

## Is *Ellipura* monophyletic? A combined analysis of basal hexapod relationships with emphasis on the origin of insects

Gonzalo Giribet<sup>a,\*</sup>, Gregory D. Edgecombe<sup>b</sup>, James M. Carpenter<sup>c</sup>,  
Cyrille A. D'Haese<sup>d</sup>, Ward C. Wheeler<sup>c</sup>

<sup>a</sup>Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA

<sup>b</sup>Australian Museum, 6 College Street, Sydney, New South Wales 2010, Australia

<sup>c</sup>Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA

<sup>d</sup>FRE 2695 CNRS, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, 45 rue Buffon, F-75005 Paris, France

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### Abstract

Hexapoda includes 33 commonly recognized orders, most of them insects. Ongoing controversy concerns the grouping of Protura and Collembola as a taxon *Ellipura*, the monophyly of Diplura, a single or multiple origins of entognathy, and the monophyly or paraphyly of the silverfish (*Lepidotrichidae* and *Zygentoma* s.s.) with respect to other dicondylous insects. Here we analyze relationships among basal hexapod orders via a cladistic analysis of sequence data for five molecular markers and 189 morphological characters in a simultaneous analysis framework using myriapod and crustacean outgroups. Using a sensitivity analysis approach and testing for stability, the most congruent parameters resolve *Tricholepidion* as sister group to the remaining Dicondylia, whereas most suboptimal parameter sets group *Tricholepidion* with *Zygentoma*. Stable hypotheses include the monophyly of Diplura, and a sister group relationship between Diplura and Protura, contradicting the *Ellipura* hypothesis. Hexapod monophyly is contradicted by an alliance between Collembola, Crustacea and Ectognatha (i.e., exclusive of Diplura and Protura) in molecular and combined analyses.

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### Introduction

The origin and evolution of insects have long captivated biologists, perhaps because insects constitute the largest group of organisms. Indeed, no other animal clade has recorded diversity remotely comparable to

that of insects. Due to the great interest in insects, most of the major lineages of the group have long been described or identified, and currently 33 orders of hexapods are commonly recognized (Naumann 1991; Klass et al. 2002). Hexapod relationships have been studied using an array of different techniques, and several phylogenetic hypotheses based on morphological cladistic (non-numerical) analyses have been proposed (e.g., Hennig 1969; Kristensen 1975, 1981, 1991, 1995,

\*Corresponding author.

E-mail address: [ggiribet@oeb.harvard.edu](mailto:ggiribet@oeb.harvard.edu) (G. Giribet).

1998; Kukulová-Peck 1991). The incorporation of explicit numerical analyses (Whiting et al. 1997; Bitsch and Bitsch 1998, 2000; Carpenter and Wheeler 1999; Beutel and Gorb 2001; Wheeler et al. 2001), and of sources of data other than morphology (Whiting et al. 1997; Carpenter and Wheeler 1999; Carapelli et al. 2000; Wheeler et al. 2001; D’Haese 2002a; Hovmöller et al. 2002; Whiting 2002; Luan et al. 2003; Ogden and Whiting 2003), have yielded more testable hypotheses. However, the relationships among the sister groups of insects, or ‘basal’ hexapods, have received little attention from the perspective of molecular data.

True insects (Ectognatha sensu Hennig or Insecta sensu Kristensen) contain about two dozen orders of winged insects (Pterygota) and two orders of primitively wingless insects, Archaeognatha (also called Microcorphya) and Zygentoma. Zygentoma is the putative sister group of the pterygote insects; these two together forming the clade Dicondylia, with Archaeognatha as its sister group. Non-insect hexapods (the so-called Entognatha) comprise the orders Protura, Collembola, and Diplura. Relationships among the entognathous hexapods, however, are not agreed upon (see reviews in Stys and Zrzavy 1994; Bitsch and Bitsch 2000).

Among basal hexapod relationships, four issues remain problematic: (1) the monophyly or paraphyly of the entognathous forms; (2) the monophyly of the so-called Ellipura or Parainsecta (Protura + Collembola); (3) the monophyly of the Diplura and the relationships among the dipluran families; and (4) the monophyly and relationships among the zygentoman families, especially with regard to the Lepidotrichidae,<sup>1</sup> a taxon based on the Baltic amber species *Lepidotrix*<sup>1</sup> *pilifera* Menge and represented in the extant fauna by the single species *Tricholepidion gertschi* Wygodzinsky, 1961. These issues have recently been investigated by Bitsch and Bitsch (1998, 2000), who used a set of detailed and explicit morphological character matrices in a parsimony framework. Carapelli et al. (2000) published the first well-sampled molecular analysis of the ‘apterygotes’ using sequence data of the nuclear encoding gene elongation factor-1 $\alpha$  and the mitochondrial ribosomal 12S rRNA (see also Zhang et al. 2001), though neither the families Lepidotrichidae nor Nicoletiidae were included in the analyses. To a lesser extent, Wheeler et al. (2001) explored basal hexapod relationships by a combined analysis of morphological and molecular (18S and 28S rRNA) data in their study of the hexapod orders, testing

the first two issues listed above. No sequence data for the zygentoman families Lepidotrichidae and Nicoletiidae, or the archaeognathan family Meinertellidae were included in those studies. Nardi et al. (2003) included data for *Tricholepidion* in their analysis of hexapod relationships based on mitochondrial genomes. Luan et al. (2003) analyzed 18S and 28S rRNA sequence data of several basal hexapods and found monophyly of Diplura as well as monophyly of Diplura + Protura (see also D’Haese 2002b). Other recent work has used molecular data to investigate basal hexapod relationships to a certain extent, although in the context of collembolan evolution (D’Haese 2002a), or higher arthropod relationships (Wheeler 1998; Edgecombe et al. 2000; Giribet and Ribera 2000; Giribet et al. 2001).

“In spite of their great morphological differences, Protura and Collembola have been put together by many authors in a particular clade, the so-called ‘Ellipura’ (=Parainsecta)” (Bitsch and Bitsch 1998, p. 356). In fact, most authors have recognized this group based on morphology (Hennig 1953, 1981; Kristensen 1975, 1981, 1995, 1998; Boudreaux 1979a; Kukulová-Peck 1987, 1991; Stys and Bilinski 1990; Stys et al. 1993; Kraus and Kraus 1994; Stys and Zrzavy 1994; Koch 1997; Kraus 1998, 2001; Ax 1999; Carpenter and Wheeler 1999; Edgecombe et al. 2000; Wheeler et al. 2001). Morphologically, Ellipura finds its most compelling support in a distinctive style of entognathy involving a linea ventralis on the underside of the head capsule (Koch 1997). Other characters cited as ellipuran apomorphies (e.g., loss of cerci or terminal filaments: Kraus 1998; reduction of antennae, compound eyes and tracheae; unpaired pretarsal claws: Ax 1999) are less obviously straightforward in their interpretation, and Bitsch and Bitsch (1998, 2000) did not find unambiguous support for Ellipura in parsimony analyses of internal anatomical and external morphological characters.

The monophyly of Diplura has also been disputed (for a review of alternative hypotheses see Stys and Zrzavy 1994). The traditional union of Campodeoidea and Japygoidea as a clade, Diplura, has recently been defended on morphological (Koch 1997; Kristensen 1998), developmental (Ikeda and Machida 1998), and molecular grounds (Carapelli et al. 2000; Giribet and Ribera 2000; Giribet and Wheeler 2001; D’Haese 2002b; Luan et al. 2003). The alternative resolution of Diplura as a grade involves Campodeoidea as being more closely related to Ellipura than to Japygoidea (Stys and Bilinski 1990; Stys et al. 1993), based on ovary structure.

The monophyly of the Zygentoma (families Lepidotrichidae, Nicoletiidae, Maindroniidae and Lepismatiidae [Atelurinae is given familial status by certain authors, while others include it within the Nicoletiidae]) is also debated. The sole extant representative of the Lepidotrichidae, *Tricholepidion gertschi*, has some

<sup>1</sup>The family-group name was first published as “Lepidothricinae” by Silvestri (1912), based on the genus spelled “*Lepidothrix*”. Carpenter (1992) indicated that the latter emendation of *Lepidotrix* Menge was unjustified, and the valid family name becomes Lepidotrichidae under the International Code of Zoological Nomenclature, Article 29.3 (ICZN 1999). *Lepidotrix*, based on *L. pilifera* Menge, 1854, is the senior synonym of *Lepidion* Menge, 1854, *Klebsia* Olfers, 1907, and *Micropa* Olfers, 1907, also from Baltic amber.

putative primitive characters not found in other zygentomans (including large abdominal sterna with posteriorly attached coxopodites, a large number of pregenital styles and eversible sacs; Kristensen 1991). *Tricholepidion* also has retained the cephalic, ligamentous endoskeleton (Boudreaux 1979a; Kristensen 1998; Staniczek 2000), and for these reasons Kristensen (1991, p. 130) considered the possibility that *Tricholepidion* is the sister group of all other Dicondylia and should be given ordinal rank. A detailed consideration of the mandible and its musculature endorsed the view that *Tricholepidion* is basal to all other Dicondylia (Staniczek 2000). This followed Kristensen's (1998) reassessment of the position of *Tricholepidion*, which concluded that *Zygentoma* (including *Tricholepidion*) is monophyletic based on (1) the presence of a widened apical segment of the labial palp; (2) obliteration of the superlingua; and (3) sperm conjugation (Wygodzinsky 1961; Wingstrand 1973; Jamieson 1987; Jamieson et al. 1999). However, the two numerical parsimony analyses where the monophyly of *Zygentoma* has been tested placed *Tricholepidion* as the sister group of the remaining Dicondylia (Bitsch and Bitsch 2000; Beutel and Gorb 2001). Recent work has contributed characters of sperm ultrastructure for *Tricholepidion* (Dallai et al. 2001a, b).

In this study, we explore the relationships of basal hexapods by performing a simultaneous analysis combining 189 morphological characters and sequence data for five molecular markers, totalling about 3.5 Kb of DNA sequence information. The molecular markers investigated are two nuclear ribosomal genes (18S rRNA and the D3 region of the 28S rRNA), a nuclear coding gene (histone H3), a mitochondrial ribosomal gene (16S rRNA), and a mitochondrial coding gene (cytochrome *c* oxidase subunit I [COI hereafter]). We have included representatives of many families of the basal hexapods, among others the zygentomans Lepismatidae, Nicoletiidae, and Lepidotrichidae (see Table 1), and several new dipluran sequences. Among various other goals this study aims to test one of the most intriguing issues in insect systematics, whether the *Zygentoma* (silverfish) constitute a natural group, including the three aforementioned families. To date, this has not been tested with molecular data.

## Materials and methods

### Terminal taxa

Members of the other major taxa of mandibulate arthropods, Myriapoda and Crustacea, are used as outgroups for rooting Hexapoda. Inclusion of myriapod as well as crustacean outgroups accommodates each of two competing hypotheses for the sister group of

Hexapoda, the Atelocerata (Kraus 1998, 2001) and Tetraconata (Dohle 2001; Richter 2002) hypotheses, respectively. In addition to Lithobiomorpha and Symphyla, the myriapod outgroups of Bitsch and Bitsch (1998, 2000), we add another chilopod terminal, *Scutigera*, so that both major clades of Chilopoda (Notostigmophora and Pleurostigmophora) are coded. Crustaceans are sampled using the cephalocarid *Hutchinsoniella*, the anostracan branchiopod *Artemia*, and the reptant malacostracan *Homarus*, which, taken together, span much of the phylogenetic scope of Crustacea.

In the morphological analysis, a single coding is used for Japygoidea (sensu Pagés 1997), represented in the analysis by Japygidae and Parajapygidae. Similarly, morphological codings for the proturan group Acerentomoidea and collembolan groups Symphypleona, Poduromorpha and Entomobryomorpha follow those of Bitsch and Bitsch (1998, 2000).

### Morphological characters

One hundred and eighty-nine morphological characters have been extracted from literature sources, mainly from studies of basal hexapods and hexapod phylogeny (Kristensen 1981, 1991, 1995, 1998; Koch 1997, 2000; Bitsch and Bitsch 1998, 2000; Kraus 1998; Machida and Ando 1998; Gereben-Krenn and Pass 1999, 2000; Pass 2000; Staniczek 2000; Beutel and Gorb 2001; Wheeler et al. 2001). The matrices published by Bitsch and Bitsch (1998, 2000) were combined with that published by Wheeler et al. (2001). Some characters were also scored differently according to studies of particular character systems cited above. Appendix A presents the character list and Appendix B the matrix.

### Molecular data

Genomic DNA samples were obtained from fresh or ethanol-preserved tissues following standard methods for DNA purification. The 18S rRNA loci were PCR-amplified in two or three overlapping fragments of about 950, 900 and 850 bp each, using primer pairs 1F-5R, 3F-18Sbi and 5F-9R, respectively. All other markers were amplified and sequenced using a single primer pair, namely 28Sa and 28Sb for 28S rRNA, 16Sar and 16Sb for 16S rRNA, LCO1490 and HCO2198 for COI, and H3aF/H3bF and H3aR/H3bR for histone H3 (Xiong and Kocher 1991; Folmer et al. 1994; Whiting et al. 1997; Colgan et al. 1998; Edgecombe et al. 2002).

Amplification was carried out in a 50 µl volume reaction, with 1.25 units of AmpliTaq<sup>®</sup> DNA Polymerase (Perkin Elmer, Foster City, CA, USA), 200 µM of dNTPs, and 1 µM of each primer. The PCR program consisted of an initial denaturing step at 94 °C for 60 s,

**Table 1.** Terminal taxa and molecular represented in the analyses

Terminal taxa		18S	28S	COI	16S	H3
CHILOPODA	<i>Lithobius obscurus/forficatus</i>	AF334271	AF334292	AF309492*	AJ270997*	AF110853
	<i>Scutigera weberi</i>	AY288689	AY288705	AY288741	AF370867	AY428835
SYMPHYLA	<i>Hanseniella</i> sp.	AF173237	AF173268	AF370839	AF370864	AF110856
	<i>Scutigera</i> sp.	AF007106	AY555527			
CEPHALOCARIDA	<i>Hutchinsoniella macracantha</i>	AF370801	AF370811	AF370852	AF370875	AF110867
BRANCHIOPODA	<i>Artemia salina/franciscana</i>	X01723*	X01723*	NC_001620*	X69067*	AF110871
MALACOSTRACA	<i>Homarus americanus</i>	AF235971*	AF370812	AF370853	AF370876	AF370819
COLLEMBOLA						
Entomobryomorpha	<i>Orchesella villosa</i>	AY555514	AY555528	AY555540	AY555550	AY555560
	<i>Folsomia candida</i>	AY555515	AY555529	AY555541	AY555551	AY555561
	<i>Tomocerus minor</i>	AY555516	AY555530			AY555562
Poduromorpha	<i>Xenylla grisea</i>	AY555517	AY555531		AY555552	AY555563
	<i>Onychiurus ambulans</i>	AY555518	AY555532	AY555542		AY555564
	<i>Tetrodontophora bielensis</i>	AY555519	AY555533	AY555543		
	<i>Neanura muscorum</i>	AY555520	AY555534	AY555544	AY555553	
	<i>Morulina verrucosa</i>	AY555521	AY555535		AY555554	AY555565
Symphyleona	<i>Sminthurinus bimaculatus</i>	AY555522	AY555536	AY555545	AY555555	AY555566
PROTURA						
Acerentomidae	<i>Acerentulus traegardi</i>	AF173233	AF005469			
Protentomidae	<i>Neocondeellum dolichotarsum</i>	AY037170*	AF416873*			
Berberentomidae	<i>Baculentulus tienmushanensis</i>	AY037169*	AF416872*			
DIPLURA						
Campodeidae	<i>Campodea tillyardi</i>	AF173234		AF370844	AF370868	AF110860
	Campodeidae sp.	AF005455	AF005471			
	<i>Lepidocampa weberi</i>	AY037167*	AF416870*			
Japygidae	<i>Catajapyx</i> sp.	AF005456				
	<i>Metajapyx</i> sp.	AY555523	AF173266			
	<i>Heterojapyx</i> sp.	AY555524	AY555537	AF370845	AF370869	AY555567
Parajapygidae	<i>Parajapyx emeryanus</i>	AY037168*	AF416871*			
ARCHAEOGNATHA						
Machilidae	<i>Dilta littoralis</i>	AF005457	AF005473	AF370847	AF370871	
	<i>Petrobius brevistylis/Petrobiinae</i> sp.	X89808*	X90665*			AF110865
Meinertellidae	<i>Machiloides</i> sp.	AY084061	AY084065	AF370846		
	<i>Allomachilis froggatti</i>	AF370788	AF370806	AY084068	AF370870	AF110864
ZYGENTOMA						
Lepidotricidae	<i>Tricholepidion gertschi</i>	AF370789	AF370807		AY555556	AF110863
Lepismatidae	<i>Lepisma</i> sp.	AF005458	AF005474	AY555546	AY555557	AY555568
	<i>Thermobia domestica</i>	AF370790	AF370808	AF370872	AF370848	
Nicoletiidae	<i>Texoreddellia texensis</i>	AY084062	AY084066			
	<i>Squamigera latebricola</i>	AY084063	AY084067	AY084070	AY084073	AY084075
EPHEMEROPTERA	<i>Callibaetis ferrugineus</i>	AF370791		AF370849	AF370873	AF370815
	<i>Choroterpes</i> sp.	AY555525	AY555538	AY555547	AY555558	AY555569
ODONATA	<i>Anax junius</i>	AY555526		AY555548	AY555559	AY555570
	<i>Libellula pulchella</i>	U65109	U65168	AF195753*	AF037180*	
DERMAPTERA	<i>Forficula auricularia</i>	X89490*			AF157313*	
	<i>Labidura riparia</i>	U65114	AY555539	AY555549	AF290383*	AY555571
ORTHOPTERA	<i>Locusta migratoria</i>	AF370793	AF370809	X80245*	X80245*	AF370817

Abbreviations: 18S = 18S rRNA; 28S = 28S rRNA, D3 region; COI = cytochrome *c* oxidase subunit I; 16S = 16S rRNA; H3 = histone H3. Asterisks denote sequences obtained from GenBank

35 amplification cycles (94 °C for 15 s, 49 °C for 15 s, 72 °C for 15 s), and a final step at 72 °C for 6 min in a GeneAmp<sup>®</sup> PCR System 9700 (Perkin Elmer). The annealing temperature to amplify the COI fragment was 46 °C.

PCR amplified samples were purified with the GENECLAN<sup>®</sup> III kit (BIO 101 Inc., Vista, CA, USA) or with the AGTC<sup>®</sup> Gel Filtration Cartridges (Edge BioSystems, Gaithersburg, MD, USA), and directly sequenced using an automated ABI Prism<sup>®</sup>

377 DNA sequencer or an ABI Prism® 3700 DNA analyzer. Cycle-sequencing with AmpliTaq® DNA polymerase, FS (Perkin-Elmer) using dye-labeled terminators (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Foster City, CA, USA) was performed in a GeneAmp® PCR System 9700 (Perkin Elmer). The sequencing reaction was carried out in a 10 µl volume reaction: 4 µl of Terminator Ready Reaction Mix, 10–30 ng/ml of PCR product, 5 pmoles of primer and dH<sub>2</sub>O to 10 µl. The cycle-sequencing program consisted of an initial step at 94 °C for 3 min, 25 sequencing cycles (94 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min), and a rapid thermal ramp to 4 °C and hold. The BigDye-labeled PCR products were isopropanol-precipitated following manufacturer protocol, or cleaned with AGTC® Gel Filtration Cartridges (Edge BioSystems). Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher™ 3.0 (Gene Codes Corporation). Complete sequences were edited in GDE (Smith et al. 1994), where they were split according to primer delimited regions and secondary structure features. All external primers were excluded from the analyses. All the new sequences have been deposited in GenBank (see accession codes in Table 1).

Molecular data have been obtained for 42 terminals (Table 1). The five molecular loci are as follows:

**18S rRNA:** The complete sequence of the small nuclear ribosomal subunit has proven to be useful in previous studies of arthropod and hexapod relationships, and has been chosen as the ‘skeleton’ of the cladogram. This locus has been sampled for 42 specimens, of a total length (excluding primers 1F and 9R) ranging from 1324 bp in the symphylian *Scutigereella* sp. to over 2330 bp in *Heterojapyx* sp. The 18S rRNA sequences have been divided into 27 fragments, according to primer regions and secondary structure features (Giribet 2001). Two of these regions showed large sequence length heterogeneity and have been excluded from the analyses.

**28S rRNA:** The D3 fragment of the large nuclear ribosomal subunit also has been used in previous analyses of arthropod and hexapod phylogeny. Here, we use sequences for 37 specimens, of a total length (excluding primers 28Sa and 28Sb) ranging between 293 and 520 bp. The fragment has been divided into four pieces, one of which has been excluded from the analyses due to large sequence length heterogeneity.

**16S rRNA:** A fragment of the mitochondrial ribosomal large subunit ranging between 475 and 540 bp has been sequenced for 27 specimens. The gene fragment has been divided into 11 fragments, all of them included in the analyses.

**COI:** A fragment of 658 bp of the mitochondrial protein coding gene cytochrome *c* oxidase subunit I has

been sequenced for 26 species. This fragment has been analyzed as a single piece due to the fact that it is a coding fragment that does not present sequence length variation.

**Histone H3:** A 327 bp fragment of the nuclear protein coding gene has been sequenced for 25 species. This fragment has been analyzed in a single piece.

In total, we have included ca. 3500 bp of sequence data per complete taxon, although missing gene fragments are found for some of the analyzed terminal taxa. In a few cases where sequences were not available for single species we have combined partitions from different taxa and use supraspecific taxa as summary terminals (see Table 1). The terminal taxon “Campodeidae sp.” is not a composite taxon, but an unidentified species of campodeid from Spain.

## Analytical methods

Morphological data (Appendices A and B) were analyzed using the parsimony programs NONA version 2.0 (Goloboff 1998) and Winclada version 1.00.08 (Nixon 2002). The search strategy used tbr (tree bisection and reconnection) branch swapping on a series of 1000 random addition replicates retaining up to 10 cladograms per replicate (commands: *h/10; mult\*1000*). Since all the replicates found cladograms of minimum length, no further search strategies were deemed necessary. Jackknife values were calculated using an approximate search with ten random addition replicates, and this procedure was repeated 1000 times. The jackknife deletion function that was employed assigns each character a probability of  $e^{-1}$  of being deleted (Farris et al. 1996; Farris 1997).

Molecular data were analyzed using the direct optimization method (Wheeler 1996) as implemented in the computer program POY (Wheeler et al. 2002). Each gene was analyzed independently and in combination with (1) all other molecular data, and (2) all available data (molecular and morphological). A parameter space of two variables (gap/transversion ratio and transversion/transition ratio) was explored, totalling twelve parameter sets analyzed per partition, and for each of the combined analyses (molecular and total evidence). Therefore 96 independent analyses were performed.

The POY analyses were run in parallel on a cluster of 128 dual-processor nodes using PVM software and the parallel version of POY (commands in effect: “-parallel-jobspernode 2-dpm-dpmacceptratio 1.5-dpmjobspernode 1”), and the cluster was subdivided into 10 subclusters (command: “-controllers 10”). Each replicate consisted of 25 starting Wagner trees generated through random addition sequence, the best of which is submitted to a combination of SPR and TBR branch

**Table 2.** Tree lengths for different partitions (RIB to MOR) at different parameter set combinations (110–441), and values of the ILD metric (see Materials and methods) for all the molecular partitions (ILD-MOL) and for the combined molecular and morphological analyses (ILD-TOT)

	RIB	COI	16S	H3	MOL	MOR	TOT	ILD-MOL	ILD-TOT
110	1179	1052	1424	373	4181	374	4621	0.0366	0.0474
111	2760	2105	2398	852	8343	374	8770	0.0273	0.0320
121	<i>4010</i>	<i>3210</i>	<i>3947</i>	<i>1261</i>	<i>12,741</i>	<i>748</i>	<i>13,585</i>	<i>0.0246</i>	<i>0.0301</i>
141	6401	5334	6829	2031	21,154	1496	22,877	0.0264	0.0344
210	1465	1052	1755	373	4848	748	5721	0.0419	0.0573
211	3083	2105	2821	852	9111	748	9945	0.0274	0.0338
221	4612	3209	4680	1261	14,162	1496	15,849	0.0282	0.0373
241	7615	5335	8254	2031	23,965	2992	27,383	0.0305	0.0422
410	1940	1052	2202	373	5948	1496	7685	0.0641	0.0809
411	3630	2105	3354	852	10,295	1496	11,955	0.0344	0.0433
421	5672	3210	5662	1261	16,484	2992	19,827	0.0412	0.0519
441	9702	5334	10145	2031	28,567	5984	35,285	0.0474	0.0592

Abbreviations: RIB=18S rRNA + 28S rRNA; COI=cytochrome *c* oxidase subunit I; 16S=16S rRNA; H3=histone H3; MOL=molecular [18S + 28S + 16S + COI + H3]; MOR=morphology; TOT=molecular and morphology partitions combined. Values for the parameter set (121) that minimizes incongruence appear in italics.

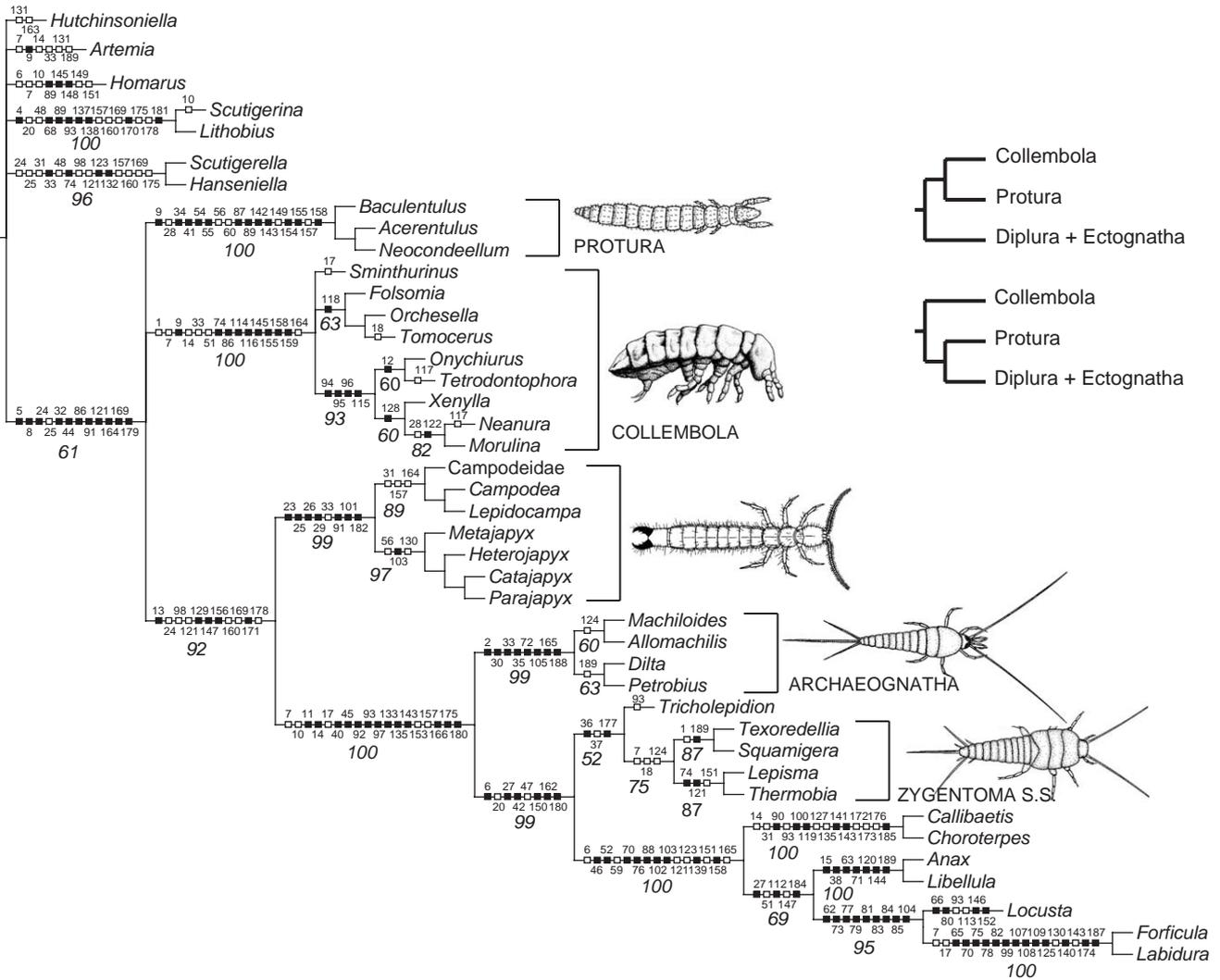
swapping (the default in POY), for a total of 20 replicates. TBR branch swapping was followed by a combination of tree-fusing (Goloboff 1999) and ratchetting (Nixon 1999) to optimize tree searches. The “-slop” and “-checkslop” commands were employed to improve cladogram cost calculations from the heuristic operations (Wheeler 2002). Each search was performed for each partition independently, as well as for the combined analysis of all data. Jackknife values were calculated with POY using 1000 random addition replicates submitted to spr (subtree pruning and regrafting) and tbr branch swapping.

Character congruence was used to choose the combined analysis that minimized incongruence among partitions (as in Wheeler 1995). A more conservative estimate of the phylogenetic hypothesis was also presented via the strict consensus of all the parameter sets (Giribet 2003). Congruence among partitions (morphological and molecular) was measured by the ILD metric (Mickey and Farris 1981; Farris et al. 1995) (Table 2). This value is calculated from the difference between the overall cladogram length and the sum of its data components:  $ILD = (\text{Length}_{\text{Combined}} - \sum \text{Length}_{\text{Individual Sets}}) / \text{Length}_{\text{Combined}}$ . Character congruence is thus used as an optimality criterion to discriminate between cladograms, the optimal cladogram being that which minimizes conflict among all the data. Criticisms of the ILD test as a measure of data set independence for combinability issues (Dolphin et al. 2000) are not relevant to our use of the metrics, since we do not use it as a randomization test. Our usage of the metric registers comparative levels of congruence among partitions when analyzed under different analytical parameters.

## Results

The morphological analysis yielded seven trees of 374 steps (CI 0.73, RI 0.93), the same trees being obtained in every single replicate performed. The strict consensus of these cladograms (Fig. 1) shows monophyly of Hexapoda, Ectognatha, Dicondylia, Pterygota, and monophyly of all the orders represented by more than one terminal, including the monophyly of the orders Diplura and Zygentoma. ‘Entognatha’ is not supported, with Diplura resolved as sister group of Ectognatha, with a jackknife frequency of 92%. Among the ‘apterygote’ orders, Protura, Collembola, Diplura, and Archaeognatha have jackknife support values of 99% or 100% (Fig. 1). Zygentoma has a jackknife frequency of 52%, with *Tricholepidion* being the sister group of the other two sampled families, Lepismatidae and Nicoletiidae. These data do not support the previously proposed possibility of zygentoman parphyly based solely on morphology. The data set is ambiguous with respect to the position of Collembola and Protura. In one minimal length topology, Ellipura is monophyletic, but an alternative, equal-length resolution places Protura as the sister group to Diplura + Ectognatha (Fig. 1). Although numerous characters optimize as basal apomorphies of Hexapoda (Fig. 1), the jackknife frequency for hexapods is low (61%).

The molecular data (Fig. 2) reject hexapod monophyly. If the trees are rooted with the Chilopoda, for the parameter set that minimizes overall incongruence between partitions (121; gap/transversion ratio = 1; transversion/transition ratio = 2), hexapods are polyphyletic. Protura + Diplura forms the sister taxon to Symphyla, the crustaceans form a clade with

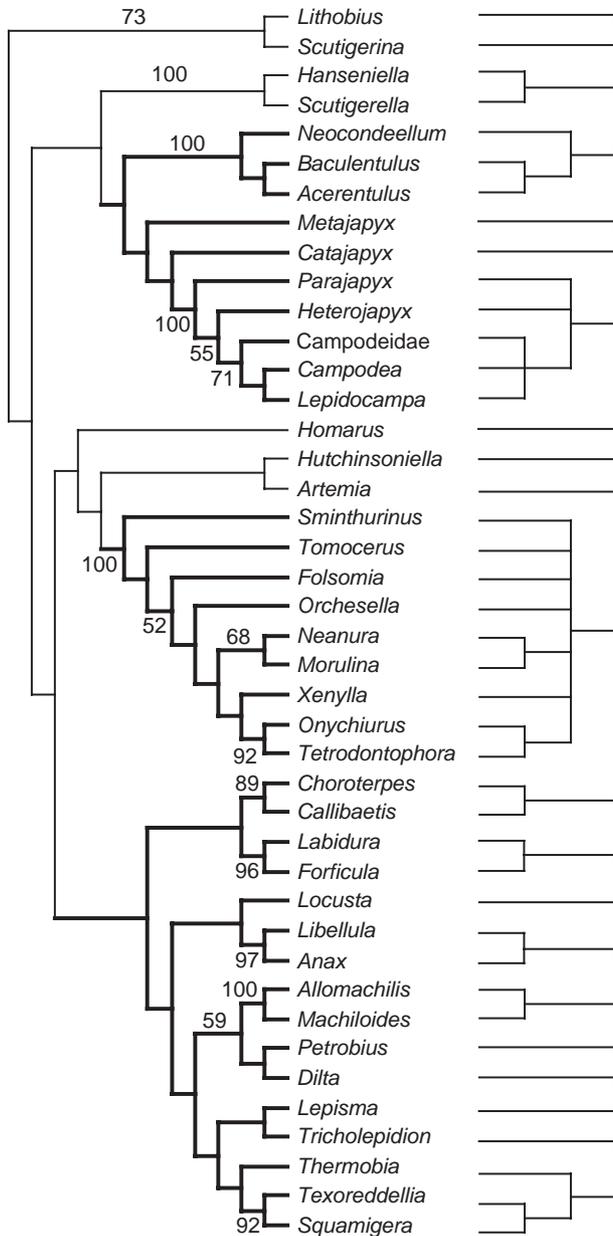


**Fig. 1.** Strict consensus of seven most parsimonious cladograms based on morphological data in Appendix B. Numbers in italics are jackknife frequencies for clades present in more than 50% of jackknife replicates. Only unambiguous character optimizations are shown. Inset shows the two equally parsimonious resolutions of basal hexapod interrelationships.

Collembola, and the Ectognatha comprise the sister group to the latter. None of these nodes receives jackknife values above 50%. The data support monophyly of Protura and Collembola under all analytical parameters explored and with 100% jackknife support each (Fig. 2), but few higher-level clades are stable to parameter variation. Relationships of ectognaths based solely on the molecular data are at odds with morphology in not finding monophyly of either Pterygota or Dicondylia. Jackknife support for the orders Diplura, Archaeognatha and Zygentoma is low (59% for Archaeognatha; <50% for the other orders), while support is considerably higher for the pterygote orders Ephemeroptera (89%), Odonata (97%), and Dermaptera (96%), although this could be an artefact of lesser sampling.

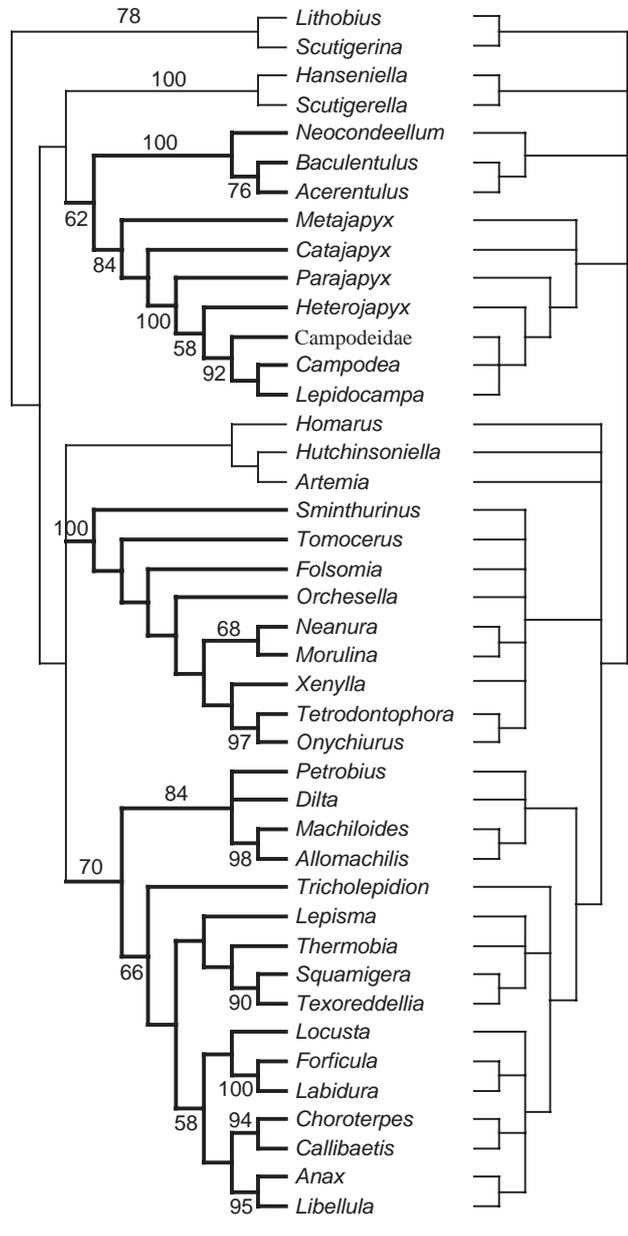
The combined analysis of all the molecular and morphological evidence (Figs. 3 and 4) still shows

polyphyly of hexapods under all analytical conditions, and recovers three main hexapod clades. One clade unites Protura + Diplura (jackknife of 62%), another contains all the collembolan species (jackknife 100%), and a third consists of the ectognathous hexapods (jackknife 70%). Resolution within Ectognatha is (Archaeognatha (*Tricholepidion* (Zygentoma s.s. + Pterygota))) under the optimal parameter set, but *Tricholepidion* and Zygentoma s.s. form a clade under most other parameter sets (Fig. 4). While Dicondylia receives a jackknife support value of 66%, support for Dicondylia to the exclusion of *Tricholepidion* is below 50%. Diplura appear monophyletic under all analytical conditions (jackknife of 84%), and in all cases, Campodeidae appear nested within a paraphyletic Japygoidea, with a clade containing *Parajapyx*, *Heterojapyx* and Campodeidae being found under all analytical conditions as well as in 100% jackknife replicates.



**Fig. 2.** Cladograms based on combined molecular data for five markers. Cladogram at left is single shortest cladogram for parameter set 121, which minimizes incongruence between the six data partitions (five genes and morphology). Cladogram at right is strict consensus of all 12 explored parameter sets. Jackknife frequencies shown for clades present in more than 50% of jackknife replicates. Hexapoda shown with bold branches.

While monophyly of Pterygota is stable to parameter variation, its jackknife support is low (58%) and its internal relationships are shown to be parameter-dependant. In this analysis, when rooted with Chilopoda, all analytical parameters find Crustacea, Collembola and Ectognatha forming a monophyletic group, thus rendering Hexapoda non-monophyletic, although jackknife support for this clade is below 50%.

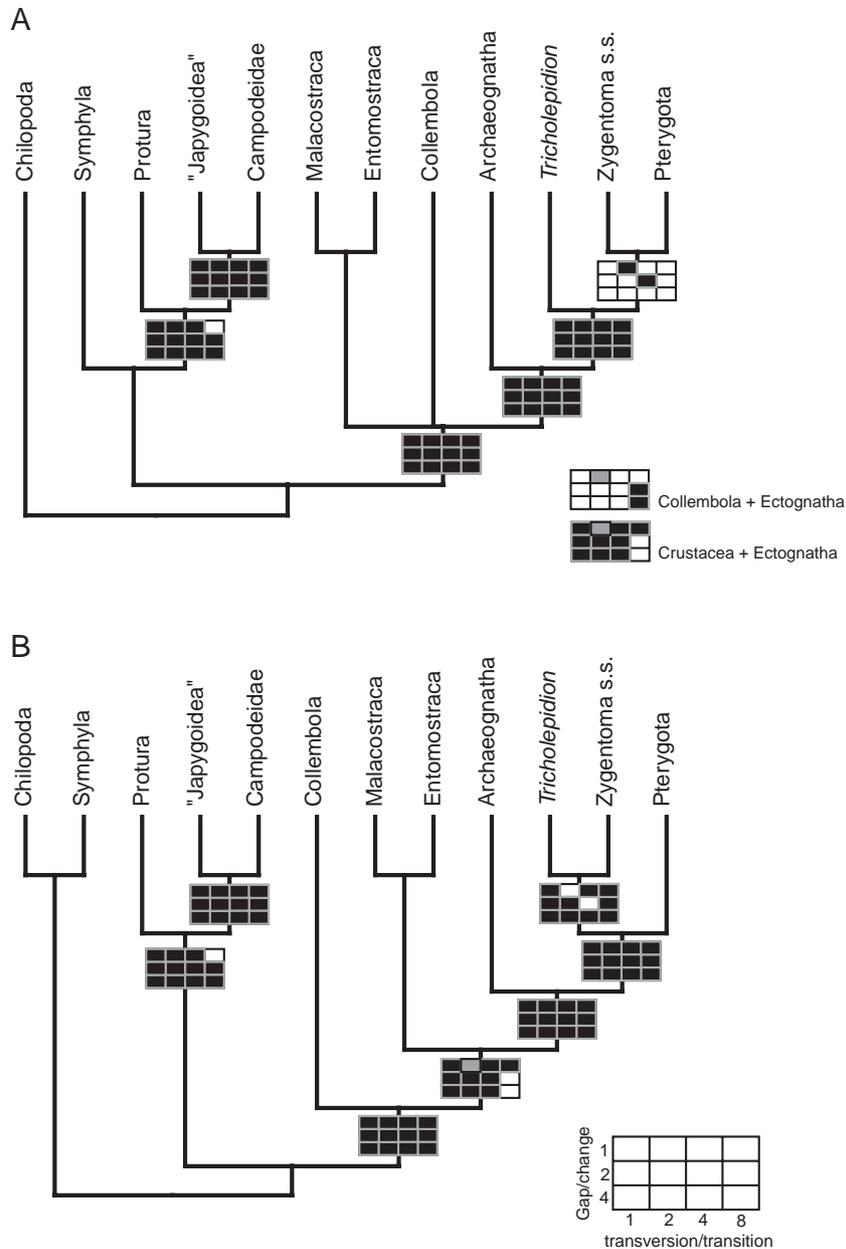


**Fig. 3.** Cladograms based on combined molecular data for five markers and morphological data in Appendix B. Cladogram at left is consensus of 4 shortest cladograms for parameter set 121, which minimizes incongruence between the six data partitions. Cladogram at right is strict consensus of all cladograms for 12 explored parameter sets. Jackknife frequencies shown for clades present in more than 50% of jackknife replicates. Hexapoda shown with bold branches.

## Discussion

### Hexapod monophyly

Monophyly of hexapods has been largely recognized by morphologists (Hennig 1969; Kristensen 1991; Kraus and Kraus 1994; Carpenter and Wheeler 1999; Bitsch and Bitsch 2000; Edgecombe et al. 2000; Koch 2000;



**Fig. 4.** Summary cladograms showing most parsimonious topologies for combined analysis of molecular and morphological data for parameter set 121 (A) and immediately suboptimal parameter set 111 (B). Monophyly of clades in 12 explored parameter sets is indicated (black square = monophyly; gray square = monophyly in some of a set of equally parsimonious resolutions; white square = non-monophyly). Cladogram A shows groups in two equally parsimonious resolutions of Crustacea + Collembola + Ectognatha.

Beutel and Gorb 2001; Giribet et al. 2001; Wheeler et al. 2001), although molecular biologists have had trouble finding data to support this hypothesis (but see Mallatt et al. 2004). Recent analyses with dense character and taxon sampling including several lineages of 'basal' hexapods have suggested hexapod monophyly (Carpenter and Wheeler 1999; Wheeler et al. 2001), although unusually autapomorphic sequences can yield patterns incongruent with morphology (Giribet et al. 2001; Nardi

et al. 2003). In the present analyses, monophyly of Hexapoda is found only for morphological data in isolation (Fig. 1), because the molecular and combined analyses nest crustaceans and symphylans within the Hexapoda when chilopods are specified as outgroups. The non-monophyly of hexapods is certainly shocking from a morphological perspective. In addition to the unique thoracic tagmosis of hexapods (ch. 64; see Appendix A), other apomorphic characters of

Hexapoda (Fig. 1) include the two corneagenous cells being primary pigment cells (ch. 5), the labium (ch. 24), the lack of *Distal-less* expression in the mandible (ch. 32), six-segmented locomotory limbs (chs. 86, 91), and provisional dorsal closure of the embryo (ch. 179). Other characters optimized as hexapod apomorphies in Fig. 1 are less straightforward in their interpretation, notably when they involve ‘losses’ of characters otherwise unique to crustaceans (median eyes fused to naupliar eyes, ch. 8) or myriapods (transverse bar fused to the posterior process of the tentorium, ch. 44). The fulcrum of hexapods appear to be unique, sharing a common position in a groove between the arthrodial membrane of the maxilla and the labium (Koch 2003). The tendency of molecular data to suggest hexapod polyphyly should be taken with caution, because it often relies on depauperate taxonomic sampling within the basal hexapods or for the outgroups. Given diversity and disparity of Crustacea, the number of crustacean terminals included in an analysis may influence the status of Hexapoda. While we have made the effort of sampling the basal hexapod lineages intensely and have employed sequence data for five loci, some of the ‘odd’ relationships are unstable to parameter variation and receive low jackknife support.

### Entognathous hexapod relationships

Among the unorthodox relationships well corroborated by our data is a relationship between Protura and Diplura (see also Carapelli et al. 2000; Giribet and Ribera 2000; Giribet and Wheeler 2001; D’Haese 2002b; Luan et al. 2003), and therefore a rejection of the Ellipura hypothesis. The clade composed of Protura and Diplura consistently falls outside of a clade uniting Crustacea with the Collembola and Ectognatha. While most analyses suggest Collembola nesting outside of the Crustacea–Ectognatha clade (as also found by Nardi et al. 2003, and Negrisolo et al. 2004), the alternative result suggesting monophyly of Collembola + Ectognatha is found in some of the resolutions under the optimal parameter set, as well as under two other analytical parameters (Fig. 4). A major difference between our analysis and that of Nardi et al. (2003) is the dense sampling within the Collembola, but yet our analyses do not permit unambiguous choice between the two hypotheses. Morphological support for a clade uniting Collembola and Ectognatha is restricted to the presence of ocelli (character 7), although this state is absent in Lepismatidae, Nicoletiidae and the fossil *Lepidotrix*. Broader sampling that includes Crustacea calls the polarity of this character into question, since many crustaceans possess homologues of ocelli (the naupliar eyes), whereas the absence of median eyes in Myriapoda may be an autapomorphic loss in that group (Ax 1999).

Monophyly of Ellipura, and to a lesser degree Entognatha, are supported by most morphological and combined morphological–molecular analyses published so far (Carpenter and Wheeler 1999; Edgecombe et al. 2000; Wheeler et al. 2001), but none have previously employed multiple representatives of all the entognathous orders. Relationships obtained here for the entognathous hexapods are mostly driven by the molecular partitions.

The monophyly of Diplura has been contested by some morphologists (Stys and Bilinski 1990; Stys et al. 1993), although more recent studies endorse the monophyly of the group (Koch 1997; Kristensen 1998), as do the data here presented (morphological, molecular and combined analyses). Morphologically, dipluran monophyly is supported by the admentum in the head capsule (ch. 23), the interlocking of the superlinguae and galea (ch. 25), reduction of the mandibular condylus and ligament (ch. 26), terminal mandibular teeth and a shovel-like pars incisivus (ch. 29), a rotating pivot joint in the ‘knee’ of the locomotory limbs (ch. 91), trochanteral femur-twisting muscles (ch. 101), and rotation of the labial Anlagen (ch. 182). Monophyly of the Campodeidae is also obtained, although the molecular data are not consistent with the monophyly of Japygoidea, and no data have been evaluated for the other dipluran groups, such as Procampodeidae, Anajapygidae, and Projapygidae. Including these families into future analyses should aid in reconstructing the phylogeny of the monophyletic Diplura.

### Ectognath relationships

The relationships among the orders of true insects (Archaeognatha + Dicondylia) are well resolved and stable. Ectognatha, Dicondylia, and Pterygota are monophyletic under all analytical conditions for the combined data (Figs. 3 and 4). *Tricholepidion* appears as sister group of Zygentoma s.s. + Pterygota in the most congruent parameter set, but the jackknife support value of the node uniting Zygentoma (excluding *Tricholepidion*) and Pterygota shows a frequency below 50%. Ten of 12 explored parameter sets support the alternative resolution of *Tricholepidion* as sister group to Zygentoma (Fig. 4). A sister group relationship between *Tricholepidion* and Zygentoma + Pterygota was raised as a possibility (Kristensen 1981) based on the retention of the intergnathal connective ligament in *Tricholepidion*. The presence of this ligament in *Tricholepidion* but not in Zygentoma was confirmed by Kristensen (1998) and Staniczek (2000), who argued for its plesiomorphic condition.

The relationships among the three pterygote lineages are unsettled due to limited taxon sampling. Although most parameter sets (including the most congruent

parameter set: Fig. 3) support monophyly of the Palaeoptera, others suggest Ephemeroptera as the most basal pterygote order. Most phylogenetic analyses of hexapod relationships have suggested paraphyly of the paleopteran orders, with Odonata as the sister group to Neoptera (Hennig 1953; Kristensen 1975; Whiting et al. 1997; Carpenter and Wheeler 1999; Beutel and Gorb 2001; Wheeler et al. 2001), although others support the monophyly of Palaeoptera (= Ephemeroptera + Odonata) (Hennig 1969; Kukalová-Peck 1983, 1991, 1992, 1998; Rasnitsyn 1998; Whiting 1998; Willmann 1998). A sister group relationship between Ephemeroptera and Neoptera has also been proposed based on the mode of sperm transfer from gonopore to gonopore (Boudreaux 1979a), and it is best supported by a recent combined analysis of morphology and molecules (Ogden and Whiting 2003). We refer readers to the latter article for a more detailed discussion of the Palaeoptera controversy.

## Concluding remarks

Relationships among apterygote hexapods have been long debated in the morphological arena, and the addition of new sources of characters, in this case molecular, can contribute to resolution of some conflicting issues. For example, the monophyly of the Diplura has been a contentious issue based on morphology (Stys and Bilinski 1990; Stys and Zrzavy 1994; Koch 1997), while molecular data generally support its monophyly (Carapelli et al. 2000; Giribet and Ribera 2000; Wheeler et al. 2001; Luan et al. 2003). Other issues, such as the status of Ellipura and Entognatha have received wide attention, both from morphologists and molecular systematists. However, the position of the enigmatic *Tricholepidion* has only received dedicated attention from a morphological standpoint (Boudreaux 1979a, b; Kristensen 1998; Bitsch and Bitsch 2000; Beutel and Gorb 2001). Although some molecular-based analyses have employed *Tricholepidion* as a terminal taxon (Edgecombe et al. 2000; Giribet et al. 2001; Nardi et al. 2003), the scope of those studies was higher arthropod relationships, and sampling within hexapods was far from optimal for attempting to resolve the position of this enigmatic species. However, the position of *Tricholepidion* remains in conflict after this study because none of the sets of data show substantial support for either of the two competing hypotheses (monophyly or paraphyly of Zygentoma). What seems clear, though, is that a sister group relationship of *Tricholepidion* to Nicoletiidae, as suggested by Wygodzinsky (1961), is not supported by morphology or molecules. Addition of the family Maindroniidae to our study might prove important to settle the issues concerning the position of *Tricholepidion*.

The most salient result of our analyses is a grouping of Protura + Diplura to the exclusion of Collembola. A proturan/dipluran clade has never been considered by morphologists but it has molecular support and is stable to parameter variation in the combined analysis of morphological and molecular data (Fig. 4). This result has been obtained in some molecular analyses employing ribosomal sequence data (e.g., Giribet and Ribera 2000; Giribet and Wheeler 2001; D'Haese 2002b), in Luan et al. (2003) even with high bootstrap support values. However, no morphological character optimizes unambiguously at the base of this node under the topologies shown in Fig. 4, and the group conflicts with a well-supported morphological grouping of Diplura and Ectognatha (Fig. 1). Finally, the position of Collembola, which has received special attention in recent publications (Nardi et al. 2003; Delsuc et al. 2003) requires caution. Our results conflict in placing Collembola ambiguously with the Ectognatha, or outside a clade of Crustacea + Ectognatha in our optimal tree (Fig. 4A), with most parameter sets favoring the latter hypothesis. The resolution of Collembola outside a Crustacean–Ectognath clade has been found in other molecular analyses (Nardi et al. 2003; Negrisolo et al. 2004). These results will need further testing using new data, especially of more comprehensively sampled crustacean diversity and including a broader representation of ectognathous hexapods. At this point the question of hexapod monophyly and the relationships among the basal hexapod lineages may require an even more expanded analysis covering a much larger diversity within the other mandibulate groups. Adding information from the complete large ribosomal subunit (Mallatt et al. 2004), and from more conserved loci, especially myosin heavy chain II (Ruiz-Trillo et al. 2002) and other protein-coding genes, may prove useful to resolve these questions.

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## Appendix A

Characters coded in the morphological data matrix. Characters 6, 25, 27, 42, 91 and 180 are additive.

1. Compound eye (Müller et al. 2003): (0) Developed; (1) Dispersed (including myriapod stemmata); (2) Absent.
2. Compound eye medial margins (Kraus 1998: ch. 29): (0) Not medially contiguous; (1) Medially contiguous.
3. Compound eye stalked, basally articulated: (0) Eye sessile; (1) Eye stalked.
4. Number of reticular cells (Paulus 1979): (0) Many; (1) Eight.
5. Primary pigment cells (Paulus 1979): (0) Corneagenous cells not primary pigment cells; (1) Corneagenous cells are two primary pigment cells.
6. Chiasmata in optic lobe (Strausfeld 1998): (0) No chiasmata (*Scutigera*: Strausfeld in Klass and Kristensen 2001); (1) Chiasma between lamina ganglionaris and medulla; (2) Two chiasmata, between lamina ganglionaris and medulla/between medulla and lobula.
7. Ocelli (Bitsch and Bitsch 2000: ch. 8): (0) Present (median eyes, including naupliar eyes); (1) Absent.
8. Median eyes fused to naupliar eyes (Lauterbach 1983): (0) Absent; (1) Present.
9. Antennal segmentation: (0) Multisegmented; (1) Reduced to a few segments; (2) Unjointed; (3) Antenna absent.
10. Antennal musculature: (0) Antennal articles individually muscled; (1) Annulated antenna lacking independently muscled articles.
11. Johnston's organ in antennal pedicel: (0) Absent; (1) Present.
12. Sensory organ of third antennal segment (D'Haese 2003: ch. 10): (0) Without papillae; (1) Hidden by well-developed papillae.
13. Postantennal organs (Bitsch and Bitsch 1998: ch. 5): (0) Present (Bellonci's organ possibly homologous: Klass and Kristensen 2001); (1) Absent.
14. Antennal vessels (Pass 1991; Bitsch and Bitsch 1998: ch. 18): (0) Pair connected to aorta; (1) Pair separate from aorta; (2) Absent.
15. Antennal ampullae indirectly compressed by pharynx movements (Pass 2000): (0) Absent; (1) Present.
16. Antennal pulsatile organs (Pass 2000): (0) Absent; (1) Pulsatile organ with fronto-pharyngeal compressor; (2) Pulsatile organ with ampullo-pharyngeal, ampullo-ampullary and ampullo-aortic dilators.
17. Head orientation (Bitsch and Bitsch 2000: ch. 3): (0) Hypognathous; (1) Prognathous; (2) Mouth directed backwards.
18. Scales: (0) Absent; (1) Present; (2) Absent on antennae.
19. Frontal line (Bitsch and Bitsch 2000: ch. 4): (0) Inverted, Y-shaped line; (1) Reduced to coronal suture; (2) Weak to absent.
20. Clypeofrontal sulcus (Bitsch and Bitsch 2000: ch. 5): (0) Absent; (1) Present.
21. Entognathy: (0) Mouthparts not retracted into pocket in head capsule; (1) Hypertrophy of pleural folds, maxillae and mandibles in separate gnathal pouches; (2) Pleural folds almost fused ventrally, maxillae and mandibles in single gnathal pouch.
22. Linea ventralis (Koch 1997; Kraus 1998: ch. 18): (0) Absent; (1) Present.
23. Admentum differentiated ventrally between prementum and oral fold on each side of head capsule (Koch 1997), developing from posterior part of mouth fold (Ikeda and Machida 1998): (0) Absent; (1) Present.
24. Labium (second maxilla bordering mouth: Kraus and Kraus 1994): (0) Second maxillae separate; (1) Fused; (2) Basally obliterated.
25. Superlinguae: (0) Absent; (1) Present; (2) Interlocking with galea (Koch 1997; Kristensen 1998).
26. Mandibular condylus and connection with head (Koch 1997): (0) Basic condylus and ligament connecting base of mandibles with lateral wall of head capsule; (1) Basic condylus and mandibular ligament reduced.
27. Mandibular articulation (Fürst von Lieven 2000; Staniczek 2000): (0) Monocondylic; (1) Dicondylic; (2) Ball-and-socket articulation between clypeal condyle and mandibular ridges.
28. Mandibular form (Kristensen 1981): (0) Broad; (1) Stylettiform.
29. Mandibular teeth (Koch 2001): (0) Lateral; (1) Terminal, forming shovel-like pars incisivus.
30. Mandible with large, stalked molar process and tapering incisor process (Koch 2001): (0) Absent; (1) Present.
31. Mandibular prosthema (Bitsch and Bitsch 2000: ch. 14): (0) Absent; (1) Present (lacinia mobilis not homologous: Richter et al. 2002).
32. *Distal-less* expression in mandible (Scholtz et al. 1998): (0) Transient expression at least; (1) No expression at any stage.
33. Maxillary palpi: (0) Smaller than thoracic leg (Crustacea: maxillary endopodal palp smaller than thoracic endopods); (1) Enlarged; (2) Reduced or vestigial; (3) Absent.
34. Lacinia (Kristensen 1981): (0) Broad; (1) Slender rod.
35. Labial (second maxillary) endites: (0) Single lobe; (1) Bilobed (Kristensen 1998: ch. 40); (2) Several lobate endites on second maxillary protopod.
36. Apical segment of labial (second maxillary) palp (Kristensen 1998: ch. 60): (0) Narrow; (1) Widened.
37. Labial (second maxillary) palp (Bitsch and Bitsch 2000: ch. 18): (0) 3 segments; (1) 4 segments;

- (2) Short, 1-segmented, or replaced by row of short setae; (3) Setigerous plate; (4) Absent; (5) 5 segments; (6) Endopod of 6 segments and terminal spine; (7) Endopodal palp with 2 segments.
38. Larval labium (second maxilla): (0) Not prehensile; (1) Prehensile.
39. Opening of ectodermally derived salivary glands (Bitsch and Bitsch 1998: ch. 8): (0) Pair, at base of second maxillae; (1) Unpaired, median.
40. Position of median salivary gland opening (Bitsch and Bitsch 1998: ch. 8): (0) Midventral groove of labium; (1) Salivarium.
41. Anterior tentorium: (0) Absent (Diplura coded following Bitsch and Bitsch 2002); (1) Ectodermal invaginations (Folsom's arms homologous: Bitsch and Bitsch 2002); (2) Fulcro-tentorium.
42. Anterior tentorial apodemes (Koch 2000): (0) Separate rod-like apodemes; (1) Anterior part of tentorial apodemes forms arched, hollow plates that approach each other mesially but remain separate; (2) Anterior tentorium an unpaired roof.
43. Posterior suspension of anterior apodemes to cranial wall (Koch 2000): (0) Absent; (1) Present.
44. Posterior process of tentorium fused anteriorly with hypopharyngeal bar and transverse bar (Bitsch and Bitsch 2002; Koch 2003): (0) Transverse bar absent; (1) Transverse bar present.
45. Posterior tentorial apodemes (Bitsch and Bitsch 2002): (0) Absent; (1) Present as metatentorium.
46. Corporotentorium: (0) Absent; (1) Present.
47. Fulturae or hypopharyngeal suspensorium (Bitsch and Bitsch 2000: ch. 19): (0) Present; (1) Absent.
48. Anterior dorsal muscle forming gnathal lobe flexor (Kluge 1999; Koch 2001): (0) Dorsal remotor forms cranial adductor; (1) Dorsal promotor (anterior dorsal muscle) forms gnathal lobe flexor.
49. Origin of ventral tentorial adductors (Staniczek 2000): (0) On intergnathal connective lamina; (1) On anterior tentorial arm (intergnathal connective lamina absent).
50. Mandibulo-hypopharyngeal muscle (Staniczek 2000): (0) Absent; (1) Present.
51. Tentorio-mandibular muscles (Kristensen 1975; Beutel and Gorb 2001: ch. 29): (0) Several bundles; (1) One thin bundle or absent.
52. Transverse stipital muscle (Kristensen 1981): (0) Present; (1) Absent.
53. Tentorio-lacinal muscle (Kristensen 1975): (0) Present; (1) Absent.
54. Protocerebrum (Bitsch and Bitsch 1998: ch. 1): (0) Two hemispheres; (1) Two dorsal lobes and two posterior lobes.
55. Deutocerebrum (Bitsch and Bitsch 1998: ch. 2): (0) Well-developed lobes, receiving antennary nerves; (1) Small lobes, without antennary nerves.
56. Tritocerebral commissures (Bitsch and Bitsch 1998: ch. 3): (0) One or two free commissures; (1) Commissure in subesophageal ganglion.
57. Epipharyngeal ganglia (François 1969; Kristensen 1991): (0) Small; (1) Enlarged.
58. Pseudocommissure of stomatogastric nervous system: (0) Without entognathous position; (1) With entognathous position.
59. Circumesophageal ring (Gereben-Krenn and Pass 1999): (0) Present; (1) Absent.
60. Cephalic excretory organs (Bitsch and Bitsch 1998: ch. 13): (0) One or two pairs of complete nephridial organs; (1) Pair of labial kidneys; (2) Absent.
61. Postoccipital ridge (Kristensen 1975; Staniczek 2000): (0) Absent; (1) Lateral; (2) Continuous.
62. Two cervical sclerites (Kristensen 1981): (0) Absent; (1) Present.
63. Lateral cervical sclerite in three pieces: (0) Absent; (1) Present.
64. Position of ambulatory legs: (0) Postcephalic segments 1–3; (1) Postcephalic segments 2–16; (2) Postcephalic segments 1–12; (3) Postcephalic segments 5–8 (second to fifth pereopods).
65. Discoid pronotum (Kristensen 1981): (0) Absent; (1) Present.
66. Pronotum overlapping propleuron: (0) Absent; (1) Present.
67. Prothorax tergum (D'Haese 2003: ch. 71): (0) Developed; (1) Reduced, without setae.
68. Trunk heterotery (Ax 1999): (0) Absent; (1) Present (long tergites on pedigerous segments 1, 3, 5, 7, 8, 10, 12 and 14).
69. Musculus scutello-postnotalis III (Beutel and Gorb 2001: ch. 59): (0) Present; (1) Absent.
70. Pleural sutures (Boudreaux 1979a): (0) Absent; (1) Not slanting forward; (2) Slanting forward.
71. Pteropleura tilted backward, notum small: (0) No; (1) Yes.
72. Trochantin (Kristensen 1981): (0) Absent; (1) Present; (2) Trochantin episternal sulcus; (3) Absent in meso- and metathorax.
73. Separate coxopleuron (Kristensen 1975): (0) Yes; (1) No.
74. Sternal part of the trunk (thorax) (Bitsch and Bitsch 2000: ch. 23): (0) Each segment includes a large sternum; (1) Sternal area divided into 2 hemisternites; (2) Mostly membranous, showing only a pair of small sclerites; (3) Y-shaped ridge on sternal plates; (4) Sternites extended rearward to form substernal laminae.
75. Mesothoracic basisternal fold (Kristensen 1975): (0) Absent; (1) Present.
76. Two pairs of wings: (0) Absent; (1) Present.
77. Wing flexion: (0) Absent; (1) Present.
78. Forewings truncate: (0) No; (1) Yes.

79. Sclerotization of forewing (Kristensen 1981): (0) Membranous; (1) Sclerotized.
80. Prominent precostal field: (0) Absent; (1) Present.
81. Basal wing brace (Kukalová-Peck 1985): (0) Absent; (1) Present.
82. Forewing media fusion: (0) Absent; (1) MA + MP; (2) MA + R.
83. Anal furrow (Hennig 1969): (0) Absent; (1) Present.
84. Enlarged hindwing vannus (Kristensen 1981): (0) Small; (1) Enlarged.
85. Pleating of vannus (Kristensen 1981): (0) Absent; (1) Present.
86. Locomotory limbs: (0) 7-segmented; (1) 6-segmented; (2) Tibiotarsus.
87. Coxa-body articulation: (0) Sternal; (1) None (arthrodial membrane); (2) Dicondylic; (3) Pleural.
88. Mobility of pleural articulation of coxa: (0) Mobile; (1) Immobile.
89. Legs I: (0) Locomotory appendages; (1) Maxillipede housing poison gland; (2) Sensory; (3) First maxillipede resembling (second) maxilla.
90. Male forelegs clasping: (0) Absent; (1) Present.
91. Jointed “knee”: (0) Absent; (1) Present; (2) Rotating pivot (Manton 1972; Kristensen 1998: ch. 14).
92. Femoro-tibial articulation: (0) Moncondylic; (1) Dicondylic.
93. Tarsi: (0) Not subdivided; (1) 3-segmented; (2) 5-segmented; (3) 2-segmented.
94. Tibiotarsal setae of T row (D’Haese 2003: ch. 88): (0) Absent; (1) Present.
95. Tibiotarsal M setae (D’Haese 2003: ch. 89): (0) Absent; (1) Present.
96. Ratio tibiotarsus:claw (D’Haese 2003: ch. 94): (0) Much more than 2; (1) Between 1 and 2.
97. Pretarsus (Beutel and Gorb 2001): (0) Retained as a small separate sclerite and attached claws; (1) Separate sclerite reduced, transformed into planta and unguitactor plate.
98. Pretarsal claws (Hennig 1969, 1981; Kristensen 1975): (0) Two; (1) One.
99. Hairy adhesive soles of two proximal tarsomeres (Beutel and Gorb 2001): (0) Absent; (1) Present.
100. Claw pad, one claw transformed into thickened attachment organ (Beutel and Gorb 2001): (0) Absent; (1) Present.
101. Thoracic leg musculature (Manton 1972; Kristensen 1991): (0) Without trochanteral femur-twisting muscles; (1) With such muscles.
102. Two coxal proprioceptor organs (Lombardo 1973; Hennig 1981) (0) Absent; (1) Present.
103. Trunk endoskeleton in each segment (Bitsch and Bitsch 2000: ch. 25): (0) Pair of lateral connective plates; (1) Pair of sternocoxal rods; (2) Complex connective endoskeleton; (3) With cuticular spiniae on connective endoskeleton; (4) Endoskeleton mainly cuticular.
104. Metaspina (Kristensen 1975): (0) Present; (1) Absent.
105. Pleural apodeme: (0) Not strongly developed in meso- and metathorax; (1) Strongly developed.
106. Pterothoracic muscles (Kristensen 1975): (0) Present; (1) Absent.
107. Indirect wing muscles (Boudreaux 1979a): (0) Developed; (1) Reduced.
108. Metathoracic tergosternal wing elevators: (0) Present; (1) Weak.
109. Metathoracic and abdominal sternum I fused: (0) Free; (1) Fused.
110. Spiracles (Bitsch and Bitsch 2000: ch. 44): (0) 6 or 7 pairs of trunk spiracles on segments with long tergites (post-maxillipede segments 3, 5, 8, 10, 12 and 14); (1) One pair, on side of head; (2) 3 pairs, on thorax only; (3) Up to 4 pairs on thorax and 7 pairs on abdomen; (4) 2 pairs of thoracic spiracles.
111. Direct spiracular musculature (Whiting et al. 1997: ch. 25): (0) Absent; (1) Present.
112. Posterior tracheation of leg (Kristensen 1975): (0) Absent; (1) Present.
113. Abdominal segmentation: (0) Abdomen of 11 segments + telson (= 12); (1) 11 segments; (2) 10 segments; (3) 6 segments.
114. Ventral tube: (0) Absent; (1) Present.
115. Shape of ventral tube (D’Haese 2003: ch. 104): (0) Cylindrical, elongate (optical section circular), longer than wide; (1) Not prominent (optical section oval), wider than long.
116. Furcula: (0) Absent; (1) Present.
117. Pseudocelli (D’Haese 2003: ch. 124 + 125): (0) Absent; (1) Present.
118. Pseudopores (Deharveng 1978; D’Haese 2003: ch. 130): (0) Absent; (1) Present.
119. Paired larval gills: (0) Absent; (1) Present, lateral.
120. Posterior tracheal larval gills: (0) Absent; (1) Present.
121. Eversible vesicles (Bitsch and Bitsch 2000: ch. 34): (0) Coxal vesicles on more than 5 abdominal segments; (1) One pair of coxal vesicles on < 5 abdominal segments; (2) Vesicles at distal part of first abdominal appendage (leglet or colophore) (Klass and Kristensen 2001); (3) Absent.
122. Sixth abdominal segment (D’Haese 2003: ch. 122): (0) Simple; (1) Bilobate.
123. Pregenital abdominal styli: (0) Proximal to coxae; (1) Reduced styli; (2) Absent.
124. Abdominal sterna (Kristensen 1998: ch. 67): (0) Large; (1) Reduced.
125. Female SVII (Hennig 1981): (0) Small; (1) Large.
126. Gonopore: (0) Near back of body, on segment XVII; (1) On sternite of trunk segment IV; (2) On

- abdominal segment XI; (3) On abdominal segment V; (4) Between abdominal segments VIII and IX; (5) Abdominal segment VIII (female), X (male); (6) On sixth pereion segment (= abdominal segment III of Hexapoda); (7) On twelfth pereion segment (= abdominal segment IX of Hexapoda); (8) On sixth pereion segment in female, eighth pereion segment in male.
127. Paired female genital openings (Kristensen 1975; Boudreaux 1979a): (0) Absent; (1) Present.
128. Anal valves (D’Haese 2003: ch. 117): (0) Subequal, not overlapping; (1) Ventral paired valves overlapping dorsal unpaired valve.
129. Cerci: (0) Absent; (1) Present.
130. Cerci forcipate, unisegmented, strongly sclerotized: (0) Absent; (1) Present.
131. Paired, articulated furcal rami: (0) Absent; (1) Present.
132. Paired terminal spinnerets (Kraus 1998: ch. 49): (0) Absent; (1) Present.
133. Caudal filament: (0) Absent; (1) Present, long; (2) Short.
134. Male median caudal filament: (0) Without sensory cones; (1) With sensory cones.
135. Ovipositor: (0) Absent; (1) Present; (2) Vestigial; (3) Third valvulae forming sheath.
136. Gonangulum in ovipositor base (Hennig 1969): (0) Absent; (1) Present.
137. Female gonopod, used to manipulate single eggs (Ax 1999): (0) Absent; (1) Present.
138. Male gonopods or gonocoxites (Bitsch and Bitsch 2000: ch. 39): (0) Pair of gonopods on trunk segment XVII; (1) Gonocoxites; (2) Absent.
139. Position of gonocoxites: (0) Fused into single segment borne by segment XI; (1) Two pairs on segments VIII and IX or only on IX; (2) Fused into subgenital plate.
140. Male styli: (0) Expressed; (1) Suppressed.
141. Male styli IX: (0) Not claspers; (1) Claspers.
142. Male parameres (Bitsch and Bitsch 2000: ch. 42): (0) Absent; (1) Pair of lateral plates on segment IX; (2) Sclerites included in phallic complex.
143. Male penes (Bitsch and Bitsch 2000: ch. 43): (0) Absent; (1) Paired penes on segment XI; (2) Paired penes on segment IX; (3) Median penis.
144. Male accessory copulatory organs: (0) Absent; (1) Present.
145. Dorsal vessel ostia (Bitsch and Bitsch 1998: ch. 17): (0) Dorsal vessel elongated, with 7–15 pairs of ostia; (1) Dorsal vessel with 3–6 pairs of ostia; (2) Short heart in posterior part of cephalothorax, with few pairs of ostia.
146. Open-ended chambers enclosing paired excurrent ostia: (0) Absent; (1) Present.
147. Dorsal vessel: (0) Rear end open, bidirectional flow; (1) Rear end closed.
148. Ventral vessel (Bitsch and Bitsch 1998: ch. 17): (0) Supraneural; (1) Subneural; (2) Absent.
149. Digestion: (0) Extracellular; (1) Intracellular (*Homarus*: Barker and Gibson 1977).
150. Crop (Bitsch and Bitsch 1998: ch. 6): (0) Absent; (1) Differentiated, posterior in esophagus.
151. Proventriculus (Bitsch and Bitsch 1998: ch. 7): (0) Absent; (1) Present.
152. Proventricular neck, before anterior enlarged portion: (0) Absent; (1) Present.
153. Gastric caeca (Bitsch and Bitsch 1998: ch. 9): (0) Absent; (1) Present.
154. Peritrophic membrane (Bitsch and Bitsch 1998: ch. 10): (0) Present; (1) Absent.
155. Pyloric region (Bitsch and Bitsch 1998: ch. 11): (0) Short, simple sphincter or valvula; (1) Sphincter followed by pyloric chamber; (2) Short, with ring of flattened cells.
156. Proctodeum (Bitsch and Bitsch 1998: ch. 12): (0) Lacking large posterior dilation; (1) Having rectal ampulla with differentiated papillae.
157. Malpighian tubules (Bitsch and Bitsch 1998: ch. 14): (0) Elongate tubules; (1) Short papillae; (2) Absent.
158. Ventral nerve cord (Bitsch and Bitsch 1998: ch. 4): (0) Long chain; (1) 3 pairs thoracic ganglia, 4–6 pairs abdominal; (2) Several ganglia fused together; (3) All abdominal ganglia fused with metathoracic.
159. Tendinous struts: (0) Absent; (1) Present, anchoring cuticle.
160. Tracheal system (Bitsch and Bitsch 1998: ch. 15): (0) Tracheae not interconnected; (1) Tracheae with transverse and longitudinal connections; (2) Absent.
161. Ovary (Bitsch and Bitsch 1998: ch. 20): (0) Elongated sacs; (1) Ovarioles.
162. Arrangement of ovarioles (Bitsch and Bitsch 1998: ch. 20): (0) Pectinate; (1) Grouped, inserted into lateral oviduct.
163. Location of ovary germarium (Bitsch and Bitsch 1998: ch. 21): (0) In ovarian wall; (1) In apical part of each egg tube.
164. Ovary (Bitsch and Bitsch 1998: ch. 22): (0) Follicles connected with ovarian epithelium; (1) Panoistic; (2) Meroistic-polytrophic.
165. Seminal receptacle or spermatheca (Bitsch and Bitsch 1998: ch. 23): (0) Dorsal; (1) Lateral at base of ovipositor; (2) Seminal receptacle on each side of head; (3) Absent.
166. Testis (Bitsch and Bitsch 1998: ch. 24): (0) Elongate sacs, sometimes fused; (1) Several follicles.
167. Arrangement of testicular follicles (Bitsch and Bitsch 1998: ch. 24): (0) Pectinate; (1) Grouped.

168. Location of testicular germ tissue (Bitsch and Bitsch 1998: ch. 25): (0) Ventral or laterodorsal; (1) Apical.
169. Form of spermatozoa (Bitsch and Bitsch 1998: ch. 26): (0) Elongate, flagellate, motile; (1) Encysted, motile at fecundation; (2) Immotile, aflagellate.
170. Spiral ridge on nucleus of sperm (Dohle 1985): (0) Absent; (1) Present.
171. Supernumary axonemal tubules (peripheral singlets): (0) Absent; (1) Present.
172. Protofilaments in wall of accessory tubules of axoneme (Dallai and Afzelius 1999, Jamieson et al. 1999): (0) 13; (1) 16.
173. Medial microtubules in axoneme (Jamieson et al. 1999): (0) Present; (1) Absent.
174. Sperm axoneme sheaths (Jamieson et al. 1999): (0) Absent; (1) Present.
175. Sperm centriole adjunct (Jamieson et al. 1999): (0) Present; (1) Absent; (2) Crystalline accessory bodies developed from the centriole.
176. Pair of elongate, cristate mitochondrial derivatives in sperm (Jamieson et al. 1999): (0) Absent; (1) Present.
177. Sperm conjugation (Kristensen 1998: ch. 62): (0) Absent; (1) Spermatozoa paired.
178. Egg cleavage (Bitsch and Bitsch 1998: ch. 30): (0) Total and equal; (1) Early total cleavage followed by superficial; (2) Superficial cleavage.
179. Dorsal closure of embryo (Machida and Ando 1998): (0) Definitive dorsal closure (dorsal covering of embryo participates in the definitive dorsal closure); (1) Provisional dorsal closure (embryonic dorsal covering degenerates without participating in definitive closure, which is exclusively derived from the embryo).
180. Amniotic cavity (Machida and Ando 1998): (0) Absent; (1) Present, open; (2) Present, closed (amnioserosal fold fuses beneath the embryo).
181. Egg tooth on embryonic cuticle of second maxilla (Dohle 1985): (0) Absent; (1) Present.
182. Rotation of labial Anlagen (Ikeda and Machida 1998): (0) Absent; (1) Present.
183. Postembryonic development: (0) Anamorphosis; (1) Epimorphosis.
184. Subimago (Kristensen 1975): (0) Present; (1) Absent.
185. Imaginal lifespan: (0) Normal, feeding; (1) Shortened, nonfeeding.
186. Habitat of nymphs: (0) Terrestrial; (1) Aquatic.
187. Female broodcare of eggs and first one or two free-living nymphs: (0) Absent; (1) Present.
188. Jumping by abdominal push: (0) Absent; (1) Present.
189. Insemination mode (Bitsch and Bitsch 1998: ch. 29): (0) Indirect, via stalked spermatophore; (1) Indirect, via droplets; (2) Indirect, via spermatophore in web; (3) Indirect, via spermatolophids; (4) Indirect, claspers; (5) Copulation.

## Appendix B

Morphological data matrix; “A” represents a 0, 1 polymorphism; “-” represents inapplicable states; “?” represents unknown states.

	1	11	21	31	41	51	61	71	81	91
<i>Hutchinsoniella</i>	2-----1-00	0-????2020	00000??000	0?0-2060--	0---0010-?	-?-00????0	000----0?0	-0-0-0----	-----?1-00	0??-----?-
<i>Artemia</i>	001100012-	0-?2-020--	00000??0-0	002-----0--	0---0010-?	-?-0000010	000----0?0	-0-?-0----	-----?1-00	0??-----?-
<i>Homarus</i>	0011020101	0-?0-020??	00000??000	000-2070--	0---0010-?	-?-0000010	0003---0?0	-0-0-0----	-----??-30	0??-----?-
<i>Scutigera</i>	0000001-01	0-00-01021	0000000000	0?0--0500-	1001000100	0000000010	0001---1?0	-000-0----	-----00-10	003---0100
<i>Lithobius</i>	10000?1-00	0-00-01021	0000000000	000--0100-	1001000100	0000000000	0001---1?0	-000-0----	-----00-10	003---0100
<i>Scutigera</i>	2-----1-00	0-00-01000	0001100000	1?3-0-40--	1001000100	0000000000	0002---0?0	-002-0----	-----00-00	000---0000
<i>Hanseniella</i>	2-----1-00	0-00-01000	0001100000	1?3-0-40--	1001000100	0000000000	0002---0?0	-002-0----	-----00-00	000---0000
<i>Acerentulus</i>	2-----1-3-	--0---1020	21021001-0	0?01002010	20?0000000	0001111112	00000000?0	-00300----	-----12-20	1000000100
<i>Neocondeellum</i>	2-----1-3-	--0---1020	21021001-0	0?01002010	20?0000000	0001111112	00000000?0	-00300----	-----12-20	1000000100
<i>Baculentulus</i>	2-----1-3-	--0---1020	21021001-0	0?01002010	20?0000000	0001111112	00000000?0	-00300----	-----12-20	1000000100
<i>Xenylla</i>	1001100010	0002--1000	2102100000	0?200-4010	?00?000000	1000001100	00000000?0	-10100----	-----21-00	1001110100
<i>Onychiurus</i>	1001100010	0102--1000	2102100000	0?200-4010	?00?000000	1000001100	00000000?0	-10100----	-----21-00	1001110100
<i>Tetrodontophora</i>	1001100010	0102--1000	2102100000	01200-4010	?00?000000	1000001100	00000000?0	-10100----	-----21-00	1001?10100
<i>Neanura</i>	1001100010	0002--1000	21021001-0	0?200-4010	?00?000000	1000001100	00000000?0	-10100----	-----21-00	1001110100
<i>Morulina</i>	1001100010	0002--1000	21021001-0	0?200-4010	?00?000000	1000001100	00000000?0	-10100----	-----21-00	1001?10100
<i>Orchesella</i>	1001100010	0002--1000	2102100000	0?200-4010	1000000000	1000001100	00000010?0	-10100----	-----21-00	1000000100
<i>Folsomia</i>	1001100010	0002--1000	2102100000	0?200-4010	1000000000	1000001100	00000010?0	-10100----	-----21-00	1000000100
<i>Tomocerus</i>	1001100010	0002--1100	2102100000	01200-4010	1000000000	1000001100	00000010?0	-10100----	-----21-00	1000000100
<i>Sminthurinus</i>	1001100010	000?--00?0	2102100000	0?200-4010	?00?000000	100000110?	00000010?0	-10100----	-----21-00	1000000100
<i>Campodeidae</i>	2-----1-00	0-10-01000	1011210010	1?20003010	0---000000	0000000000	00000000?0	-10300----	-----10-00	200---0000
<i>Campodea</i>	2-----1-00	0-10-01000	1011210010	1?20003010	0---000000	0000000000	00000000?0	-10300----	-----10-00	200---0000
<i>Lepidocampa</i>	2-----1-00	0-10-01000	1011210010	1?20003010	0---000000	0000000000	00000000?0	-10300----	-----10-00	200---0000
<i>Catajapyx</i>	2-----1-00	0-10-01010	1011210010	0?20002010	0---000000	0000010000	00000000?0	-10300----	-----10-00	200---0000
<i>Metajapyx</i>	2-----1-00	0-10-01010	1011210010	0?20002010	0---000000	0000010000	00000000?0	-10300----	-----10-00	200---0000
<i>Heterojapyx</i>	2-----1-00	0-10-01010	1011210010	0?20002010	0---000000	0000010000	00000000?0	-10300----	-----10-00	200---0000
<i>Parajapyx</i>	2-----1-00	0-10-01010	1011210010	0?20002010	0---000000	0000010000	00000000?0	-10300----	-----10-00	200---0000
<i>Dilta</i>	0101100001	1-11000120	0001100001	0?10100011	1110100000	0000000000	1000000000	-30000----	-----13000	111---1000
<i>Petrobius</i>	0101100001	1-11000120	0001100001	0?10100011	1110100000	0000000000	1000000000	-30000----	-----13000	111---1000
<i>Machiloides</i>	010???0001	1-1???0220	0001100001	0?10100011	1110100000	00000000?0	1000000000	-30000----	-----13000	111---1000
<i>Allomachilis</i>	010???0001	1-1???0220	0001100001	0?10100011	1110100000	00000000?0	1000000000	-30000----	-----13000	111---1000
<i>Tricholepidion</i>	000?10001	1-1???0001	0001001000	0?00011011	1210101000	0010000??1	20000000?0	-?0000----	-----13000	112---1000
<i>Lepisma</i>	0001111-01	1-11000101	0001001000	0100011011	1210101011	0010000001	2000000000	-10400----	-----13000	111---1000
<i>Thermobia</i>	0001111-01	1-11000101	0001001000	0100011011	1210101011	0010000001	2000000000	-10400----	-----13000	111---1000
<i>Texoreddellia</i>	2-----1-01	1-1???0101	0001001000	0?00011011	1210101011	00100000?1	2000000000	-10000----	-----13000	111---1000
<i>Squamigera</i>	2-----1-01	1-1???0101	0001001000	0?00011011	1210101011	00100000?1	2000000000	-10000----	-----13000	111---1000
<i>Callibaetis</i>	000?120001	1-12--0001	0001101000	1?00000011	1210111011	0100000012	2000000011	0000010000	1100013101	112---1001
<i>Choroterpes</i>	000?120001	1-12--0001	0001101000	1?00000011	1210111011	0100000012	2000000011	0000010000	1100013101	112---1001
<i>Anax</i>	0001120001	1-11100001	0001002000	A?00000111	1210111011	1110000012	2010000011	1000010000	1100013100	111---1000
<i>Libellula</i>	0001120001	1-11100001	0001002000	A?00000111	1210111011	1110000012	2010000011	1000010000	1100013100	111---1000
<i>Forficula</i>	000???1-01	1-11011001	0001002000	0?00000011	1210111011	1110000012	21001000A2	0210111110	0211113100	111---1010
<i>Labidura</i>	000???1-01	1-11011001	0001002000	0?00000011	1210111011	1110000012	21001000A2	0210111110	0211113100	111---1010
<i>Locusta</i>	0001120001	1-11020001	0001002000	0100000011	1210111011	1110000012	2100010001	0110011011	0111113100	112---1000

## Appendix B (continued)

	101	111	121	131	141	151	161	171	181
<i>Hutchinsoniella</i>	00??-0-0	--0-0000-3-20061-0-	100-0-02--	-000??-0	0-10??2?02	0-1??0-?20	0-1??0-?20	----100???	?-0?0-00?
<i>Artemia</i>	00??-0-0	--0-0000-3-2?071-0-	100-0-02--	-0000012?0	0-10002002	0-0??0-?20	0-0??0-?20	----1000?0	0-000-005
<i>Homarus</i>	00??-0-0	--0-0000-3-20081-0-	000-0-02--	-00020?11?	1?10002?02	0-0??0-?20	0-0??0-?20	----1000?0	0-000-00?
<i>Scutigera</i>	0000-0-0	00-0-00000-3-20000-0-	000-0-10--	-000001000	0-00000000	0-0000-001	0-0000-001	0-000002?0	1-0000001
<i>Lithobius</i>	0000-0-0	00-0-00000-3-20000-0-	000-0-10--	-000001000	0-00000000	0-0000-001	0-0000-001	0-00000200	1-0000002
<i>Scutigera</i>	0010-0-01	00-0-00000-0-00010-0-	010-0-02--	-0000?00?	?000?0000	0-0020-?00	0-0000-?00	0-00000000	000000000
<i>Hanseniella</i>	0010-0-01	00-0-00000-0-00010-0-	010-0-02--	-0000?00?	?000?0000	0-0020-?00	0-0000-?00	0-00000000	000000000
<i>Acerentulus</i>	00200-0-0	--0-00000-2010020?0-	000-0-010-	-110001210	0-01101202	0-1130-110	0-1130-110	0-10000???	?0000000?
<i>Neocentulellum</i>	00200-0-0	--0-00000-2010020?0-	000-0-010-	-110001210	0-01101202	0-1130-1??	0-1130-1??	??????????	?0000000?
<i>Baculentulus</i>	00200-0-0	--0-00000-2010020?0-	000-0-010-	-110001210	0-01101202	0-1130-1??	0-1130-1??	??????????	?0000000?
<i>Xenylla</i>	00200-0-0	--31110000-202003010-	000-0-02--	-000101200	0-00202312	0-0230-010	0-0230-010	0-00110010	001000000
<i>Onychiurus</i>	00200-0-0	--31110000-202003000-	000-0-02--	-000101200	0-00202312	0-0230-010	0-0230-010	0-00110010	001000000
<i>Tetradontophora</i>	00200-0-0	--31110000-202003000-	000-0-02--	-000101200	0-00202312	0-0230-010	0-0230-010	0-00110010	001000000
<i>Neanura</i>	00200-0-0	--31110000-212003010-	000-0-02--	-000101200	0-?0202312	0-0230-010	0-0230-010	0-00110010	001000000
<i>Morulina</i>	00200-0-0	--31110000-212003010-	000-0-02--	-000101200	0-?0202312	0-0230-010	0-0230-010	0-00110010	001000000
<i>Orchesella</i>	00200-0-0	--31010100-202003000-	000-0-02--	-000101200	0-00202?12	0-0230-010	0-0230-010	0-00110010	001000000
<i>Folsomia</i>	00200-0-0	--31010100-202003000-	000-0-02--	-000101200	0-00202312	0-0230-0??	0-0230-0??	??????????	001000000
<i>Tomocerus</i>	00200-0-0	--31010100-202003000-	000-0-02--	-000101200	0-00202312	0-0230-0??	0-0230-0??	??????????	001000000
<i>Sminthurinus</i>	00200-0-0	--31010000-202003000-	000-0-02--	-000101200	0-0-0?2?23?2	0-0230-110	0-0230-110	0-00110010	001000000
<i>Campodeidae</i>	10200-0-02	0020-00000-0-10040-10	000-0-02--	-000000200	0-00011000	0-1230-1??	0-1230-1??	??????????	?01000000
<i>Campodea</i>	10200-0-02	0020-00000-0-10040-10	000-0-02--	-000000200	0-00011000	0-1230-100	0-1230-100	1000110210	011000000
<i>Lepidocampa</i>	10200-0-02	0020-00000-0-10040-10	000-0-02--	-000000200	0-00011000	0-1230-1??	0-1230-1??	????????210	011000000
<i>Catajapyx</i>	10300-0-03	0020-00000-0-10040-11	000-0-011-	-000000200	0-0?0?2001	101130-100	10001102??	01100000?	01100000?
<i>Metajapyx</i>	10300-0-03	0020-00000-0-10040-11	000-0-011-	-000000200	0-0?0?2001	101130-100	10001102??	01100000?	01100000?
<i>Heterojapyx</i>	10300-0-03	0020-00000-0-10040-11	000-0-011-	-000000200	0-0?0?2001	101130-1??	101130-1??	??????????	??100000?
<i>Parajapyx</i>	10300-0-03	0020-00000-0-10040-11	000-0-011-	-000000200	0-0?0?2001	101130-1??	101130-1??	??????????	??100000?
<i>Dilta</i>	00201-0-04	0010-00000-0-10050-10	001000110	0230000200	0-10010000	101110100	1100210111	001000011	001000011
<i>Petrobius</i>	00201-0-04	0010-00000-0-10050-10	001000110	0230000200	0-10010000	101110100	1100210111	001000011	001000011
<i>Machiloidea</i>	00201-0-04	0010-00000-0-11050-10	0010100110	0030000200	0-10010000	101110100	101110100	??????????	??1000010
<i>Allomachilis</i>	00201-0-04	0010-00000-0-11050-10	0010100110	0030000200	0-10010000	101110100	101110100	??????????	??1000010
<i>Tricholepidion</i>	00200-0-04	1010-00000-0-10050-10	0011101110	0230000201	0-??010001	1111311100	1100211???	??1000002	??1000002
<i>Lepisma</i>	00200-0-04	1010-00000-1-11050-10	0010101110	0230000201	1010010001	1111311100	1100211212	001000002	001000002
<i>Thermobia</i>	00200-0-04	1010-00000-1-11050-10	0010101110	0230000201	10?0010001	1111311100	1100211212	001000002	001000002
<i>Texoredellia</i>	00200-0-04	1010-00000-0-11050-10	0011101110	0230000201	0-?0010001	111131110?	111131110?	??????????	??1000003
<i>Squamigera</i>	00200-0-04	1010-00000-0-11050-10	0011101110	0230000201	0-?0010001	111131110?	111131110?	??????????	??1000003
<i>Callibaetis</i>	014000004	0020-00010-3-20051-10	00100-0120	1220000201	1010010101	1111011100	1010200212	001011005	001011005
<i>Choroterpes</i>	014000004	0020-00010-3-20051-10	00100-0120	1220000201	1010010101	1111011100	1010200212	001011005	001011005
<i>Anax</i>	0140010004	1120-00001-3-20050-10	0020110120	0231001201	1010010101	1111011100	1100210212	001101004	001101004
<i>Libellula</i>	0140010004	1120-00001-3-20050-10	0020110120	0231001201	1010010101	1111011100	1100210212	001101004	001101004
<i>Forficula</i>	014101114	1120-00000-3-20150-11	000-2-0121	-200001201	1010001201	1111011100	1101210212	001100105	001100105
<i>Labidura</i>	014101114	1120-00000-3-20150-11	000-2-0121	-200001201	1010001201	1111011100	1101210212	001100105	001100105
<i>Locusta</i>	0141010004	1110-00000-3-20050-10	000-310120	02330011201	1110010101	1111011100	1100210212	001100005	001100005

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