suggests that it is a secondary conductor. The majority of the comparisons supports an interpretation as a primary conductor, but they come from closely related species, thus are not independent.

Examining alternative correspondences during tree search

An exhaustive evaluation of alternative correspondences should examine, at least implicitly, all the possible correspondences for each phylogenetic tree (Wheeler, 1996; Wheeler et al., 2006). The rudimentary approach used here involves the generation of alternative alignments, which are submitted to regular parsimony analysis. The procedure is inefficient for the purpose of searching optimal hypotheses, because the alignments are produced blindly, without any feedback from optimality values.

In the case presented here the terminals with three sclerites have fixed correspondences, as a means to limit the possible number of homology lines (see inapplicables above). Terminals with one sclerite produce three possible combinations (the sclerite can be either C1, C2 or pPMA) (Table 2). Similarly, terminals with two sclerites produce three combinations as well (C1 and C2, C2 and pPMA, C1 and pPMA). Four species known only from females produce only missing entries.

In total, there are 85 terminals with one or two sclerites (Fig. 14); hence there are 3^{85} possible alignments, too

Table 2
Terminals with one or two sclerites are aligned in three different ways

Alignment type	Correspondences		
	One sclerite	Two sclerites	Three sclerites
0	C1	C2, C1	C2, C1, pPMA
1	C2	C2, pPMA	C2, C1, pPMA
2	pPMA	C1, pPMA	C2, C1, pPMA

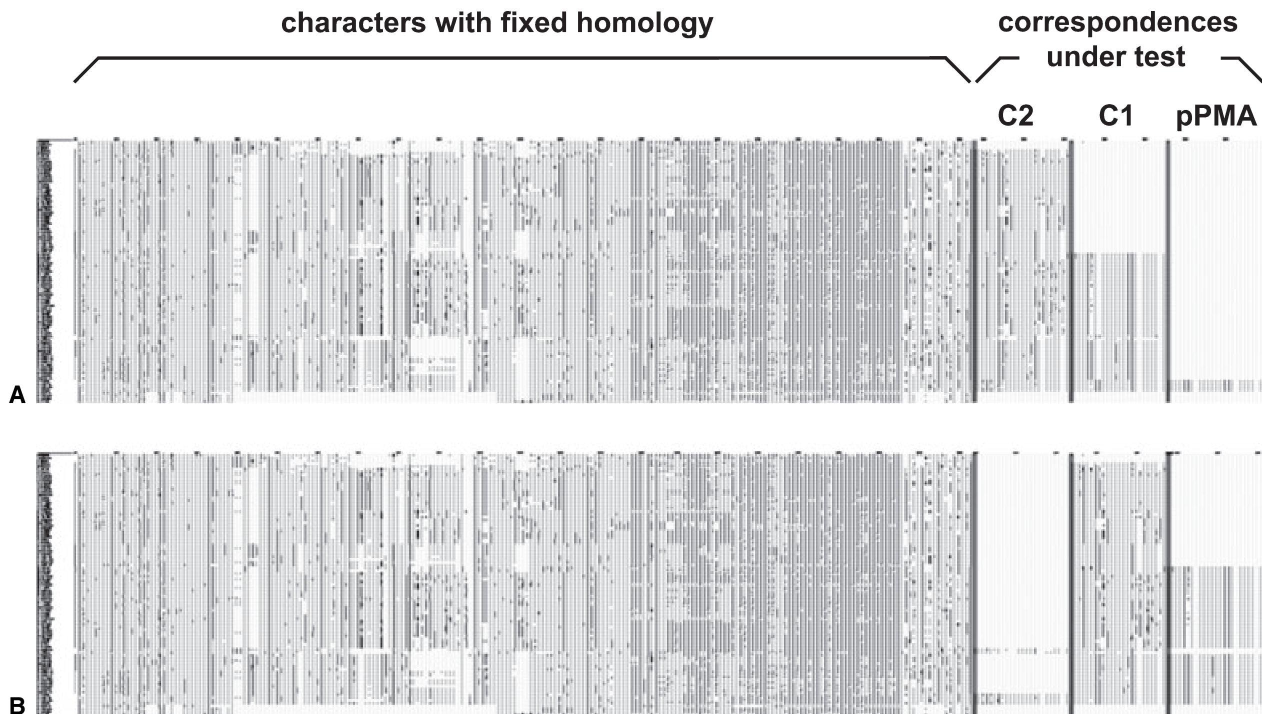


Fig. 14. Schematic view of the data set. A. Unaligned data set. B. The shortest alignment found here, one of the 6561 alignments analyzed.

many for exhaustive enumeration. To reduce the problem, several terminals were grouped in eight blocks that changed correspondences coordinately (Fig. 14B), thus producing only $3^8 = 6561$ alignments. These eight groups (Appendices 1, 4) are defined by being closely related according to the previous analyses (Ramírez, 2003; Ramírez et al., 2004), and by having very similar morphology in the apical sclerites of the copulatory organ. With such similarity in morphology, it is unlikely that heterogeneous correspondences inside each group will produce shorter trees; this reduction of the problem is considered realistic. If the combinatorial approach is inefficient for searching optimal trees, it is however, useful for experimental purposes, as for evaluating the increase in tree length that specific correspondences imply (see below). The new versions of POY under preparation (Ward Wheeler, personal communication) will be able to analyze dynamic correspondences of morphological structures.

Each of the 6561 alignments was analyzed with parsimony under equal weights with TNT (Goloboff et al., 2003–06) using five replicates of random addition sequences plus TBR, followed by 50 iterations of the parsimony ratchet (Nixon, 1999a), keeping up to two optimal trees on each replicate (commands "ratchet: iter 50; mult = replic 5 tbr ratchet hold 2;"). Pilot tests indicated that these search parameters are sufficiently aggressive to hit the minimum length several times. This small set of best trees (typically four to eight trees) was saved for each alignment. For calculation of consensus the set of optimal trees was expanded by TBR swapping of this reduced set. The alignments were produced and analyzed with specific scripts made for TNT, using an unaligned matrix as source. The graphic mapping of characters on the consensus (Fig. 15) is represented as the union of the optimizations over 4000 equally parsimonious trees ("common mapping" in TNT). The data set was edited and maintained with Winclada (Nixon, 1999b).

Results

The tree length over the 6561 alignments examined ranged between 969 and 1045 steps. Only one alignment produced the optimal length of 969 steps, its strict consensus is shown on Fig. 15.

The original interpretation of correspondences used in Ramírez (2003) produces trees of 972–975 steps, depending on the interpretation of the outgroups (Table 3). This is at least three steps longer than the best length found here. These extra steps are produced by interpreting the large conductor of *Josa* and Gayennini as a C2, and a small sclerite present in the same groups as a C1. The present analysis suggests that these sclerites are better interpreted as a C1 and pPMA, respectively. The correspondences found here as most parsimonious were considered as a second reasonable

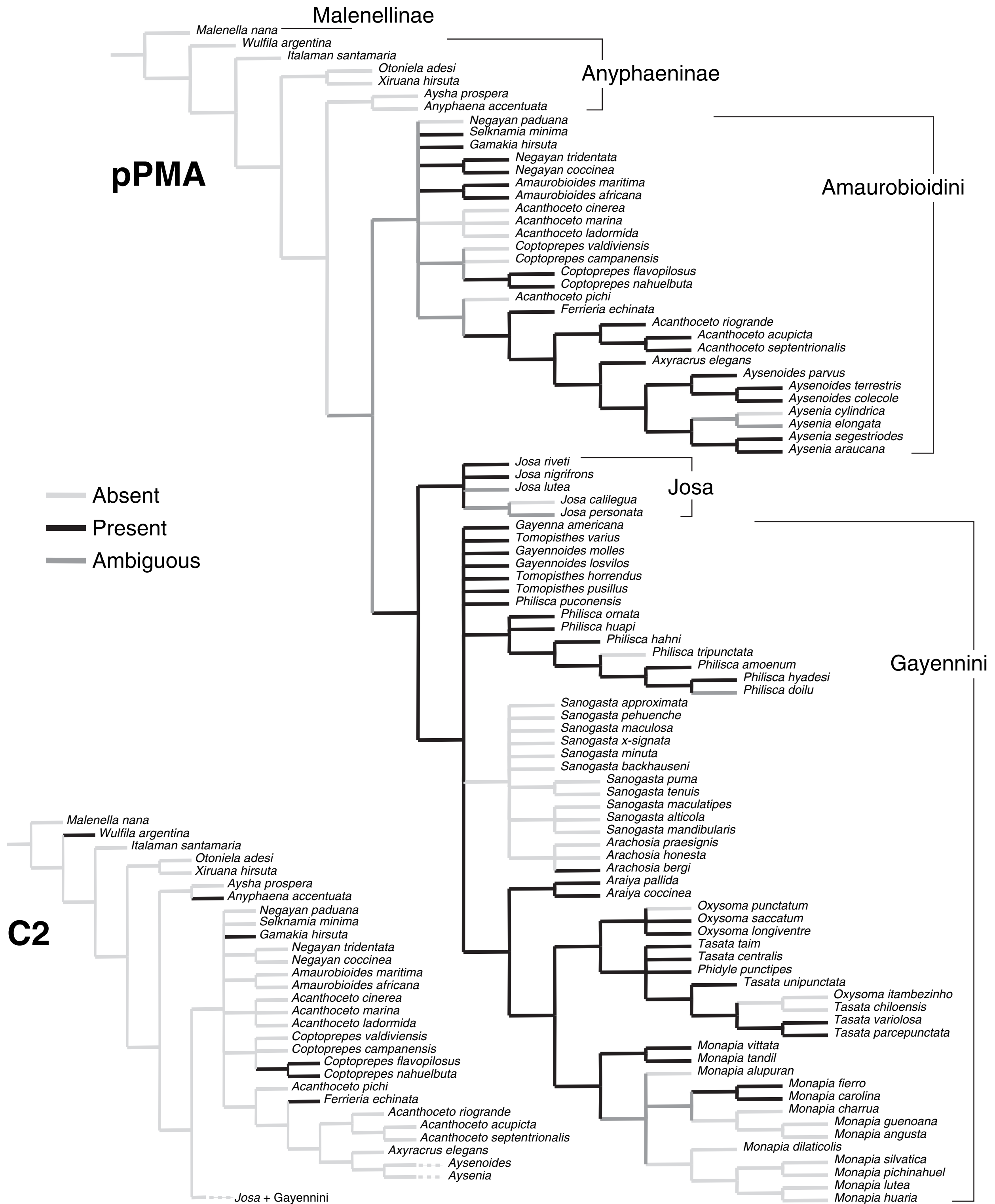


Fig. 15. Strict consensus of 4000 trees from the optimal alignment (969 steps), with common mapping of the prolateral cusp of paramedian apophysis (pPMA, top) and secondary conductor (C2, bottom). The primary conductor (C1) is present in all known males except the outgroups *Aysha* and *Italaman*.

Table 3
Correspondences for the blocks of species analyzed under dynamic homology, for four alignments: the optimal found here, and three suboptimal alignments with the preferred interpretation in Ramírez (2003), with three different interpretations for outgroups

	Optimal	Ramírez (2003) not considering outgroups		
Length	969	972	973	975
0. Amaurobioidini with two sclerites	C1.pPMA	C1.pPMA	C1.pPMA	C1.pPMA
1. Amaurobioidini with two sclerites, one of them a "massive" conductor	C1.pPMA	C1.pPMA	C1.pPMA	C1.pPMA
2. Amaurobioidini with one sclerite	C1	C1	C1	C1
3. Josa with one sclerite	C1	C2	C2	C2
4. Josa with two sclerites	C1.pPMA	C2.C1	C2.C1	C2.C1
5. Gayennini with one sclerite	C1	C2	C2	C2
6. Gayennini with two sclerites	C1.pPMA	C2.C1	C2.C1	C2.C1
7. Outgroups with one sclerite	C1	C1	C2	pPMA
Alignment number	6226	6076	6075	6077

possibility by Ramírez (2003, p. 50). The consensus of the optimal trees (Fig. 15) is very similar to the consensus found in Ramírez (2003, Fig. 5) under equal weights, even if the anatomical interpretations are quite different. The results for all alignments are available at http://www.cladistics.org/journal/data.

The optimality of alternative anatomical interpretations

So far we have concentrated on finding the set (or sets) of optimal homology correspondences and the trees they imply. Once the correspondences are integrated into the quantitative evaluation of phylogenetic hypotheses, it is possible to assign optimality values to further schemas of correspondences, other than the optimal. The length difference gives an idea of the decay in optimality for an alternative interpretation X:

$$\text{Length difference}_{\text{Interpretation X}} = \text{Best length}_{\text{Interpretation X}} - \text{Best length}_{\text{All Interpretations}}$$

For example, in Ramírez (2003) the large conductor of Gayennini (Groups 5 and 6, Appendix 1) was interpreted as a C2. Constraining the alignment of this conductor as a C2 in Groups 5 and 6, while permitting rearrangements in the second sclerite, and also in all sclerites in the rest of the terminals, produces trees of only one extra step (970 steps, alignment 6105). Similarly, interpreting the small sclerite present in some *Josa* (Group 4, Appendix 1) as a C1 (again as in Ramírez, 2003), produces trees of 3 extra steps (972 steps, in alignment 6076).

The two outgroup terminals that were allowed dynamic correspondences are *Otoniella* and *Xiruana* (Group 7). They have one sclerite, optimally aligned as a C1, although the general morphology is quite distant compared with any of the ingroup terminals. Not surprisingly, the alternative interpretation of their only sclerite as a C2 implies one extra step only (alignments 6105, 6225).

As expected, more radical reinterpretations produce more extra steps. For example, if any of the Amaurobioidini with one or two sclerites (Groups 0, 1, and 2) is constrained to have a C2, this implies at least nine extra steps (several alignments). Forcing the unique sclerite of Amaurobioidini Group 2 to be a pPMA, which is clearly untenable in morphological grounds, produces at least 17 extra steps. Mixed interpretations for similar taxa are also unparsimonious. As explained above, the large conductor of Gayennini could be a C2 with only one extra step. However, a heterogeneous interpretation within Gayennini (C1 for Group 5, C2 for Group 6, or vice versa) imposes a minimum of 19 extra steps.

Bremer support for anatomical interpretations

Similarly as for group support, we can estimate the Bremer support for a given anatomical interpretation as the minimum decay in optimality when such interpretation does not hold:

$$\text{Bremer support}_{\text{Interpretation Y}} = \text{Best length}_{\text{Not Interpretation Y}} - \text{Best length}_{\text{Interpretation Y}}$$

For example, the support for the interpretation of the unique sclerite of Amaurobioidini Group 2 as a C1 is nine steps, because the closer suboptimal with a different interpretation is alignment 2946, interpreted as a C2, and implies 978 steps. Similarly, the massive conductor of *Negayan*, *Selknamia*, and some *Acanthoceto* (Group 1) as a C1 has a Bremer support of nine steps (closer suboptimal interpreted as a C2, in alignment 2946). This support measure of differential homology schemes can be used in general for other non-morphological data as well.

Homoplasy and implied weights

In Ramírez (2003) I used estimations of stability and congruence for the election of the method of analysis,

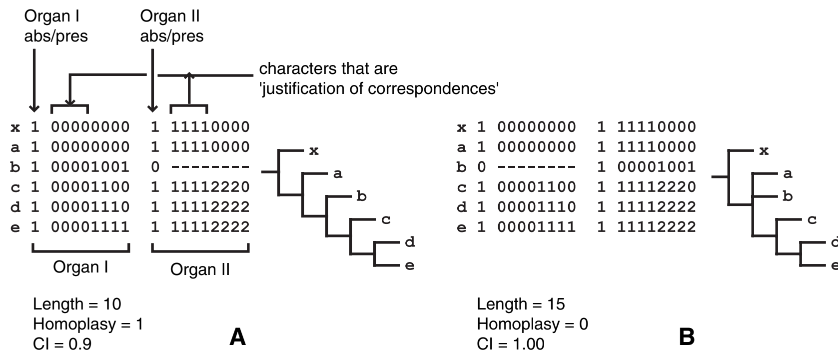


Fig. 16. Hypothetical example of six species scored for two Organs I and II. (A) In Species b the Organ II is missing; four positional and compositional characters (the "justification of correspondences") support the correspondence of the unique structure as an organ of the type I. (B) If the unique organ in Species b is aligned as an Organ II, against all positional and compositional characters, it produces a longer tree, but without homoplasy; implied weighting will prefer this alignment and tree.

finally preferring implied weights against homoplasy (Goloboff, 1993), with a mild weighting strength (constant of concavity $k = 6$ as implemented in PeeWee; Goloboff, 1993–97). Unfortunately, it is not possible to use the same method here. As noted by Wheeler (1996) for direct optimization of molecular sequences, in the absence of fixed correspondences it is not possible to define meaningful homoplasy levels (see also Kluge and Grant, 2006), although these values could be calculated for entire fragments (Wheeler et al., 2006, p. 88). The same effect occurs here. When the morphological alignments are analyzed under implied weights, the optimal fit value is obtained when the conductor of two outgroup terminals (*Otoniella* and *Xiruana*; Group 7, Appendix 1) are aligned with the pPMA of Amaurobioidinae, and some Amaurobioidini (Group 0) have a C2, while other Amaurobioidini (Groups 1 and 2) have a C1. This is a bizarre anatomical interpretation that produces 18 extra steps. Implied weighting prefers this odd alignment because it slightly reduces the homoplasy here and there, at the expense of creating several "synapomorphies" or autapomorphies in otherwise invariant characters of C2 and pPMA. These are precisely the positional and compositional characters that justify the correspondences of sclerites.

The effect can be schematized with a simpler hypothetical example (Fig. 16). Although the second alignment is longer by five steps, the minimum number of steps is increased in six, thus producing a net reduction of homoplasy of one step. The idea of weighting against homoplasy is preferring the trees that concentrate homoplasy in the more homoplastic characters. Dynamic homology would open a further possibility of concentrating homoplasy in the more homoplastic morphological regions. However, under dynamic homologies the current definition of homoplasy would lead to the maximization of autapomorphies, which is far from the original aim of weighting against homoplasy.

Discussion

As a result of the dynamic homology approach, the correspondences are optimized within each local homology problem, and tailored to each phylogenetic tree. By doing this, the separate step of determination of primary homology has disappeared; homology is no longer primary—it is a result of the phylogenetic analysis (Grant and Kluge, 2004). It is opportune here to go back to the traditional procedures in the establishment of primary homology, and check where their operations and concepts have gone in the dynamic schema.

First of all, even under dynamic homology, most of the morphology will probably be analyzed under static correspondences; the dynamic schema will typically be used for specific structures of debatable homology. This is a simplification of the problem, rather than a rejection of the idea of applying dynamic homology to whole organisms. Static correspondences can be thought as extreme cases where the context characters justifying those correspondences add up to very large transformation costs, when alternative correspondences are tried. This is analogous to accepting the homology of genes or chromosomes, while testing the correspondences of nucleotides. The correspondences within local homology problems are solved independently of each other; their only interaction is the phylogenetic tree, which is common to all the characters. We can think of a local problem as a very complex character that needs a special optimization algorithm (cf. Wheeler, 1999). The differences between static and dynamic approaches to homology correspondences are thus restricted to what happens inside each local problem.

In the traditional schema with static correspondences, primary homology is determined by recourse to comparative anatomy. A dynamic homology approach takes into account that there are many possible pairwise comparisons between terminals, and that related-

ness of terminals is an important factor in those comparisons. More specifically, what comparative anatomy does is establishing correspondences between the anatomical contexts where the structures under study occur, literally the "alignment" of morphology (Stevens, 2000, p. 88). The arguments derived from application of the criterion of topology, including connections, are homology correspondences themselves: structures are homologous because they *share* a specific topological configuration relative to other parts of the organism, specific connections with other structures, and a common architecture; "positions, as well as parts, can correspond" (Ghiselin, 2006, p. 96). Sometimes these homology criteria contradict each other, or are inconsistent across all terminals, thus defining a local homology problem. By being homologies themselves, the justifications of correspondences can be traced back to ancestors, and when they vary, their evolutionary transformations can be minimized.

The present approach is more demanding in comparative anatomy studies than the traditional justification of primary homologies: it requires not only the list of observations that support and contradict a given schema of correspondences, but also those that support and contradict all other alternative schemas under test. Furthermore, it requires that the relevant observations in support of one or another correspondence be expressed as character states, with explicit hypotheses of evolutionary transformations. As proposed here, dynamic homology for morphology requires all the usual data of the traditional approach, and some more. The comparative anatomy data that are the foundation of primary homology under static correspondences becomes the source and justification of transformation costs for several alternative correspondences at the same time under dynamic correspondences. In the dynamic schema, the primary data (the comparative anatomy) is still well separated from the conclusions (the correspondences and relationships).

A relation with total evidence

The idea of producing phylogenetic hypotheses from all available evidence in a simultaneous analysis (Eernisse and Kluge, 1993; Nixon and Carpenter, 1996) plays both as a source and a solution for the homology problem. The principle of using all available characters implies that characters proposed for closely related species are used as well for higher rank phylogenies. Many of the finely grained characters used to distinguish between closely related species are details of organs and structures that are so radically changed between distant taxa that their correspondences are difficult to trace, and the character states hardly make sense across all terminals. Thus, total evidence implies dealing with homology problems. The solution that total evidence brings to the homology problem is conceptually interesting. In dynamic homology, the phylogenetic tree and the homology correspondences are calculated simultaneously, such that if the tree changes, the correspondences may change as well. Because the phylogenetic tree comes from the interaction of multiple data sources, all data sources are potentially relevant to deciding on homology correspondences. In other words, the support or falsification of a given hypothesis of homology may come from a mixture of different character systems, including characters unrelated to the specific structures under test. In this way DNA sequences are relevant for the homology of fingers, and fossils are relevant for the homology of nucleotides.

Reciprocal illumination

The process of building a system of homology correspondences classically involves feedback from previous or preliminary results (classifications and phylogenetic analyses), both within the time frame of a given study, and in the long term within the scientific community. This informal feedback is usually referred as an iterative process of "reciprocal illumination" (Hennig, 1966), assisting the anatomical interpretations and the taxon sampling. We can distinguish two general kinds of reciprocal illumination. In one, previous or preliminary results help the learning and understanding of complex anatomical structures, in an informal, even creative or inspirational way. The primary homology statements can be evaluated on the light of resulting or competing phylogenetic trees, and some errors in scoring and in interpretations can be corrected. This process is common to all morphological practice, and pertains to the context of discovery rather than the analysis or justification. A second, more involved kind is the evaluation of a few alternative schemes of correspondences on the light of the resulting trees (when more than one scheme is defensible) and favoring the correspondences that produce less homoplasy. This second operation is basically integrated within dynamic homology, although this method tries to examine all the alternatives, not only the ones suggested by a few preliminary trees.

Pragmatic effects of dynamic homology in morphology

It is useful to distinguish between the conceptual and the pragmatic implications of using dynamic homology for morphology. In conceptual terms dynamic homology is illuminating, because it helps understand the foundation of hypotheses of correspondences, and clarifies the interplay between homology and relationships. In pragmatic terms, we should consider if the additional work required for using dynamic homologies is justified by the expected results. The limited experience so far suggests that the effect on relationships may

not be significant. For example, in the classic example of homology of fingers in the hand of birds and other theropods, the alternative correspondences have no effect in the relationships, which are strongly supported by other character systems (Wagner and Gauthier, 1999). The example case used here points to the same direction: there are alternative schemas of correspondences that are quite different in anatomical terms, but imply just a few extra steps, on very similar trees. Moreover, where the trees differ, it is in weakly supported groups: for example, the consensus from the alternative alignment number 6076 (Table 3) differs from Fig. 15 in eight groups, with Bremer support of only one step (in seven groups), or two (in one group).

The issue is totally different if the main purpose is not finding the relationships, but reconstructing the evolution of particular characters, where assuming the correspondences a priori may easily lead to unparsimonious reconstructions. For example, according to the interpretation in Ramírez (2003), in the clade *Josa* + Gayennini there was a switching from the primary to the secondary conductor as the main sclerite protecting or guiding the embolus. In the more parsimonious reconstructions found here, this function is consistently accomplished by the primary conductor, which is more apically placed in *Josa* + Gayennini.

By integrating the data from comparative anatomy explicitly as characters, this approach can be seen as a more realistic test of homology. The characters used to support correspondences can in principle include evidence from gene expression, regulation and signaling, and all the fine anatomical studies. As occurs with all phylogenetic estimations, these tests should be considered crude approximations: There are good theoretical reasons to believe that morphology fits only imperfectly in the all or nothing correspondences imposed by a phylogenetic matrix (e.g., Sattler, 1984, 1992).

Conclusions

The method proposed here employs the same elements of comparative anatomy that were traditionally used to justify and argument about correspondences. It is demanding in the sense that requires that the justification of correspondences, derived from the so-called "homology criteria", are expressed as characters, and the characters must be expressed in a way that make sense over multiple structures at the same time. Because the names of morphological structures often presuppose correspondences, and the descriptive language itself is built for fixed homologies, expressing characters that may work under multiple correspondences requires an intense exercise of abstraction. The rewards for doing this are several. First, characters are explicit and invite a detailed scoring in all terminals, not just the two or three species used to justify correspondences; and second, shifting hypotheses of homology is illuminating, because modifying the correspondences makes evident the reasons to keep these correspondences together. Dynamic homologies bring the discussion of correspondences into the same quantitative field where phylogenetic hypotheses are compared. This makes it possible to construct a rigorous test of homology correspondences, with precise specification of alternatives, and an objective criterion to choose among them.

The problems exposed here with inapplicables and implied weights are evident under dynamic homology, although not exclusive to it. They call attention to anomalies in the tenets of phylogenetic analyses: Inapplicable characters are not logically independent (De Laet, 2005), and the difficulty of defining homoplasy complicates the traditional justification of parsimony as minimization of ad hoc hypotheses (Kluge and Grant, 2006). In the face of these anomalies, we may still expect significant changes in the way phylogenies are inferred.

A further significant issue that dynamic homology brings to our attention is the specification of possible transformation events, and their relative cost. Characters represent possible evolutionary transformations. In static homology, the derivation of a character from observations, with all its complexity, is relatively straightforward: homologous structures in two organisms show different conditions, hence at some point in evolution there must have occurred some transformation. Under dynamic homology the range of evolutionary transformations is much wider, because we can consider events that destroy, create, duplicate and move structures around in the organism. When all transformations are given the same cost, including some radical events, as are insertions and deletions of large sequence fragments or body parts, a phylogenetic analysis produces trivial reconstructions maximizing independent origin of structures. On the other hand, the list of evolutionary events that can be imagined is very large, and many of them are just fantastic speculations. The events that are considered are only the ones that, for some biological reason, we consider plausible. This brings to the forefront experimental biology as a source of potential evolutionary transformations that morphologists and systematists can consider for phylogenetic reconstruction (e.g., Collazo, 2000; Vergara-Silva, 2003; Richter and Olsson, 2006).

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Appendix 1. Groups of terminals

Groups of terminals used for the generation of the alignments. Within each group all terminals shift correspondences coordinately.

Species	Block for alignments	Figure
Amaurobioides africana	0. Amaurobioidini with two sclerites	
Amaurobioides maritima		
Axyracrus elegans		
Aysenoides colecole		Fig. 7
Aysenoides parvus		
Aysenoides terrestris		
Aysenia araucana		
Aysenia segestrioides		
Acanthoceto acupicta	1. Amaurobioidini with two sclerites, one of them a "massive" conductor	
Acanthoceto riogrande		
Acanthoceto septentrionalis		
Negayan coccinea		
Negayan tridentata		
Selknamia minima		
Acanthoceto cinerea	2. Amaurobioidini with one sclerite	
Acanthoceto ladormida		
Acanthoceto marina		
Acanthoceto pichi		
Aysenia cylindrica		
Coptoprepes campanensis		
Coptoprepes valdiviensis		
Negayan paduana		
Josa calilegua	3. *Josa* with one sclerite	Fig. 9
Josa nigrifrons	4. *Josa* with two sclerites	Fig. 8
Josa riveti		
Arachosia honesta	5. Gayennini with one sclerite	
Arachosia praesignis		
Monapia alupuran		
Monapia angusta		
Monapia charrua		
Monapia dilaticollis		
Monapia guenoana		

Appendix (*Continued*).

Species	Block for alignments	Figure
Monapia huaria		
Monapia lutea		
Monapia pichinahuel		
Monapia silvatica		
Oxysoma itambezinho		
Oxysoma punctatum		Fig. 11
Philisca tripunctata		
Sanogasta alticola		
Sanogasta approximata		
Sanogasta backhauseni		
Sanogasta maculatipes		
Sanogasta maculosa		
Sanogasta mandibularis		
Sanogasta minuta		
Sanogasta pehuenche		
Sanogasta puma		
Sanogasta tenuis		
Sanogasta x-signata		Fig. 13
Tasata chiloensis		
Arachosia bergi	6. Gayennini with two sclerites	
Araiya coccinea		
Araiya pallida		
Gayenna americana		Fig. 12
Gayennoides losvilos		
Gayennoides molles		
Monapia carolina		
Monapia fierro		
Monapia tandil		
Monapia vittata		
Oxysoma longiventre		
Oxysoma saccatum		
Philisca amoenum		
Philisca hahni		
Philisca huapi		
Philisca hyadesi		
Philisca ornata		
Philisca puconensis		
Phidyle punctipes		
Tomopisthes horrendus		
Tomopisthes pusillus		
Tomopisthes varius		Fig. 10
Tasata centralis		
Tasata parcepunctata		
Tasata taim		
Tasata unipunctata		
Tasata variolosa		
Otoniela adesi	7. Outgroups with one sclerite	
Xiruana hirsuta		
Coptoprepes flavopilosus	8. Amaurobioidini with all three sclerites	Fig. 5
Coptoprepes nahuelbuta		
Ferrieria echinata		Fig. 6
Gamakia hirsuta		Fig. 4
Aysenia elongata	Male unknown	
Josa lutea		
Josa personata		
Philisca doilu		
Aysha prospera	All three sclerites absent	
Italaman santamaria		
Anyphaena accentuata	Outgroup with fixed homology (C1, C2)	
Wulfila argentina		
Malenella nana	Outgroup with fixed homology (fleshy C1)	

Appendix 2. Morphology and characters

In Amaurobioidinae, several sclerites can arise from the distal inflatable membrane (distal hematodocha): embolus, median apophysis, a primary (C1), and a secondary conductor (C2), and the paramedian apophysis (PMA) (Figs 4–13). The PMA may have several cusps; the principal and more conservative is the retrolateral cusp (rPMA). Some terminals may have a further prolateral cusp (pPMA), more or less separated from the rPMA. The rPMA and pPMA are considered independent sclerites here, even if in most cases they arise from a common sclerotized plate. Of those apical sclerites, only the correspondences of C1, C2 and pPMA are ambiguous, while the rest are more easily interpreted. The embolus is easily identified because it bears the sperm duct; the median apophysis and rPMA occur in conservative positions and shapes. In general, sclerites are easily individuated when they arise from areas of unsclerotized, flexible membrane. In Amaurobioidinae the embolus is conservatively inserted on membranous areas, but the areas around the other sclerites appear sclerotized in some species, such that their individuation may be controversial.

The reference configuration

In this data set, four species of the tribe Amaurobioidini (*Ferrieria echinata*, Fig. 6; *Gamakia hirsuta*, Fig. 4; *Coptoprepes flavopilosus*, Fig. 5; and *C. nahuelbuta*) have the complete complement of three apical sclerites for which correspondences will be tested. The four species have a similar conformation, such that the correspondences of sclerites are reasonably clear. I used those species as reference for names and description of the typical configuration. Because only three kinds of sclerites are considered for this local homology problem (i.e., three positions or homology lines), these four species are also the reference against which the sclerites of terminals having only one or two sclerites are aligned. *Ferrieria* and *Gamakia* are monotypic genera, but in *Coptoprepes* there are species with one, two, or three sclerites.

Primary conductor (C1)

A typical C1 arises between the base of the embolus and the prolateral margin of the tegulum (char. 299), and bears a canal where the embolus fits; hence its name. This conformation of C1 is most conserved in the tribe Amaurobioidini. The canal usually ends in a beak-shaped tip.

Secondary conductor (C2)

A typical C2 occurs on the apical-dorsal region of the distal hematodocha, and is closely related, often fused to some degree, to a sclerotized stripe of the tegulum where the sperm duct runs (SD, Figs 4–6; char 298). It has a shallow canal fitting the most distal stretch of the embolus.

Prolateral cusp of the paramedian apophysis (pPMA).

In the unexpanded copulatory organ, the PMA arises between the retrolateral margin of the tegulum and the median apophysis, and has a well defined retrolateral cusp, the rPMA. Between PMA and tegulum there is a tightly folded membrane that becomes inflated during hydraulic expansion (Ramírez, 2003, figs 33, 42C, 45C, 50D, 56B, 60B and 63C). In the typical configuration, while this membrane inflates, the prolateral side of the PMA is articulated with the C1. The area close to this articulation often bears a separate cusp, the pPMA. Both pPMA and rPMA often arise from a common sclerotized plate (char. 301).

Other configurations

Amaurobioidini

All members of the tribe except the four referred above lack a well-defined C2. Some of them have a shallow outgrowing of the tegular area where a C2 should be found (Fig. 7). The genera *Amaurobioides*, *Axyracrus*, *Aysenoides*, *Selknamia*, and some species of *Aysenia* and *Negayan* have a pPMA; the remaining genera lack it. Two species (*Negayan paduana*, Ramírez, 2003, Fig. 50D; *Aysenia cylindrica*) have a shallow ridge that may represent a relic of pPMA; for the purpose of this analysis they were scored as absent. There are two morphs of *Acanthoceto pichi*, probably separate species (Ramírez, 2003, p. 75), differing in the presence or absence of a pPMA. For the present analysis I used the morph without pPMA.

Gayennini

The conspicuous conductor of Gayennini, identified as C2 in Ramírez (2003) arises centrally from the distal hematodocha, and extends up to the distal-dorsal margin of the tegulum (Figs 10–13). In some species the conductor is tightly connected, even fused, with the distal-dorsal tegulum. This conductor often bears a well-defined canal where much of the embolus fits. Several scattered species of Gayennini have an additional, small and simple sclerite arising just behind the rPMA. On close examination, there is a sclerotized stripe going from this small sclerite up to the articulation between embolus and tegulum (Fig. 12), and for this reason it was interpreted as a C1 in Ramírez (2003). According to the same interpretation, all Gayennini lack a pPMA. A result of this analysis is that this small sclerite is better interpreted as a pPMA, and the large conductor as a C1 (Figs 10 and 12). Many Gayennini lack a pPMA (Figs 11 and 13).

Josa

The copulatory organ of *Josa* is rather homogeneous within the genus, but quite different from those of Gayennini and Amaurobioidini (Figs 8 and 9). There is a large, complex conductor that bears a canal, but the embolus does not fit the canal, at least in its unexpanded position. The embolus has a large basal process in close contact with the conductor. Because the conductor and the basal embolar process occupy most of the distal hematodocha, in some species it is difficult to tell whether the conductor arises close to the articulation between embolus and tegulum, or not. In *Josa nigrifrons* the conductor is, however, well separated from the embolus (Fig. 8). This large sclerite was interpreted as a C2 in Ramírez (2003). Two species of *Josa* have an outgrowth fused to the prolateral tegulum, interpreted as a C1 in Ramírez (2003). As with Gayennini, a result of this analysis is that this small outgrowth is better interpreted as a pPMA, and the large conductor as a C1 (Fig. 8).

Possible correspondences

In the reference species the C2 arises close to the dorsal-distal, or prolateral-distal margin of the tegulum, and the pPMA arises close to the rPMA; the C1 lies in between (Figs 4–6). The dubious correspondences of sclerites always involve the alternative identification of C1–C2, and C1–pPMA, hence the sclerites were linearly ordered, as apical–prolateral–retrolateral = C2–C1–pPMA. When two sclerites are present, the most retrolateral is either pPMA or C1, and the most prolateral is either C1 or C2 (i.e., no "inversions" considered). When only one sclerite is present, it is evidently a C1 or C2; in this experiment it was allowed also to align with the pPMA as well, to illustrate the effect of suboptimal correspondences.

Characters used in the local homology problem

The C1, C2 and pPMA were scored for the same conditions, irrelevant to their correspondences; hence character states are referred to an unspecified "Sclerite X" (SclX). If a particular sclerite is aligned with the C2, then SclX becomes C2, and so on. Correspondences with character numbers in Ramírez (2003) are given on Appendix 3. The numeration of the characters should be considered arbitrary, and corresponds with the un-aligned sclerites in the data set, available at http://www.cladistics.org/journal/data. Characters followed by an asterisk are new.

296. SclX: *(0) absent; (1) present*. Ramírez (2003) interpreted the C2 as present even when it was only represented as a ridge. After closer examination it seems that the simple ridges found in *Amaurobioides*, *Aysenia*, some species of *Acanthoceto* (*A. pichi, A. cinereus, A. marinus, A. ladormida*), *Coptoprepes* (*C. campanensis, C. valdiviensis*), and *Aysenoides* (*A. terrestris, A. colecole*) are unlikely to be separate sclerites. These cases are scored as absent here.

297*. SclX relative to ventral tegulum: *(0) separate* (e.g., pPMA in Figs 10), *(1) firmly fused* (e.g., pPMA in Fig. 8).

298. SclX relative to dorsodistal tegulum: *(0) free from dorsodistal tegulum; (1) fused to dorsodistal tegulum.*

299*. SclX origin: *(0) between embolus and prolateral tegulum* (e.g., C1 in Fig. 6); *(1) apical tegulum* (e.g., C1 in Fig. 12, C2 in Fig. 6).

300*. Relative size of SclX: *(0) well defined, large* (e.g., the C1 in all terminals); *(1) small* (e.g., the pPMA when present).

301*. SclX relative to rPMA: *(0) separated by membranous area; (1) connected by sclerotized area.*

302. Canal on SclX: *(0) absent; (1) present* (e.g., C1 in Figs 4–7).

303. Extension of canal on SclX: *(0) short; (1) deep, long, arising under the rPMA,* Gayenna *type.*

304. Prolateral process on SclX: *(0) absent; (1) elongate, rounded lobe,* Arachosia *type*; *(2) flattened lobe, directed basally,* Tasata *type; (3) thin lobe crossing the canal*, Negayan *type*. (Unordered.)

305. SclX divided by a membranous area: *(0) undivided; (1) totally divided by a membranous area, retrolateral to the canal.*

306. SclX membranous area prolateral to the canal: *(0) absent; (1) present.*

307. Origin of SclX relative to the contiguous prolateral sclerite: *(0) separate; (1) SclX arising on top of the contiguous prolateral sclerite*.

308. Globose retrolateral basal lobe on SclX: *(0) absent; (1) present*. Only present in *Negayan coccinea*. After reexamination, the lobe in *Coptoprepes flavopilosus* and *C. nahuelbuta* is interpreted as a pPMA.

309. Translucent vertical lamina on SclX: *(0) absent; (1) present*.

310. Apex of SclX relative to base of median apophysis (MA): *(0) separate from base of MA; (1) close to base of MA*.

311. Separation of SclX from dorsodistal tegulum: *(0) narrow to fused; (1) wide retrolateral membranous area*.

312. Dentate prolateral ridge or lobe on SclX: *(0) absent; (1) present*.

313. Apex of SclX in Gayennini: *(0) apical*; *(1) median or basal, the C2 extended in a ridge beyond the apex*. The "apex" is identified as the end of the canal, often beak-shaped. This character is specific for an area in the large conductor of Gayennini; it is scored as inapplicable outside the tribe.

314. Membranous lobe on SclX. (*0) absent; (1) present, an outgrowth of the unsclerotized area dividing the C2*.

315. Denticles on prolateral portion of SclX: *(0) absent; (1) present.*

316. Teeth on SclX apex, regularly disposed, pointing backward: *(0) absent; (1) present.*

317. Denticles on retrolateral portion of SclX: *(0) absent; (1) present.*

318. Shape of base of retrolateral portion of SclX in Gayennini: *(0) thick; (1) wide, thin, translucent.* This character is specific for an area in the large conductor of Gayennini; it is scored as inapplicable outside the tribe.

319. SclX *Josa* type: *(0) absent, simple shapes; (1) present, with hypertrophied crescent-shaped sector.*

Characters in Ramírez (2003) that were redefined

Characters 70–72 in Ramírez (2003) are now referred to rPMA.

Character 68 in Ramírez (2003). Shape of PMA: *(0) one short cusp*; *(1) two or more short cusps*; *(2) thick, simple, and elongate, type* Philisca; (*3) slender, type* Monapia *or* Sanogasta; (*4) bifid*. (Unordered.) State 1 refer to the presence of pPMA (recoded as char. 296 above); there may be additional, small cusps, that are recoded as char. 70 (see below). States 2–4 are conditions of the main, retrolateral cusp of the PMA (rPMA), as found in Gayennini (''coding details within Gayennini sacrificed the presumed homology of their conspicuous, projecting PMA'', p. 18). Recoded as:

69. Retrolateral cusp of the PMA (rPMA): *(0) absent; (1) present.*

70. Additional cusps besides the pPMA and rPMA: *(0) absent; (1) present.*

71. rPMA shape: *(0) short; (1) conspicuous, protruding.*

72. shape of protruding rPMA: *(0) thick, simple, and elongate, type* Philisca; (*1) slender, type* Monapia *or* Sanogasta; (*2) bifid*. (Unordered.)

Character 75 in Ramírez (2003). Primary conductor (C1): *(0) absent; (1) present, without canal*; *(2) with a canal where the embolus fits*; *(3) massive, with canal.* (Ordered.) Recoded as char. 296 (see above); after reexamination, state 3 seemed ambiguously defined, and it was not considered here.

Character 93 in Ramírez (2003). Shape of the relic of C1 in Gayennini: *(0) conical*; *(1) acute*; *(2) thin, rounded.* Reexamination of specimens showed that there are many intermediate shapes, and the character was eliminated.

Data set notes

In all the data set files posted with the additional materials in http://www.cladistics.org/journal/data for further details, ''?'' means either missing or inapplicable; this is so because the first versions of the data set were maintained and exported from Nona formats. In the alignments (1–6561), the sclerites analyzed under dynamic correspondences are repeated tree times. The first block (chars. 224–295) corresponds with the correspondences as in Ramírez (2003). The second (chars. 296–367) are the unaligned sclerites. The third is the specific alignment; only this last block is active, together with the characters with static correspondences.

For managing the data and interacting with the scripts, there are controlling lines that appear as lines of ''terminals'' at the end of the data set, and controlling columns as ''characters''. Those are always inactive. The original characters in Ramírez (2003) and Ramírez et al. (2004) that were reinterpreted are still in the data set, but inactive.

There are some corrections to the data set in Ramírez et al. (2004). The most significant are the consistent filling with missing entries of male characters when males are unknown. The original maintenance of the data set using the command ''match'' of PeeWee-Nona inadvertently helped to introduce many of those mistakes.

Appendix 3. Character equivalences

Characters here	Characters in Ramírez (2003)
69	68*
70	68*
71	68*
72	68*
73	70 =
74	71 =
75	72 =
296	68*, 75*, 79*
297	+
298	79*
299	+
300	+
301	+
302	84*
303	84*
304	77, 81*
305	85
306	86
307	69
308	73
309	76
310	78
311	80
312	82
313	83
314	87
315	88
316	89
317	90
318	91
319	92

*Partial equivalence; +, added here; =, same, reworded as rPMA.

Appendix 4. Aligned data set

Portion of the data set with dynamic correspondences, from the optimal alignment (number 6226). (a = [01]; b = [02]) See complete data set with the additional material in http://www.cladistics.org/journal/data/.

		C2					C1					pPMA				
	Group	296	300	305	310	315	296	300	305	310	315	296	300	305	310	315
Malenella nana	-	0---	-----	-----	-----	-----	1--0	-----	-----	-----	-----	0---	-----	-----	-----	-----
Aysha prospera	-	0---	-----	-----	-----	-----	0---	-----	-----	-----	-----	0---	-----	-----	-----	-----
Italaman santamaria	-	0---	-----	-----	-----	-----	0---	-----	-----	-----	-----	0---	-----	-----	-----	-----
Otoniela adesi	7	0---	-----	-----	-----	-----	1000	0?0?0	00000	010-0	000-0	0---	-----	-----	-----	-----
Xiruana hirsuta	7	0---	-----	-----	-----	-----	1011	0?100	00000	000-0	000-0	0---	-----	-----	-----	-----
Josa calilegua	3	0---	-----	-----	-----	-----	100a	0a11?	00000	010-0	000-1	0---	-----	-----	-----	-----
Sanogasta approximata	5	0---	-----	-----	-----	-----	1011	00100	00000	00010	00000	0---	-----	-----	-----	-----
Oxysoma punctatum	5	0---	-----	-----	-----	-----	1001	00102	00000	01010	10100	0---	-----	-----	-----	-----
Sanogasta pehuenche	5	0---	-----	-----	-----	-----	1011	00100	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta maculosa	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta maculatipes	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta alticola	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta mandibularis	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Arachosia praesignis	5	0---	-----	-----	-----	-----	1011	00110	00000	00000	00000	0---	-----	-----	-----	-----
Arachosia honesta	5	0---	-----	-----	-----	-----	1011	00111	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta puma	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta tenuis	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta x-signata	5	0---	-----	-----	-----	-----	1001	0011?	00000	00010	00000	0---	-----	-----	-----	-----
Sanogasta minuta	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Sanogasta backhauseni	5	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	0---	-----	-----	-----	-----
Philisca tripunctata	5	0---	-----	-----	-----	-----	1001	0010b	00000	01100	10000	0---	-----	-----	-----	-----
Oxysoma itambezinho	5	0---	-----	-----	-----	-----	1001	00102	00000	00??0	10?00	0---	-----	-----	-----	-----
Tasata chiloensis	5	0---	-----	-----	-----	-----	1001	00102	00000	00110	10000	0---	-----	-----	-----	-----
Monapia alupuran	5	0---	-----	-----	-----	-----	1001	000-?	10000	000?1	00010	0---	-----	-----	-----	-----
Monapia charrua	5	0---	-----	-----	-----	-----	1001	000-?	10000	000?1	00010	0---	-----	-----	-----	-----
Monapia guenoana	5	0---	-----	-----	-----	-----	1001	000-1	10000	000?1	00010	0---	-----	-----	-----	-----
Monapia angusta	5	0---	-----	-----	-----	-----	1001	000-?	10000	000?0	00010	0---	-----	-----	-----	-----
Monapia dilaticollis	5	0---	-----	-----	-----	-----	1001	0010b	10000	000?1	00110	0---	-----	-----	-----	-----
Monapia silvatica	5	0---	-----	-----	-----	-----	1001	0010b	10000	000?1	00110	0---	-----	-----	-----	-----
Monapia pichinahuel	5	0---	-----	-----	-----	-----	1001	0010b	10000	000?1	00010	0---	-----	-----	-----	-----
Monapia lutea	5	0---	-----	-----	-----	-----	1001	00100	10000	000?1	00100	0---	-----	-----	-----	-----
Monapia huaria	5	0---	-----	-----	-----	-----	1001	00100	10000	000?1	00100	0---	-----	-----	-----	-----
Acanthoceto pichi	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Acanthoceto cinerea	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Acanthoceto marina	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Acanthoceto ladormida	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Coptoprepes valdiviensis	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Coptoprepes campanensis	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Negaya paduana	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Aysen cylindrica	2	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	0---	-----	-----	-----	-----
Josa riveti	4	0---	-----	-----	-----	-----	100a	0a110	00000	010-0	000-1	1100	110-0	00000	110-0	000-0
Josa nigrifrons	4	0---	-----	-----	-----	-----	1001	0011?	00000	010-0	000-1	1100	110-0	00000	110-0	000-0
Gayenna americana	6	0---	-----	-----	-----	-----	1001	00110	00000	01000	00000	1000	100-0	00000	110-0	000-0

Appendix 4. Aligned data set

(*Continued*)

		C2					C1					pPMA				
	Group	296	300	305	310	315	296	300	305	310	315	296	300	305	310	315
Oxysoma saccatum	6	0---	-----	-----	-----	-----	1001	00102	01000	00010	10100	1000	100-0	00000	110-0	000-0
Tasata variolsa	6	0---	-----	-----	-----	-----	1001	00102	00000	00110	10000	1000	110-0	00000	110-0	000-0
Monapia vittata	6	0---	-----	-----	-----	-----	1001	000-0	10000	000?1	10000	1000	100-0	00000	110-0	000-0
Philisca hyadesi	6	0---	-----	-----	-----	-----	1001	000-b	00000	01110	00010	1000	110-0	00000	110-0	000-0
Tomopisthes varius	6	0---	-----	-----	-----	-----	1001	0010b	00000	01000	11000	1000	100-0	00000	110-0	000-0
Araiya pallida	6	0---	-----	-----	-----	-----	1001	00100	00000	00010	11000	1000	100-0	00000	110-0	000-0
Gayennoides molles	6	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	1000	100-0	00000	110-0	000-0
Gayennoides losvilos	6	0---	-----	-----	-----	-----	1001	00110	00000	00000	00000	1000	1?0-0	00000	110-0	000-0
Arachosia bergi	6	0---	-----	-----	-----	-----	1011	00111	00000	00000	00000	1000	110-0	00000	110-0	000-0
Tomopisthes horrendus	6	0---	-----	-----	-----	-----	1001	00100	00000	01010	11000	1000	1?0-0	00000	110-0	000-0
Tomopisthes pusillus	6	0---	-----	-----	-----	-----	1001	0010b	00000	01010	11000	1000	1?0-0	00000	110-0	000-0
Philisca puconensis	6	0---	-----	-----	-----	-----	1001	00100	00000	01010	00000	1000	1?0-0	00000	110-0	000-0
Philisca ornata	6	0---	-----	-----	-----	-----	1001	00100	00000	01000	a0010	1000	1?0-0	00000	110-0	000-0
Philisca huapi	6	0---	-----	-----	-----	-----	1001	00100	00000	01000	00010	1000	100-0	00000	110-0	000-0
Philisca hahni	6	0---	-----	-----	-----	-----	1001	00100	00000	01110	100?0	1000	1?0-0	00000	110-0	000-0
Philisca amoenum	6	0---	-----	-----	-----	-----	1001	0010b	00000	01110	1a0?0	1000	110-0	00000	110-0	000-0
Araiya coccinea	6	0---	-----	-----	-----	-----	1001	00100	00000	00010	11000	1000	1?0-0	00000	110-0	000-0
Oxysoma longiventre	6	0---	-----	-----	-----	-----	1001	00102	00000	00010	10100	1000	100-0	00000	110-0	000-0
Tasata taim	6	0---	-----	-----	-----	-----	1001	00102	00000	00110	a0000	1000	1?0-0	00000	110-0	000-0
Tasata unipunctata	6	0---	-----	-----	-----	-----	1001	00102	00000	00110	a0000	1000	1?0-0	00000	110-0	000-0
Tasata parcepunctata	6	0---	-----	-----	-----	-----	1001	00102	10000	00110	10000	1000	1?0-0	00000	110-0	000-0
Tasata centralis	6	0---	-----	-----	-----	-----	1001	00102	10000	01?10	00000	1000	1?0-0	00000	110-0	000-0
Phid punctipes	6	0---	-----	-----	-----	-----	1001	0010?	10000	0a0?0	000?0	1000	1?0-0	00000	110-0	000-0
Monapia tandil	6	0---	-----	-----	-----	-----	1001	000-?	10000	000?1	10000	1000	1?0-0	00000	110-0	000-0
Monapia fierro	6	0---	-----	-----	-----	-----	1001	0010?	10000	000?1	10010	1000	1?0-0	00000	110-0	000-0
Monapia carolina	6	0---	-----	-----	-----	-----	1001	0010?	10000	000?1	00000	1000	1?0-0	00000	110-0	000-0
Anypha accentuata	-	1001	000-0	??000	01???	?00?0	1000	00110	00000	010-0	001-0	0---	-----	-----	-----	-----
Wulfila argentina	-	1000	0?0-?	??000	01??0	000?0	1000	0?0-?	000?0	01?-?	???-0	0---	-----	-----	-----	-----
Negaya tridentata	1	0---	-----	-----	-----	-----	1000	00113	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Acanthoceto riogrande	1	0---	-----	-----	-----	-----	1000	00110	00000	110-0	000-0	1000	110-0	00100	110-0	000-0
Acanthoceto acupuicta	1	0---	-----	-----	-----	-----	1000	00110	00000	110-0	000-0	1000	110-0	00100	110-0	000-0
Acanthoceto septentrionalis	1	0---	-----	-----	-----	-----	1000	00110	00000	110-0	000-0	1000	110-0	00100	110-0	000-0
Selknamia minima	1	0---	-----	-----	-----	-----	1000	00113	00000	110-0	000-0	1000	110-0	00100	110-0	000-0
Negaya coccinea	1	0---	-----	-----	-----	-----	1000	00113	00010	010-0	000-0	1000	110-0	00100	110-0	000-0
Amauro maritima	0	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	1000	110-0	00100	110-0	000-0
Amauro africana	0	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	1000	110-0	00100	110-0	000-0
Axyracrus elegans	0	0---	-----	-----	-----	-----	1000	00100	00000	?10-0	000-0	1000	110-0	00000	110-0	000-0
Aysenia segestrioides	0	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Aysenia araucana	0	0---	-----	-----	-----	-----	1000	00110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Aysenoides parvus	0	0---	-----	-----	-----	-----	1000	00110	00000	110-0	000-0	1000	110-0	00000	110-0	000-0
Aysenoides terricola	0	0---	-----	-----	-----	-----	1000	00110	00001	010-0	000-0	1000	110-0	00100	110-0	000-0
Aysenoides colecole	0	0---	-----	-----	-----	-----	1000	00110	00001	010-0	000-0	1000	110-0	00100	110-0	000-0
Ferrieria echinata	-	1011	10100	00000	000-0	000-0	1000	00110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Coptoprepes flavopilosus	-	1001	00100	00000	010-0	000-0	1000	00110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0

Appendix 4. Aligned data set

(*Continued*)

		C2					C1					pPMA				
	Group	296 |	300 |	305 |	310 |	315 |	296 |	300 |	305 |	310 |	315 |	296 |	300 |	305 |	310 |	315 |
Coptoprepes nahuelbuta	–	1001	00a?0	00000	000-0	000-0	1000	0?110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Gamakia hirsuta	–	1011	10100	00000	000-0	000-0	1000	00110	00000	010-0	000-0	1000	110-0	00000	110-0	000-0
Aysen elongata	–	????	?????	?????	?????	?????	????	?????	?????	?????	?????	????	?????	?????	?????	?????
Josa personata	–	????	?????	?????	?????	?????	????	?????	?????	?????	?????	????	?????	?????	?????	?????
Josa lutea	–	????	?????	?????	?????	?????	????	?????	?????	?????	?????	????	?????	?????	?????	?????
Philisca doilu	–	????	?????	?????	?????	?????	????	?????	?????	?????	?????	????	?????	?????	?????	?????