Blackwell Publishing

Cladistics 21 (2005) 131–142

Cladistics

www.blackwell-synergy.com

Phylogeny of the *Pantomorus–Naupactus* complex based on morphological and molecular data (Coleoptera: Curculionidae)

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Accepted 18 January 2005

Abstract

The *Pantomorus–Naupactus* complex is a Neotropical group of broad-nosed weevils (Coleoptera: Curculionidae) including several parthenogenetic species usually assigned to the genera *Naupactus* Dejean, *Pantomorus* Schoenherr, *Asynonychus* Crotch, *Aramigus* Horn, *Eurymetopus* Schoenherr and *Graphognathus* Buchanan. Sixteen species were studied to test hypotheses on the monophyly of these genera, and on the origin of the parthenogenetic lineages. A matrix of 30 morphological characters and 999 positions of the Cytochrome Oxidase I gene, was analyzed with separate partitions and simultaneously, under equal and implied weights, and with different transversion/transitions costs. The ILD test indicates that the incongruence between the molecular and morphological data is not significant. Under equal weights, the molecular data resulted in a single tree and morphology in 34 trees; under implied weights morphology gave a different tree, and under TV:TS ≥4:1 molecular and combined analyses resulted in the same optimal tree. According to the latter, *Naupactus* includes *Graphognathus*, and is thus paraphyletic and basal regarding remaining genera, *Pantomorus* is polyphyletic and includes *Aramigus* and *Asynonychus*, and *Eurymetopus* is monophyletic. The species in which apomictic parthenogenesis has been verified (*Aramigus tessellatus*, *Asynonychus cervinus* and *Graphognathus lecuoloma*), belong to different clades of the *Pantomorus-Naupactus* complex, with basal sexual relatives.

The genera *Pantomorus* Schoenherr and *Naupactus* Dejean (Curculionidae: Entiminae: Naupactini) are naturally distributed in the Neotropical Region, having their highest diversity in the tropical and subtropical areas of South America (Lanteri and O'Brien, 1990; Lanteri and Morrone, 1995). *Naupactus* is usually associated with environments having trees and shrubs, where the adults feed on leaves and other green parts of the plants, whereas *Pantomorus* species are mostly distributed in steppes and prairies, feeding on grasses (Lanteri et al., 2002a,b). Some of the latter are apomictic parthenogenetic, and have been introduced into other continents besides South America, becoming serious pests of agriculture (Lanteri and Normark, 1995; Hardwick et al., 1997; Normark and Lanteri, 1998; Mander et al., 2003).

The majority of the species traditionally classified in *Naupactus* have well-developed elytral humeri and metathoracic wings, whereas in *Pantomorus* the humeri are reduced or absent, and the membranous wings are vestigial. Buchanan (1939) coined the term *Pantomorus–Naupactus* complex (*P-N* complex) and stated that "until all species can be critically studied the wing and humeral characters must be used for dividing these two vaguely defined genera". Lanteri and Morrone (1995), and Lanteri and Normark (1995) proposed that *Naupactus* is probably paraphyletic and *Pantomorus* an artificial genus, including several independent lineages associated with similar environments, that might have evolved from different groups of *Naupactus*.

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Other genera indigenous to South America, such as *Aramigus* Horn, *Atrichonotus* Buchanan, *Asynonychus* Crotch, *Graphognathus* Buchanan, and *Eurymetopus* Schoenherr, are also part of the *Pantomorus-Naupactus* complex (Lanteri and O'Brien, 1990; Lanteri and Díaz, 1994; Lanteri and Marvaldi, 1995; Lanteri and Morrone, 1995). The taxonomic status of these genera has changed through time. Some authors treated them as independent taxa, and others as synonyms of *Pantomorus* or *Naupactus* (see Wibmer and O'Brien, 1986; Alonso-Zarazaga and Lyal, 1999; Morrone, 1999). The numerous nomenclatural changes that have taken place within the *Pantomorus–Naupactus* complex illustrate the difficulties in their delimitation and in the correct placement of their species. As an example, *Aramigus tessellatus* (Say) has also been classified as *Asynonychus*, *Pantomorus*, *Naupactus* and *Eurymetopus*.

Most previous phylogenetic analyses have referred to a single genus or a species group of the *P-N* complex (Lanteri, 1990, 1995; Lanteri and O'Brien, 1990; Lanteri and Díaz, 1994; Lanteri and Morrone, 1995; Normark and Lanteri, 1998). The main goal of this paper was to undertake a first comprehensive cladistic approach to this informal group of genera, in order to test hypotheses on their monophyly and to contribute to the study of the origin of the parthenogenesis. For this reason we selected some species representative of the diversity in South America, and a set of morphological and molecular characters analyzed through different strategies of parsimony analysis. This way we attempt to highlight some taxonomic and evolutionary issues within this group of weevils, which are of great economic importance.

Materials and methods

Sampling and storage of specimens

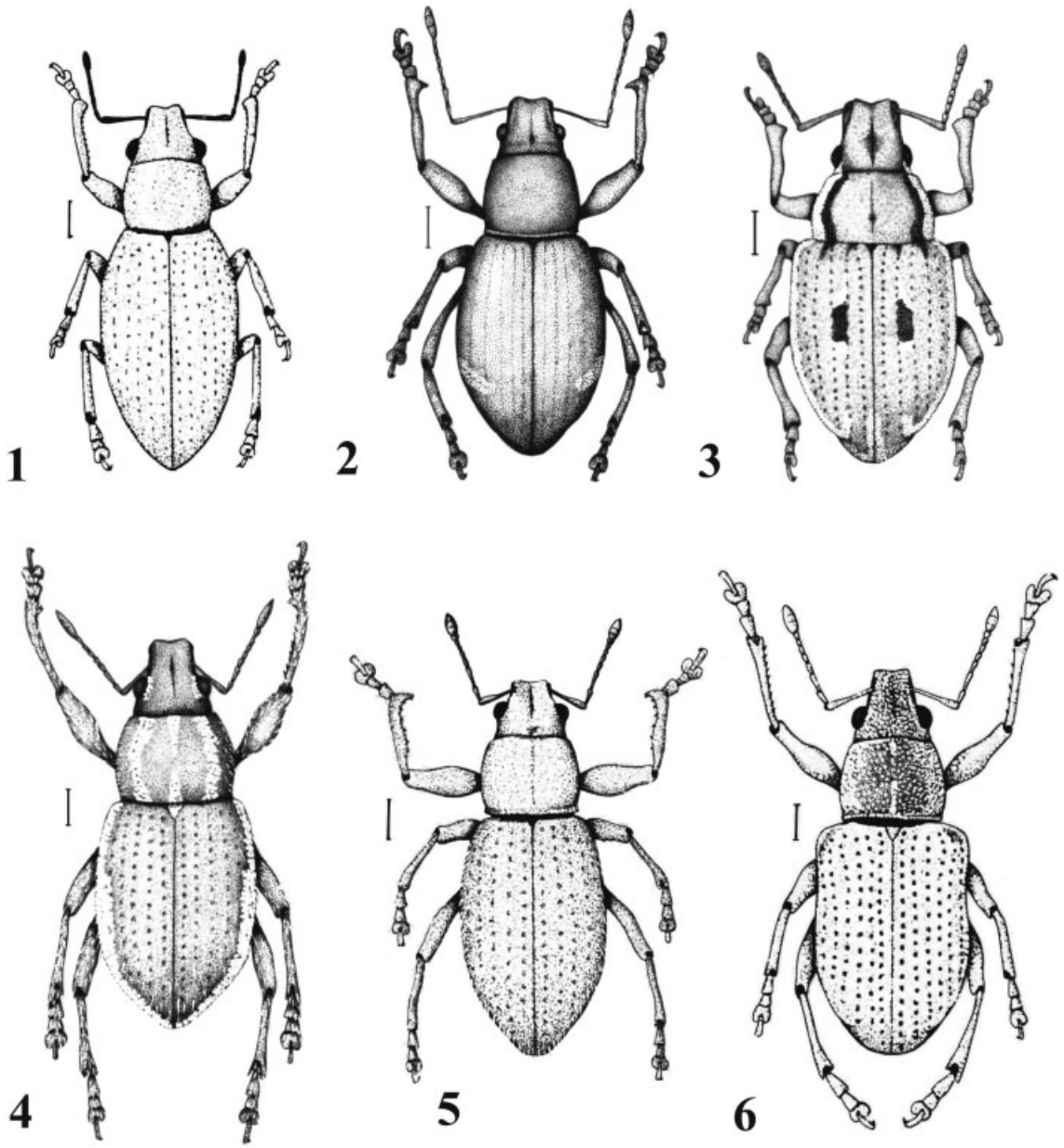
Sixteen exemplar taxa representative of the diversity of the *Pantomorus–Naupactus* complex in South America were studied. They have been assigned to the genera *Aramigus* (type sp.) (Fig. 1), *Asynonychus* (type sp.) (Fig. 2), *Eurymetopus* (two spp., one is the type) (Fig. 3), *Graphognathus* (two spp., one is the type) (Fig. 4), *Pantomorus* (four spp.) (Fig. 5) and *Naupactus* (six spp.) (Fig. 6). Although *Asynonychus* and *Graphognathus* are currently considered synonyms of *Naupactus* (see Alonso-Zarazaga and Lyal, 1999), they are herein treated as independent genera, to facilitate the discussion on their taxonomic status. Unfortunately it was not possible to amplify sequences of *Atrichonotus* Buchanan, another South American genus considered part of the *Pantomorus–Naupactus* complex (Lanteri and O'Brien, 1990). One species of *Teratopactus* Heller, *T. nodicollis* (Boheman), was used as the outgroup. The list of all species studied and their distributions are shown in Appendix 1, as well as some taxonomic information on the genera.

Molecular data: DNA isolation, PCR amplification and sequencing

Total genomic DNA was extracted from single weevils following the protocol of Reiss et al. (1995). The Cytochrome Oxidase I (COI) mitochondrial gene was amplified using the following primers designed by Normark (1994, 1996a) and Lunt et al. (1996): S1718 (5′-GGA GGA TTT GGA AAT TGA TTA GTT CC-3′); A2442 (5′GCT AAT CAT CTA AAA ATT TTA ATT CCT GTT GG-3′); S2336 (5′-GGA TTA YTA GGA TTT GTH GTW TGR GCY CA-3′); A2831 (5′-TCC TAT TAR WGA RAT TAA TCT TCC RAT TG-3′) and Uea5 (5′-AGT TTT AGC AGG AAT TAC TAT-3′); Uea8 (5′-AAA AAT GTT GAG GGA AAA ATG TTA-3′). Amplification was carried out in a 50 μL. volume reaction with 50–100 ng of DNA used as template, 0.5 μM of each primer, 50 μM of each DNTP, 3.5 mM $MgCl_2$, 2.5 units of Taq polymerase and buffer 1× provided by Promega. The reaction was performed in a thermal cycler (Techne, City, State) under the following conditions: the first period of denaturation was 94 °C for 6 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 46–50 °C (depending on the species) for 1.30 min, and extension at 72 °C for 1.30 min. Final extension at 72 °C for 5 min terminated the reactions. A negative control with no template was included for each series of amplifications to eliminate the possibility of contamination. Double-stranded PCR products were separated by electrophoresis on a 1% agarose gel with TAE buffer containing 0.5 mg/mL of ethidium bromide. The bands were excised from the gel and the DNA was purified with a QUIAquick Gel Extraction Kit (Quiagen Inc.). Samples were run on a PE Biosystem automated 377 sequencer.

The sequences obtained have been entered into GenBank under the accession numbers AY770383–89 and AY790875–82. The complete sequence of *N. verecundus* and *N. xanthographus* were already in GenBank (acc. no. AF211490–91), as well as the first half of the sequences of *N. dissimulator* (acc. no. AF211489), and the second half of *Aramigus tessellatus* (acc. no. U25534) (Normark, 1996a; Sequeira et al., 2000).

To avoid the possibility of the amplification of COI pseudogenes (Bensansson et al., 2000), sequences were translated according to the invertebrate mitochondrial genetic code and examined, using as reference amino acid sequences obtained for several insect orders (Lunt et al., 1996). A copy was assumed to be mitochondrial if it contained no frameshifts or stop codons (Sorenson and Fleischer, 1996; Zhang and Hewitt, 1996). Since no

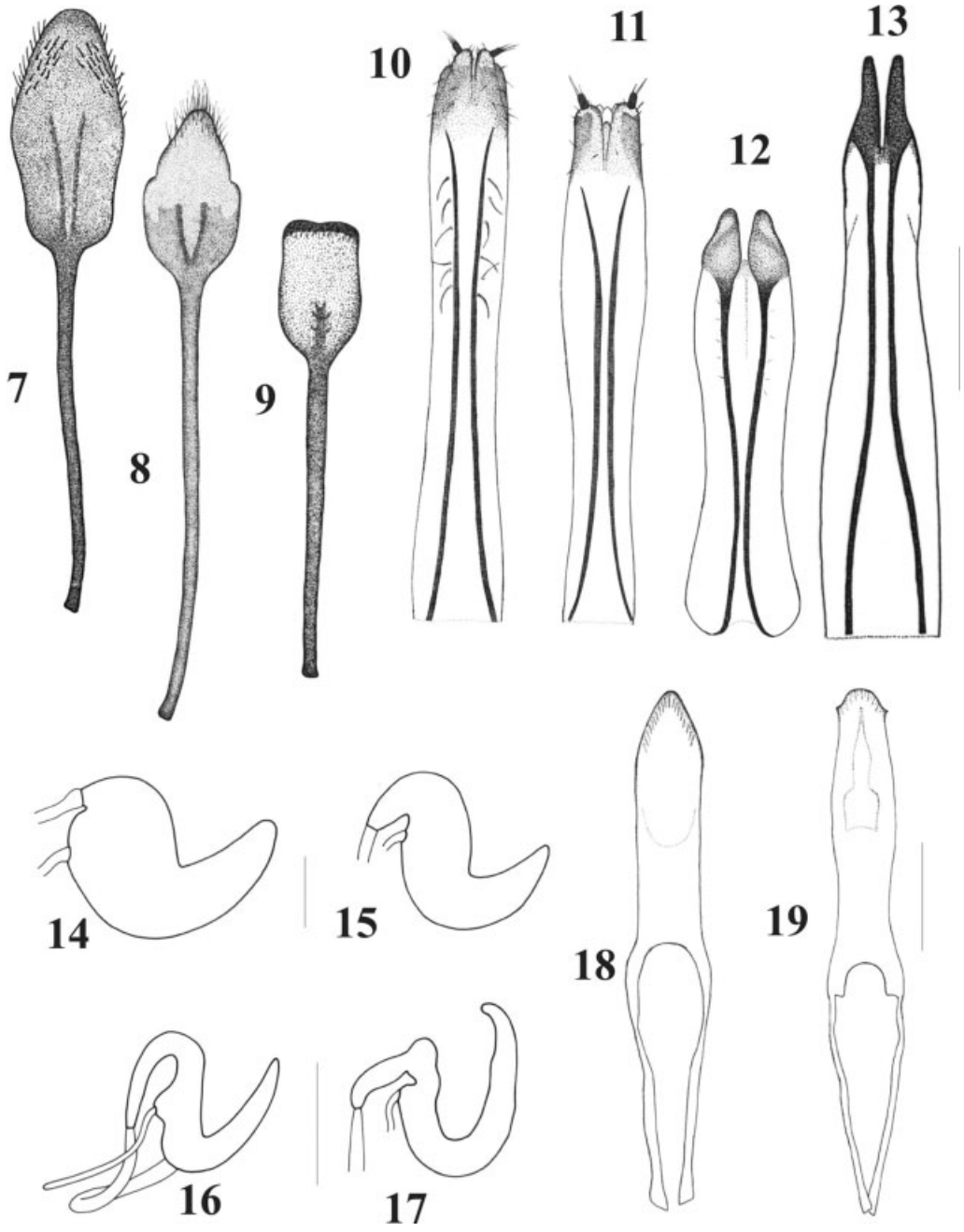


Figs 1–6. Habitus of species representative of the diversity within the *Pantomorus–Naupactus* complex: 1, *Aramigus tessellatus* (Say); 2, *Asynonychus cervinus* (Boheman); 3, *Eurymetopus fallax* Boheman; 4, *Graphognathus leucoloma* (Boheman); 5, *Pantomorus auripes* Hustache; 6, *Naupactus cinereidorsum* Hustache. Scales: 1 mm.

insertion/deletion events were apparent between the sequences, and all of them were of similar length, they were aligned using ClustalV (Higgins and Sharp, 1989). Nucleotide diversity was estimated using MEGA ver. 2.1 (Kumar et al., 2001).

Morphological data: sampling and coding of characters

Morphological features of the 16 species and the outgroup were studied and compared. The characters chosen for the phylogenetic analyses and their states are listed in Appendix 2. From a total of 30 characters, 23 belong to external morphology (character 1–23), six to female genitalia (character 24–29) (Figs 7–17), and one to male genitalia (Figs 18,19). Eighteen of these characters have two states and 12 are multistates. The data matrix of the coded data for the 17 species is shown in Appendix 2.



Figs 7–19. Characters of the genitalia in the *Pantomorus–Naupactus* complex. 7–9, Sternite VIII of female: 7, oval; 8, subrhomboidal; 9, subpentagonal. 10–13, Ovipositor of female: 10, with line of setae on each side of baculi, hemisternites moderately sclerotized, styli present; 11, idem, line of setae absent; 12, idem, hemisternites strongly sclerotized, short, styli absent; 13, idem, hemisternites long. 14–17, Spermathecae of female: 14, with vestigial nodulus; 15, with conical nodulus; 16, with tubular nodulus lacking basal prominence; 17, with tubular nodulus having basal prominence. 18–19, Aedeagi of male: 18, with pointed apex; 19, with arrow-pointed apex. Scales: 1 mm (Figs 7–13–18,19), 0.25 mm (Figs 14,15), 0.50 mm (Figs 16,17).

Phylogenetic analysis

Both data sets were analyzed separately and combined, or simultaneously (Nixon and Carpenter, 1996), using different strategies of weighting: equal weights, with NONA ver. 2.0 (Goloboff, 1999); implied weights, with PIWE ver. 3.0 (Goloboff, 1997) and differential TV/TS transformation costs, with SPA ver. 1.9 (Goloboff, 1998). Morphological and molecular characters were treated as nonadditive. Six values of transver-

sion/transition ratio (TV/TS) were explored for molecular and combined data sets, to avoid using just one "a priori" arbitrary ratio (sensitivity analysis *sensu* Wheeler, 1995). The different costs tested were: TV/TS: 1/0, 2/1, 4/1, 8/1 and 10/1.

The heuristic search procedure consisted of "TBR branch swapping" applied to a series of 10 random addition sequences, retaining 10 cladograms per replicate (h/10, mult*10). When all the replicates had found optimal trees, no further search strategies were adopted; in those cases in which the shortest trees had not been found in all replicates, additional swapping was applied (max*) to attempt to enumerate all the cladograms present in the islands found.

Branch supports were estimated using Bremer support (Bremer, 1994); values up to seven extra steps were used to find suboptimal trees (bs 7), and up to 10 000 trees were retained in the memory buffer. Morphological characters were optimized using the fast option of WinClada ver. 1.00.08 (Nixon, 2002). Congruence among morphological and molecular data sets was estimated by the "Incongruence Length Difference" test ILD (Farris et al., 1994).

Results

Molecular data

A 999 bp segment of the mtDNA COI gene corresponding to positions 260–1258 was obtained and aligned for the 17 species studied. The alignments of the translated sequences show the same distributional patterns of variation, and of amino acid sequences, within the conserved regions of the COI gene as those published by Lunt et al. (1996). These patterns, along with the absence of stop codons or frame shifts, exclude the possibility of having amplified a pseudogene (Sorenson and Fleischer, 1996; Zhang and Hewitt, 1996; Bensansson et al., 2000).

From the 999 bp, 291 parsimony informative characters resulted. The total proportions of nucleotides are 37.8% T, 17.2% C, 29.7% A, and 15.3% G, with a strong A + T bias (67.5%) as in other Curculionidae (Langor and Sperling, 1997).

When analyzed under equal weights, the molecular data resulted in a single MP tree 1242 steps long (CI = 0.44 and RI = 0.31). In the analysis under implied weights, different trees were obtained when applying different concavity constants (K = 1 to K = 6), although the same single MP tree (= tree under equal weights) was retrieved for those constants that weighted less against homoplasic characters (K = 4 to K = 6; CI = 0.44 and RI = 0.31).

When transitions had no cost (TV/TS 1/0) the analysis resulted in four different cladograms, but under differential costs of 2/1–10/1 a single tree was obtained, with the same topology for TV/TS ≥ 4/1 (CI = 0.44, RI = 0.30), and higher values of support for most groups (Fig. 20, right). In this tree *N. cinereidorsum* was the most basal and the remaining species form a clade with high values of Bremer support (≥ 7). Other groups with high support were: *N. verecundus*- (*G. leucoloma*- *G. minor*); *N. xantographus*- *N. navicularis*; *N.dissimulator*- *A. cervinus, P. ruizi*- *P. auripes*, and *E. birabeni*- *E. fallax*. Most of these groups are also well supported in the cladogram under equal weights, and they are supported in the consensus of all the trees obtained for different K constants in the analysis under implied weights.

Morphological data

When analyzed as nonadditive and under equal weights, the morphological data resulted in 34 different MP trees 74 steps long (CI = 0.62 and RI = 0.66). In the consensus of all these trees the clades are: *G. leucoloma–G. minor*, *E. birabeni–E. fallax*, and *N. dissimulator* (*N. xanthographus–N. navicularis*). The remaining species form a polytomy with the clades just mentioned.

If character 15 (reduction of the humeri) is deleted from the matrix, the number of the resulting most parsimonious cladograms is reduced to four, demonstrating that this feature, related to flightlessness, produces homoplasy and makes it more difficult to find a single topology. In the consensus of these four cladograms the only species having well developed humeri, *N. cinereidorum*, is part of a basal polytomy; however, the best supported clades in the morphological tree under implied weights are present (see below).

When the whole matrix was analyzed under implied weights, the morphological data resulted in a single MP tree for all the concavity constants (K = 1 to K = 6) (CI = 0.61 and RI = 0.65) (Fig. 20, left). The best supported clades in the molecular tree under transformation costs TV/TS ≥ 4/1 (Bremer values ≥ 7) are also supported in this morphological tree, except the sister group pair *N. dissimulator–A. cervinus*; however, some of these clades change their relative position (Fig. 20).

Combined data

According to the results of the ILD test (Farris et al., 1994), the molecular and morhological characters are not significantly incongruent ($P = 0.1890$).

When analyzed under equal weights, the combined data matrix resulted in a single MP tree 1328 steps long (CI = 0.45 and RI = 0.33). The clades with the highest support are the same as in the molecular tree under TV/TS ≥ 4/1, but the relative positions of some groups change. For example, *N. cinereidorsum* is not basal regarding the remaining species but follows the group *N. verecundus*- *G. leucoloma*- *G. minor*; and *Eurymetopus*

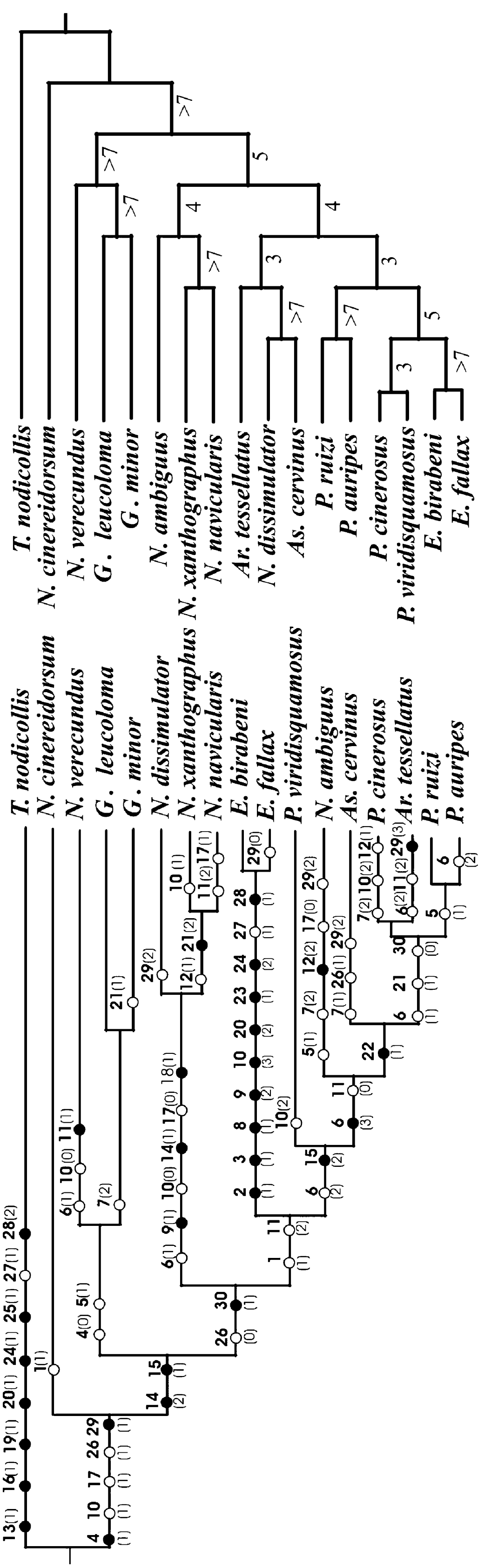


Fig. 20. Left: Most parsimonious cladogram obtained from morphological data analyzed under implied weights, with fast optimization of characters. Right: Most parsimonious cladogram based on COI sequences under transformation cost TV/TS ≥4/1 (= combined under the same transformation costs). Bremer support values under branches. Numbers of characters according to Appendix 2; apomorphies in black and homoplasies in white.

is not at the tip of the cladogram but in the middle, the same as in the morphological tree under implied weights.

Under implied weights the combined data resulted in a different tree for each concavity constant (K = 1 to K = 6), and in the consensus of all these trees the main clades retrieved are the best supported in the analysis under equal weights. When applying TV/TS = 2/1, two different cladograms were obtained, but for transformation costs of TV/TS = 1/0 and TV/TS ≥ 4/1 the combined data resulted in a single tree (CI 0.44 and RI = 0.32), coincident with that of the molecular tree under the same transformation costs (Fig. 20, right).

Discussion and conclusions

When applying equal weights, morphological characters produce less homoplasy than molecular characters (CI = 0.62 and RI = 0.66, versus CI = 0.44 and RI = 0.31), but the latter are more decisive (one single MP tree versus 34 MP trees). The number of competing morphological hypotheses was reduced by weighting the less homoplasious characters, and the values of branch support for the molecular tree were improved weighting transversions over transitions. For these reasons the best solution for the molecular data results from the analysis under TV/TS = or > 4/1 (Fig. 20, right) and the optimal morphological solution comes from the analysis under implied weights (Fig. 20, left). The preferred topology comes from the combined analysis under the just mentioned transformation costs because it is coincident with the optimal molecular tree (Fig. 20 right), and the clades recovered are better supported than in the remaining analyses.

The placement of the majority of the species traditionally assigned to *Naupactus* near the root of the trees (Fig. 20), corroborates the hypothesis proposed by Lanteri and Morrone (1995) and Lanteri and Normark (1995), according to which this genus would be paraphyletic regarding the remaining of the *Pantomorus*–*Naupactus* complex.

The basal species *N. cinereidorsum* is a typical *Naupactus* with a bisinuate elytral base and well developed humeri and metathoracic wings. In the remaining species the elytral base is either slightly bisinuate or straight, and the humeri become reduced or absent, same as the metathoracic wings (char. 15; Fig. 20, left). The group that follows *N. cinereidorsum* in the phylogenetic sequence (*N. verecundus* (*G. leucoloma*–*G. minor*)) is recovered in both the molecular and morphological trees (Fig. 20). Lanteri and Marvaldi (1995) established the synonymy of *Naupactus* and *Graphognathus*, a decision well justified by the results of the present cladistic analysis. In the same paper these authors described the "*Naupactus leucoloma* species group" for *G. leucoloma*, *G. minor*, *G. peregrinus*

(Buchanan), *N. albolateralis* Hustache and *N. tucumanensis* Hustache. According to our cladograms, *N. verecundus* would also be part of the "*leucoloma* group", since its relationship with the pair *G. leucoloma–G. minor* is supported in all the analyses with high Bremer values.

The third clade of the cladograms also contains species usually assigned to *Naupactus*: *N. ambiguus* (*N. xanthographus–N. navicularis*) in the molecular tree (Fig. 20, right), and *N. dissimulator* (*N. xanthographus–N. navicularis*) in the morphological tree (Fig. 20, left). The latter group is well justified by morphology, especially by the presence of a pair of subapical turbercles on the elytra (char. 18). However, in the molecular tree *N. dissimulator* is the sister species of *Asynonychus cervinus*. These two species share numerous molecular synapomorphies and have a similar spermatheca (char. 29). The sister group relationship *N. xanthographus–N. navicularis* is justified by the slightly transversal pronotum and the enclosed corbel plates (chars. 12 and 21), and by several molecular characters.

Eurymetopus is a very distinct genus, whose species are strongly supported as related based on both molecules and morphology, including several external and genitalic characters of the female (chars. 2–3, 8–10, 20, 23–24, 27–28). However, the position of *Eurymetopus* is not stable, since in the molecular tree it is at the tip (Fig. 20, right), and in the selected morphological tree is between *Naupactus* and *Pantomorus* (Fig. 20, left).

Most species usually assigned to *Pantomorus* based on morphological characters, such as the reduced or absent humeri (char. 15.1 and 15.2), are placed between the basal *Naupactus* and *Eurymetopus* (Fig. 20, right), or from the latter taxon to the tip of the cladogram (Fig. 20, left). In the former case *Pantomorus* is paraphyletic (char. 15.2 is a synapomorphy) and in the second it is polyphyletic because it includes species of *Aramigus*, *Asynonychus* and *Naupactus* (char. 15.2 is an homoplasy). These results help to demonstrate that the traditional concept of *Pantomorus* (Buchanan, 1939; Hustache, 1947; Wibmer and O'Brien, 1986) corresponds to an artificial group. For example, *Pantomorus sensu* Buchanan (1939) included the species *Graphognathus leucoloma, G. minor, Asynonychus cervinus* and *Aramigus tessellates*; and *Asynonychus sensu* Hustache (1947) included *P. viridisquamosus*, *N. ambiguus*, *A. cervinus* and *A. tessellatus*. The unstable position of most of these species in the cladograms is consistent with their numerous taxonomic and nomenclatural changes, i.e., in the molecular tree *A. tessellatus* is near the pair *N. dissimulator–Asynonychus cervinus,* whereas in the morphological tree it is closer to *Pantomorus cinerosus*.

Despite its artificial condition, within the genus *Pantomorus* it is possible to discern some natural groups. For example the "*auripes* species group" includes five species (Lanteri et al., 1991; Lanteri, 1995), two of them forming a well supported clade (*P. ruizi–Pauripes*), although with different positions in the molecular and morphological trees (Fig. 20).

A possible solution for the taxonomic problem of the *Pantomorus–Naupactus* complex, consistent with the results of the present cladistic analysis, would be to consider the whole group as a single genus. The transference of the South American *Pantomorus* to *Naupactus* by Morrone (1999) goes in this direction, however, it is a partial solution, because the author conserved the name *Pantomorus* for the 35 species from Mexico and Central America. These species would also represent derived lineages of the Mexican *Naupactus*, as well as those currently assigned to *Phacepholis*, a genus from North America (see Lanteri, 1990). To keep *Naupactus* monophyletic, not only *Asynonychus*, *Graphognathus* and South American *Pantomorus* should be considered synonyms of *Naupactus*, but also *Aramigus*, *Eurymetopus*, and probably *Atrichonotus* and *Alceis* Billberg (the latter was reinstated by Wibmer and O'Brien (1986) and would be a senior synonym of *Naupactus*). This decision has some pros and cons, and should wait for more detailed studies. Meanwhile, the optimal phylogenetic hypothesis herein obtained helps to recognize natural groups and to progress in the proposal of a natural classification for the *Pantomorus–Naupactus* complex.

The recognition of monophyletic groups within the *P-N* complex is significant, not only from the taxonomic point of view, but also for theoretical reasons related to the study of the parthenogenesis in animals. In this respect, the cladistic analyses test hypotheses on the independent origin of parthenogenesis within a given group (Lanteri and Normark, 1995; Lanteri, 1995; Normark and Lanteri, 1998).

Apomitic parthenogenesis is very common in broad-nosed weevils of the subfamily Entiminae, and its occurrence has been confirmed in over 60 species from several tribes (Tomiuk and Loeschcke, 1992; Saura et al., 1993; Lanteri and Normark, 1995; Normark, 1996b). This kind of reproduction has been demonstrated by cytogenetic studies and/or by rearing, for three species studied herein: *G. leucoloma*, *A. tessellatus* and *A. cervinus* (Normark, 1996b; Hardwick et al., 1997; Mander et al., 2003), but it is suspected for several other species and/or lineages of the *P-N.* complex (Lanteri and Normark, 1995).

All parthenogenetic weevils are flightless, since flight muscles are reduced or absent, the same as elytral humeri and metathoracic wings, but not all flightless weevils reproduce parthenogenetically. Thus, fightlessness seems to be a pre-condition for the acquisition of parthenogenesis within Entiminae, being present in all South American *Pantomorus* and in species assigned

to *Aramigus*, *Asynonychus*, *Eurymetopus* and *Graphognathus*. Among the species studied herein, only the basal *N. cinereidorsum* has a full capacity for flight, the remainder being partially to completely flightless. Moreover, only in the populations of *N. cinereidorsum*, *N. verecundus*, *N. xanthographus* and *N. dissimulator* males are well represented. In the remaining species, males are either scarce or unknown, suggesting some kind of alteration in their sexual reproduction.

For theoretical purposes, it is important to know if parthenogenetic lineages of animals have conspecific or congeneric sexual relatives, or if there are clades entirely composed of parthenogens (Lanteri and Normark, 1995; Normark and Lanteri, 1998). Within the *P-N* complex, the verified parthenogens would represent derived lineages of different species with geographic parthenogenesis (Vandel, 1928). This means that there are some bisexual populations in the areas of original distribution (= subtropical forests of South America) and parthenogenetic lineages in recently colonized areas (prairies of South America and of other continents where they have been introduced and became established). For example, within the "*leucoloma* species group" the basal *N. verecundus* is bisexual, *N. leucoloma* exhibits geographic parthenogenesis, and *N. minor* is probably strictly parthenogenetic (Lanteri and Marvaldi, 1995; Lanteri and Normark, 1995).

In the clade *N. dissimulator–A. cervinus*, which is supported only by COI sequences, the former species is bisexual and the latter shows geographic parthenogenesis (Lanteri, 1993). The sexual populations of both sister species are partially sympatric in the subtropical forests of Argentina and Brazil, but the parthenogenetic lineages of *A. cervinus* have been introduced and become established in several countries far from its original area of distribution (Lanteri and Normark, 1995; Lanteri et al., 2002a; Mander et al., 2003).

Aramigus tessellatus is a complex species with one sexual and several highly divergent parthenogenetic lineages, most of them triploid (Normark, 1996a,b; Normark and Lanteri, 1998). Within *Aramigus* there are sexual species, i.e., *A. globoculus* Lanteri and *A. intermedius* Lanteri, and parthenogenetic species, i.e., *A. conirostris* (Hustache), which is a pentaploid (Lanteri and Díaz, 1994; Normark and Lanteri, 1996, 1998). In our molecular tree *A. tessellatus* is in the same clade as *Asynonychus cervinus*, but in the morphological tree it lies at the tip of the cladogram, close to other species with unknown males (*P. cinerosus*, *P. ruizi* and *P. auripes*).

It is interesting to note that the intracellular maternally transmitted bacterium *Wolbachia pipientis* has been found in *A. tessellatus* by Warren et al. (1995) and more recently in *A. cervinus* by one member or our research group (Rodriguero et al., 2004). This parasite induces reproductive alterations in a variety of arthropods, such as parthenogenetic development in the infected hosts, conversion of infected genetic males into functional females, and cytoplasmic incompatibility (Huigens et al., 2000). The distribution of *Wolbachia* throughout the *P-N* complex, and the effects of this cytoplasmically inherited parasite in the reproductive systems of its species, are one of our current research goals, along with the possible coevolution of the hosts and the bacterial strains. For this reason, this contribution represents one step forward for the identification of monophyletic groups within a taxon of weevils of great importance for studies of evolutionary biology.

Acknowledgments

We wish to express our appreciation to all the individuals that helped to collect the material for molecular studies: María Laura De Wysiecki and Carlos Lange (Museo de La Plata, Argentina) and Jerson Guedes Caruss (Universidad Federal do Santa María, Brazil), to Pablo Goloboff for a critical reading of the manuscript, and to the "Consejo Nacional de Investigaciones Científicas y Técnicas" (CONICET), Fundación Antorchas and Universidad de Buenos Aires, for their financial support.

References

Alonso-Zarazaga, M.A., Lyal, C.H.C., 1999. A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera). Entomopraxis S.C.P., Spain.

Bensansson, D., Zhang, D., Hewitt, G.M., 2000. Frequent assimilation of mitochondrial DNA by grasshopper nuclear genomes. Mol. Biol. Evol. 17, 406–415.

Bremer, K., 1994. Branch support and tree stability. Cladistics, 10, 235–304.

Buchanan, L.L., 1939. The species of *Pantomorus* of America North of Mexico. US Dept. Agric. Misc. Publ. 341, 1–39.

Farris, J.M., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics, 10, 315–319.

Goloboff, P., 1997. PIWE: Parsimony and Implied Weights, Version 3.0. Published by the Author, Tucumán, Argentina.

Goloboff, P., 1998. SPA: Sankoff Parsimony Analysis, Version 1.9. Published by the Author, Tucumán, Argentina.

Goloboff, P., 1999. NONA, Version 2.0. Published by the Author, Tucumán, Argentina.

Hardwick, S., Armstrong, K.F., Wratten, S.D., Prestidge, R.A., 1997. Genetic comparison of whitefringed weevil species and populations by RAPD-PCR. Proceedings of the 50th N. Z. Plant Protection Conference. 1997, 327–332.

Higgins, D.G., Sharp, P.M., 1989. Fast and sensitive multiple sequence alignments on a microcomputer. Computer Applic Biosci. 5, 151–153.

Huigens, M.E., Luck, R.F., Klaassen, R.H.G., Maas, M.F.P.M., Timmermans, M.J.T.N., Stouthamer, R., 2000. Infectious parthenogenesis. Nature, 405, 178–179.

Hustache, A., 1947. Naupactini de l'Argentine et des régions limitrophes (Col. Curculion.). Rev. Soc. Entomol. Argent. 13, 3–146.

Kumar, S., Tamura, K., Jakobsen, I., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics, 17, 1244–1245.

Langor, D.W., Sperling, F.A.H., 1997. Mitochondrial DNA sequence divergence in weevils of the *Pissodes strobi* species complex (Coleoptera: Curculionidae). Insect Mol. Biol. 6, 255–265.

Lanteri, A.A., (dir.), 1994. Bases para el control integrado de los gorgojos de la alfalfa. De la Campana Ediciones, La Plata.

Lanteri, A.A., 1984. Revisión sistemática del género *Eurymetopus* Schoenherr (Coleoptera: Curculionidae) mediante la aplicación de técnicas numéricas. Rev. Soc. Entomol. Argent. 43, 247–281.

Lanteri, A.A., 1986. Revisión del género *Asynonychus* Crotch (Coleoptera: Curculionidae). Rev. Asoc. Cs. Nat. Litoral, 17, 161–174.

Lanteri, A.A., 1990. Systematic revision and cladistic analysis of *Phacepholis* Horn. Southwest. Entomol. 15, 179–204.

Lanteri, A.A., 1993. La partenogénesis geográfica y la sinonimia de *Asynonychus cervinus* (Boheman) y. *A. Godmanni* Crotch (Coleoptera: Curculionidae). Rev. Soc. Entomol. Argent. 52, 100.

Lanteri, A.A., 1995. Análisis cladístico y evolución de la partenogénesis en el grupo de *Pantomorus auripes* (Coleoptera: Curculionidae). Rev. Soc. Entomol. Argent. 54, 17–24.

Lanteri, A.A., Díaz, N.B., 1994. Systematic study and cladistic analysis of the genus *Aramigus* (Coleoptera: Curculionidae). Trans. Am. Entomol. Soc. 120, 113–144.

Lanteri, A.A., Guedes, J.C., Parra, J.R.P., 2002a. Weevils injurious for roots of citrus in São Paulo, Brazil. Neotrop Entomol. 31, 561–569.

Lanteri, A.A., Loiácono, M.S., 1990. Systematic study of the *Pantomorus viridisquamosus* species group (Coleoptera: Curculionidae). Insecta Mundi, 4, 1–10.

Lanteri, A.A., Loiácono, M.S., del Coscarón, M., C., Díaz, N.B., 1991. Estudio sistemático del grupo de especies afines a *Pantomorus auripes* Hustache (Coleoptera: Curculionidae). Rev. Soc. Entomol. Argent. 49, 3–16.

Lanteri, A.A., Marvaldi, A.E., 1995. *Graphognatus* Buchanan, a new synonym of *Naupactus* Dejean, and systematics of the *N. leucoloma* species group (Coleoptera: Curculionidae). Coleopts. Bull. 49, 206–228.

Lanteri, A.A., Marvaldi, A.E., Suárez, S.M., 2002b. Gorgojos de la Argentina y sus plantas huéspedes. Tomo I: Apionidae y Curculionidae. Publications Especial, Society of Entomology Argentina, Argentina.

Lanteri, A.A., Morrone, J.J., 1995. Cladistics of the *Naupactus leucoloma* species group, *Atrichonotus*, and *Eurymetopus* (Coleoptera: Curculionidae). Rev. Soc. Entomol. Argent. 54, 99–112.

Lanteri, A.A., Normark, B.B., 1995. Parthenogenesis in the tribe (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 88, 722–731.

Lanteri, A.A., O'Brien, C.W., 1990. Taxonomic revision and cladistic analysis of *Atrichonotus* Buchanan (Coleoptera: Curculionidae). Trans. Am. Entomol. Soc. 116, 697–725.

Lunt, D.H., Zhand, D.X., Szymura, J.M., Hewitt, G.M., 1996. The insect cytochrome oxidase I gene evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol. Biol. 5, 153–165.

Mander, C.V., Phillips, C.B., Glare, T.R., Chapman, R.B., 2003. Preliminary assessment of COI and ITS! Sequence variation in Fuller's rose weevil. NZ. Plant Protection, 56, 190–193.

Morrone, J.J., 1999. The species of Entiminae (Coleoptera: Curculionidae) ranged in America south of the United States. An. Inst Biol. UNAM, Ser. Zool. 76, 99–168.

Morrone, J.J., Lanteri, A.A., 1991. (90). Ubicación sistemática y variación intraespecífica de *Pantomorus ruizi* (Bréthes) (Coleoptera: Curculionidae). Rev. Soc. Entomol. Argent. 49, 17–26.

Nixon, K.C., 2002. WinClada, v1.00.08. Published by the author, Ithaca, NY.

Nixon, K., Carpenter, J.M., 1996. On simultaneous analysis. Cladistics, 12, 221–241.

Normark, B.B., 1994. Phylogeny and evolution of parthenogenesis in the *Aramigus tessellatus* complex (Coleoptera: Curculionidae). PhD Dissertation, Cornell University, Ithaca, NY.

Normark, B.B., 1996a. Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): Evidence from mitochondrial DNA sequences. Evolution, 50, 734–745.

Normark, B.B., 1996b. Polyploidy of parthenogenetic *Aramigus tessellatus* (Coleoptera: Curculionidae). Coleopts. Bull. 50, 73–79.

Normark, B.B., Lanteri, A.A., 1996. *Aramigus uruguayensis* (Coleoptera: Curculionidae), a new species based on mitochondrial DNA and morphological characters. Entomol. News, 107, 311–316.

Normark, B.B., Lanteri, A.A., 1998. Incongruence between morphological and mitochondrial DNA characters suggests hybrid origins of parthenogenetic weevil lineages (genus *Aramigus*). Syst. Biol. 47, 475–494.

O'Brien, C.W., Wibmer, G., 1982. Annotated checklist of the weevils (Curculionidae *sensu lato*) of North America, Central America, and the West Indies (Coleoptera: Curculionoidea). Mem. Am. Entomol. Inst. 34, i–ix, 1–382.

Reiss, R.A., Schwert, D.P., Ashworth, A.C., 1995. Field preservation of Coleoptera for molecular genetics analyses. Environ. Entomol. 24, 716–719.

Rodriguero, M.S., Guzman, N.V., Lanteri, A.A., Confalonieri, V.A., 2004. Presencia de *Wolbachia* en poblaciones de *Asynonychus cervinus* (Coleoptera, Curculionidae): origen de la partenogénesis telitóquica en una especie bisexual. J. Basic Appl. Genet, 16, 140.

Saura, A., Lokki, J., Suomalainen, E., 1993. Origin of polyploidy in parthenogenetic weevils. J. Theor. Biol. 163, 449–456.

Sequeira, A., Lanteri, A.A., Scataglini, M.A., Confalonieri, V.A., Farrell, B., 2000. Are flightless *Galapaganus* weevils older than the Galápagos Islands they inhabit? Heredity, 85, 20–29.

Sorenson, M.D., Fleischer, R.C., 1996. Multiple independent transposition of mitochondrial DNA control region sequences to the nucleus. Proc. Natl Acad. Sci. USA, 93, 15239–15243.

Tomiuk, J., Loeschcke, V., 1992. Evolution of parthenogenesis in the *Otiorhynchus scaber* complex. Heredity, 68, 391–397.

Vandel, A., 1928. La parthénogénèse géographique: contribution à l'étude biologique et cytologique de la parthénogénèse naturelle. Bull. Biol. France Belgique, 61, 93–125.

Warren, J.H., Zhang, W., Guo, L.R., 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc. R. Soc. Lond. B, 261, 55–71.

Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.

Wibmer, G.I., O'Brien, C.W., 1986. Annotated chechlist of the weevils (Curculionidae *sensu* lato) of South America (Coleoptera: Curculionidae). Mem. Am. Entomol. Inst. 39, i–xvi, 1–563.

Zhang, D.X., Hewitt, G.M., 1996. Nuclear integrations: challenges for mitochondrial DNA markers. TREE, 11, 251.

Appendix 1

Species studied, alternative generic placements, complete taxonomic references, and geographic distributions.

Species studied	Generic placements	Taxonomic references	Geographical distributions
Teratopactus nodicollis (Boheman 1833)	*Naupactus*	Lanteri et al. (2002a)	Argentina, Brazil, Paraguay and Uruguay.
Aramigus tessellatus (Say 1824)	*Asynonychus* *Eurymetopus* *Naupactus* *Pantomorus*	Lanteri and Díaz (1994); Normark and Lanteri (1998)	Argentina, Brazil and Uruguay. Introduced into Chile, Mexico and USA.
Asynonychus cervinus (Boheman 1840)	*Aramigus* *Naupactus* *Pantomorus*	Lanteri (1986), Lanteri et al. (2002a,b)	Argentina, Brazil, Paraguay and Uruguay. Introduced into Chile, West Indies, USA, Europe, Hawaii, Azores, Australia and New Zealand.
Eurymetopus birabeni Kuschel 1945		Lanteri (1984)	Argentina and Uruguay. Introduced into Australia.
Eurymetopus fallax Boheman (1840)		Lanteri (1984)	Argentina, Brazil and Uruguay.
Graphognathus leucoloma (Boheman 1840)	*Pantomorus* *Naupactus*	Lanteri and Marvaldi (1995) (as *Naupactus*)	Argentina, Brazil and Uruguay. Introduced into Chile, Perú, USA, South Africa, Australia and New Zealand.
Graphognathus minor (Buchanan 1942)	*Pantomorus* *Naupactus*	Lanteri and Marvaldi (1995) (as *Naupactus*)	Argentina and Uruguay. Introduced into USA.
Naupactus ambiguus Boheman 1840	*Asynonychus* *Pantomorus*	Lanteri et al. (2003)	Argentina, Brazil, Paraguay and Uruguay.
Naupactus cinereidorsum Hustache 1947		Lanteri (1994), Lanteri et al. (2002a,b)	Argentina and Uruguay.
Naupactus dissimulator Boheman 1840		Lanteri et al. (2002a,b)	Argentina, Brazil, Paraguay and Uruguay.
Naupactus navicularis Boheman 1840		Lanteri et al. (2002a)	Argentina and Brazil.
Naupactus verecundus Hustache 1947		Lanteri (1994), Lanteri et al. (2002a,b)	Argentina, Brazil and Paraguay. Introduced into Chile.
Naupactus xanthographus (Germar 1824)		Lanteri (1994), Lanteri et al. (2002a,b)	Argentina, Brazil, Paraguay and Uruguay. Introduced into Chile.
Pantomorus auripes Hustache 1947	*Naupactus*	Lanteri et al. (1991), Lanteri (1995)	Argentina and Brazil.
Pantomorus cinerosus (Boheman 1833)	*Naupactus*	Lanteri (1994), Lanteri et al. (2002a,b)	Argentina, Brazil and Uruguay.
Pantomorus ruizi (Brèthes 1925)	*Asynonychus* *Naupactus*	Morrone and Lanteri (1991)	Argentina and Chile.
Pantomorus viridisquamosus (Boheman 1859)	*Asynonychus* *Naupactus*	Lanteri and Loiácono (1990)	Argentina, Brazil and Uruguay. Introduced into Mauritius (Africa).

Genera, type species, species numbers and taxonomic references.

Genera and type species	Species number and references	Alternative taxonomic treatments
Aramigus Horn Type sp. *A. tessellatus* (Say)	8 spp. (Lanteri and Díaz, 1994; Morrone, 1999)	Subgenus or synonym of *Pantomorus* (Buchanan, 1939; Wibmer and O'Brien, 1986)
Asynonychus Crotch Type sp. *A. cervinus* (Boheman)	1 spp. (Morrone, 1999).	Subgenus or synonym of *Pantomorus* (Buchanan, 1939; Wibmer and O'Brien, 1986) Synonym of *Naupactus* (Alonso-Zarazaga and Lyal, 1999).
Eurymetopus Schoenherr Type sp. *E. fallax* Boheman	7 spp. (Lanteri, 1984; Morrone, 1999)	
Graphognathus Buchanan Type sp. *G. leucoloma* (Boheman)	3 spp. (Wibmer and O'Brien, 1986).	Subgenus of *Pantomorus* (Buchanan, 1939). Synonym of *Naupactus* (Lanteri and Marvaldi, 1995; Morrone, 1999; Alonso-Zarazaga and Lyal, 1999)
Naupactus Dejean Type sp. *N. rivulosus* (Olivier)	168 spp. (O'Brien and Wibmer, 1982; Wibmer and O'Brien, 1986)	Does not include species traditionally assigned to *Pantomorus*.

Appendix 1
Continued

Genera and type species	Species number and references	Alternative taxonomic treatments
	215 spp. (Morrone, 1999).	Includes South American species traditionally assigned to *Pantomorus*, and not congeneric with its type species.
Pantomorus Schoenherr. Type sp. *P. albosignatus* Boheman	80 spp. (O'Brien and Wibmer, 1982; Wibmer and O'Brien, 1986)	Includes North, Central and South American *Pantomorus*, and species classified as *Aramigus*, *Asynonychus*, *Phacepholis* Horn and *Galapaganus* Lanteri.
	35 spp. (Morrone, 1999).	Only includes species from Mexico and Central America.

Appendix 2

List of morphological characters, character states and codes.

Characters of the vestiture

1. Setae of the rostrum: directed towards rostral sulcus (0); arteriorly directed (1).
2. Vestiture of the antennae: composed of setae (0); composed of scales (1).
3. Elytral vestiture: composed of contiguous scales and fine setae (0); composed of imbricate scales and seta-like scales (1).
4. Elytral scales: covering the whole elytral surface (0); leaving two or three stripes naked of scales (1).
5. Elytral setae: recumbent to suberect, short (0); erect, medium-length to long (1).
6. Scutellum: squamose (0); setose (1); glabrous (2); inconspicuous (3).

External morphology

7. Eyes: convex (0); slightly convex (1); strongly convex (2).
8. Rostrum: with lateral keels (0); without lateral keels (1).
9. Scape: moderately broad, reaching to slightly exceeding hind margin of eye (0); slender, largely exceeding hind margin of eye (1); stout, not reaching hind margin of eye (2).
10. Funicular article 2, twice to three times as long as article 1 (0); slightly longer than article 1 (1); as long as article 1 (2); slightly shorter than article 1 (3).
11. Pronotum: moderately rugose (0); strongly tuberculate (1); smooth (2).
12. Pronotum: transversal (0); slightly transversal (1); strongly transversal (2).
13. Pronotum: lacking lateral tubercles (0); with lateral tubercles (1).
14. Elytral base: bisinuate (0); slightly bisinuate (1); straight (2).
15. Humeri: very broad (0); reduced (1); absent (2).
16. Tubercle on humeri: absent (0); present (1).
17. Elytral intervals: slightly convex (0); flat (1).
18. Pair of elytral subapical tubercles: absent (0); present (1).
19. Front coxae: contiguous (0); separate from each other (1).
20. Denticles on inner margin of tibiae: only on front tibiae (0); on three pairs of tibiae (1); lacking denticles on all tibiae (2).
21. Corbels of hind tibiae: open (0); slightly enclosed (1); enclosed (2).
22. Combs of hind tibiae: dorsal comb about as long as apical comb (0); dorsal comb longer than apical comb (1).
23. Tarsites 1 and 2: very elongate (0); tarsite 1 slightly elongate and tarsite 2 transversal (1).

Female genitalia

24. Sternite VIII of female: subrhomboidal (0); suboval (1); subpentagonal (2).
25. Ovipositor: with a pair of ventral baculi, only (0); with a pair of ventral baculi and a pair of dorsal baculi (1).
26. Line of setae on each side of ventral baculi: absent (0); present (1).
27. Styli: present (0); absent (1).
28. Hemisternites: slightly sclerotized, nail-like (0); moderately sclerotized, claw-like, short (1); strongly sclerotized, claw-like, long (2).
29. Nodulus of spermatheca: vestigial (0); conical, short (1); tubular, long, lacking posterior protuberance (2); tubular, long, with posterior protuberance (3).

Male genitalia

30. Apex of aedeagus: acute (0); arrow pointed (with pair of lateral points) (1).

Data matrix for the 30 morphological characters. Unknown character states are denoted by "?".

Teratopactus nodicollis
00000 00000 00100 10011 00011 01200
Aramigus tessellatus
10010 20001 20022 01000 11000 00030
Asynonychus cervinus
10010 31001 00022 01000 01000 10021
Eurymetopus birabeni
11110 00123 20021 01002 00120 0111?
Eurymetopus fallax
11110 00123 20021 01002 00120 0110?
Graphognathus leucoloma
00001 01001 00021 01000 00000 10010
Graphognathus minor
00001 01001 00021 01000 10000 1001?
Naupactus ambiguus
10011 32001 02022 00000 00000 00001
Naupactus cinereidorsum
10010 00001 00000 01000 00000 10010
Nsupactus dissimulator
00010 10010 00011 00100 00000 00021
Naupactus navicularis
00010 10010 21011 01100 20000 0001?
Naupactus verecundus
00001 10000 10021 01000 00000 10010
Naupactus xanthographus
00010 10011 01011 00100 20000 00011
Pantomorus auripes
10011 20001 00022 01000 11000 0001?
Pantomorus cinerosus
10010 12002 01022 01000 11000 0001?
Pantomorus ruizi
10011 10001 00022 01000 11000 0001?
Pantomorus viridisquamosus
10010 20002 20022 01000 00000 00011