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The Identification of Concerted Convergence in Insect Heads Corroborates Palaeoptera

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Abstract.—The relationships of the 3 major clades of winged insects—Ephemeroptera, Odonata, and Neoptera—are still unclear. Many morphologists favor a clade Metapterygota (Odonata+Neoptera), but Chiastomyaria (Ephemeroptera+Neoptera) or Palaeoptera (Ephemeroptera+Odonata) has also been supported in some older and more recent studies. A possible explanation for the difficulties in resolving these relationships is concerted convergence—the convergent evolution of entire character complexes under the same or similar selective pressures. In this study, we analyze possible instances of this phenomenon in the context of head structures of Ephemeroptera, Odonata, and Neoptera. We apply a recently introduced formal approach to detect the occurrence of concerted convergence. We found that characters of the tentorium and mandibles in particular, but also some other head structures, have apparently not evolved independently, and thus can cause artifacts in tree reconstruction. Our subsequent analyses, which exclude character sets that may be affected by concerted convergence, corroborate the Palaeoptera concept. We show that the analysis of homoplasy and its influence on tree inference can be formally improved with important consequences for the identification of incompatibilities between data sets. Our results suggest that modified weighting (or exclusion of characters) in cases of formally identified correlated cliques of characters may improve morphology-based tree reconstruction. [Character clique; convergent evolution; Chiastomyaria; Ephemeroptera; homoplasy; Metapterygota; morphology; mouthparts; Odonata; phylogeny.]

Within winged insects (Pterygota), systematists distinguish 3 major clades: Ephemeroptera (mayflies), Odonata (damselflies and dragonflies), and Neoptera (all remaining winged insects; Fig. 1). The monophyly of each of the 3 groups is generally accepted and supported by rich sets of morphological and molecular data (Rehn 2003; Carapelli et al. 2006; Klass 2009; Ogden et al. 2009; Simon et al. 2009; Meusemann et al. 2010). The relationships, however, are still unresolved (Kristensen 1981; Klass 2009). All 3 possible topologies have been proposed: (a) Palaeoptera (Ephemeroptera plus Odonata; Fig. 1a) has been advocated based on characters of the wing venation and articulation (Hennig 1969; Brauckmann and Zessin 1989; Kukalová-Peck 1997, 2008; Bechly et al. 2001; Haas and Kukalová-Peck 2001; Wheeler et al. 2001; Hovmöller et al. 2002; Soldán 2003; Willkommen and Hörnschemeyer 2007); (b) Metapterygota (Odonata plus Neoptera; Fig. 1b) is supported by characters of the mandibles and tracheal system and also by molecular data (Kristensen 1981; Staniczek 2000, 2001; Wheeler et al. 2001; Ogden and Whiting 2003; Terry and Whiting 2005; Beutel and Gorb 2006; Pass et al. 2006); and (c) Chiastomyaria (Ephemeroptera plus Neoptera; Fig. 1c) is supported by the presumably apomorphic mode of direct sperm transfer, the pterothoracic locomotor system dominated by indirect flight muscles, and molecular analyses based on rRNA genes and expressed sequence tag (EST) data (Matsuda 1970; Carle 1982; Kjer 2004; Mallatt and Giribet 2006; Simon et al. 2009).

Why is the Reconstruction of the Early Evolution of Winged Insects such a Challenge?

The sister group of Pterygota is Zygentoma (the silverfish) and both groups together form a clade Dicondylia (Fig. 1a–c). Because silverfish are primarily wingless, homology assessments of thoracic skeletal elements and muscles related to flight are problematic, and consequently character polarization within the early pterygote lineages is ambiguous. This also applies to sperm transfer, which changed from an indirect external mode (Zygentoma and Archaeognatha) to a direct transfer through an intromittent organ (Ephemeroptera and Neoptera). Odonata evolved a secondary copulatory apparatus at abdominal segments II and III and exhibit a unique form of "indirect" sperm transfer completely different from the condition in all other insects. Again, robust homology hypotheses and character polarizations covering winged and wingless groups are impossible (Witte and Doring 1999), even though more data became available in recent years (Klass 2008; Matushkina 2008a, 2008b; Dallai et al. 2011). Due to this situation, most of the aforementioned arguments for either Chiastomyaria or Palaeoptera are affected by unclear homology assessments and character polarization.

In contrast, the Metapterygota hypothesis is supported by mandibular characters with widely accepted homology and polarity assessment (Staniczek 2001). Nevertheless, it has been shown that characters of the

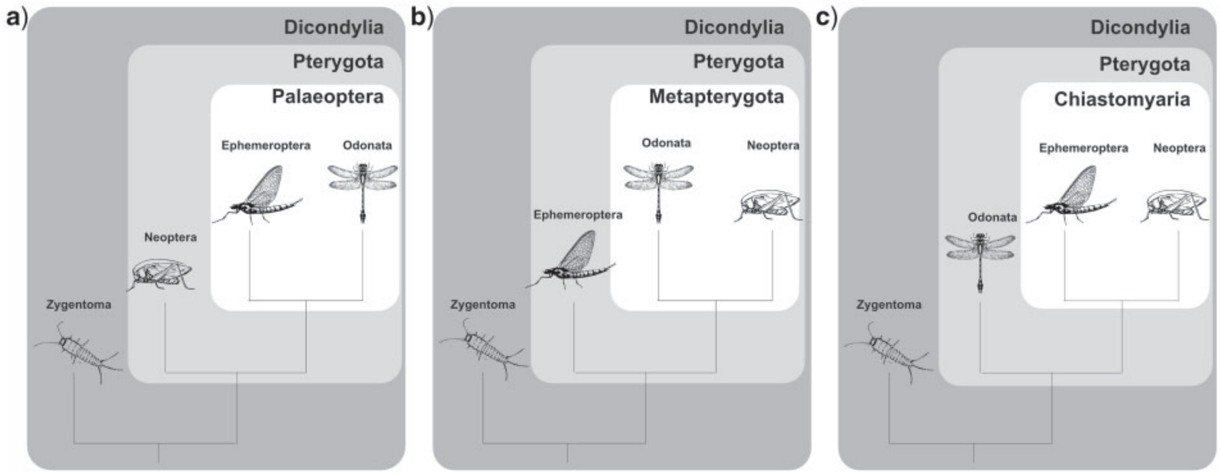

FIGURE 1. The most frequently encountered hypotheses concerning relationships of Ephemeroptera, Odonata, and Neoptera. a) Palaeoptera (Odonata+Ephemeroptera); b) Metapterygota (Odonata+Neoptera); and c) Chiastomyaria (Ephemeroptera+Neoptera).

entire head including all mouthparts and the head capsule do not support this hypothesis (Blanke et al. 2012). It turns out that formerly proposed presumptive synapomorphies in the literature (loss of certain head muscles and sutures) are in fact not groundplan features of Odonata, and data from the literature on seemingly well-known and important taxa such as Zygentoma are ambiguous. Examples are the conflicting statements of Chaudonneret (1950) and Staniczek (2000) regarding the presence of a subgena in *Thermobia* (Zygentoma) which is generally considered an important structure in the context of the evolution of the mandibular articulation.

Dealing with Homoplasy

Phylogenetic hypotheses based on morphological and molecular characters frequently contradict each other (Giribet et al. 2001; Giribet and Edgecombe 2012; Trautwein et al. 2012) although remarkable congruence has been reached in many areas (Wiegmann et al. 2009; Beutel et al. 2011). Consequently, the robustness of tree reconstruction techniques and the signal strength of molecular data and morphological characters become essential aspects of phylogenetic analyses (Wägele and Mayer 2007; Letsch et al. 2010). Basically, causes for high statistical support despite incongruence between results have to be investigated.

One potential reason for extensive morphological homoplasy among lineages is a phenomenon called concerted convergence (Patterson and Givnish 2002). This describes a process in which several traits, for instance, the character complexes "wings," "mouthparts," or "genitalia," are exposed to the same shared set of environmental conditions or functional requirements. In each of these cases, a given selective pressure might influence the whole character system such that the evolution of many individual characters occurs in a "concerted" manner. In phylogenetic analyses, this can result in an artificially increased number of presumptive synapomorphies, which are in fact not independent, and consequently in clades with unjustified support.

As a solution to this problem, Holland et al. (2010) proposed to identify groups—or cliques—of characters evolving in a concerted manner. Applying permutation tests of character compatibility (Fig. 2), Holland et al. (2010) were able to detect cliques of mutually compatible characters in water birds and demonstrated the impact of this phenomenon on phylogenetic inference.

In this study, we analyze the possible homoplasy of cephalic characters and concerted convergence obscuring the earliest divergences within Pterygota. We show that a cephalic character state matrix used to reconstruct the early evolution of winged insects contains considerable evidence of concerted convergence, which negatively affects the results of phylogenetic analyses. We address whether (i) character groups show concerted convergence and (ii) how these characters influence tree inference.

DATA

Due to the inherent problems of homology and polarity of thoracic and abdominal characters, the data assembled here are exclusively based on features of the head. The taxon sampling covers Archaeognatha, Zygentoma, Ephemeroptera, Odonata, and 12 orders of Neoptera including all major polyneopteran clades (Table 1). The matrix is composed of a total of 139 characters including 19 characters of the head capsule, 6 labral characters, 22 antennal characters, 13 tentorial characters, 13 mandibular characters, 17 maxillary characters, 33 labial characters, and 16 characters of the hypopharynx, salivarium, and fore gut. A character discussion is presented in Blanke et al. (2012). Although the focus of this study is the Palaeoptera problem it was necessary to include a wide taxon sampling of Neoptera as well. As it is currently impossible

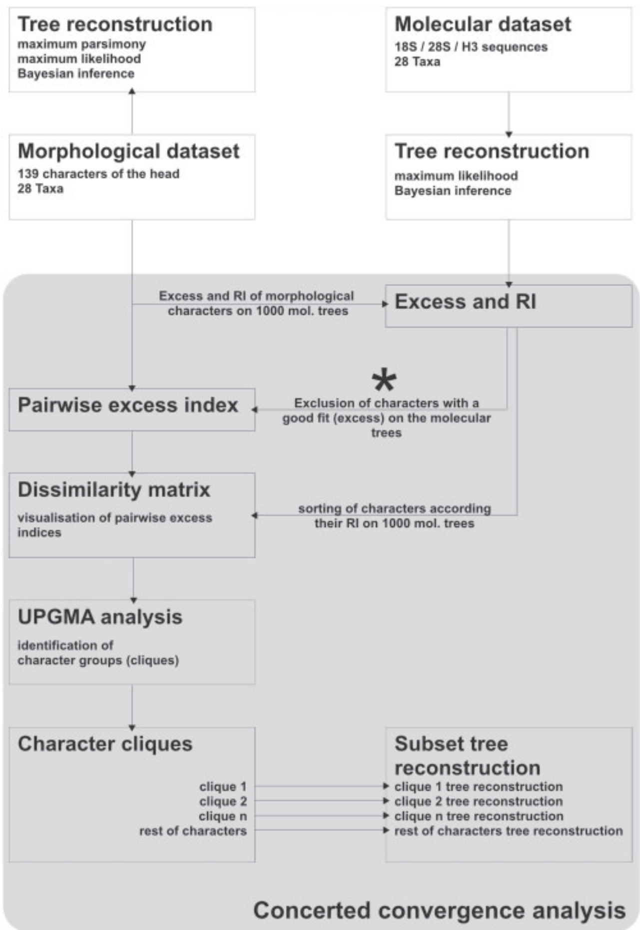

FIGURE 2. Principal workflow of the analysis to identify concerted convergence conducted by Holland et al. (2010), which is adapted herein. The asterisk indicates the analysis step of Holland et al. (2010) that is not followed in this contribution. Note that initial tree reconstructions are independent from the concerted convergence analysis and have therefore no effect on clique generation. For further explanations see text.

to define a cephalic groundplan for Neoptera, the homology hypotheses implied by the present character matrix have been carefully evaluated across a wide range of neopteran taxa. Moreover, the reliability of our concerted convergence analysis partly depends on the relationships within Neoptera. Additionally, we compiled a molecular data set with a corresponding taxon selection in which we used 18S and 28S rRNA genes and sequences of the protein-coding gene Histone H3 (Table 1). All sequences were downloaded from NCBI Genbank. Taxa were only included if represented by at least 2 genes. We considered 18S sequences with at least 1700 base pairs (bp), 28S sequences with at least 1400 bp, and complete or nearly complete sequences of Histone H3. If sequence data of a certain taxon were not publicly available or did not match our selection criteria, we chose sequences of a species of a different genus but within the same insect order (Table 1).

Definitions

Several terms related to the analytical steps proposed by Holland et al. (2010) are frequently used throughout this article. These are briefly defined as follows:

> Clique: a set of mutually pairwise compatible characters.
>
> Compatible: characters are compatible if they can be displayed on the same tree without homoplastic changes. Note that pairwise compatibility guarantees overall compatibility of a set of characters for 2-state characters but not for multistate characters.
>
> Concerted convergence: the convergent evolution of groups of characters.
>
> Dissimilarity: a measure, $d(i,j)$, of the difference between 2 objects i and j, that is symmetric, that is, $d(i,j)=d(j,i)$, and non-negative, that is, $d(i,j)\geqslant 0$, and where $d(x,x)=0$.
>
> Excess index: the extra number of character changes required to explain a character on a given tree above the minimum number possible (the number of character states -1).
>
> Pairwise excess index (Holland et al. 2010): the dissimilarity between 2 characters i and j is defined as the difference between the parsimony score of the most parsimonious tree constructed using only that pair of characters and the minimum possible parsimony scores for i and j. Thus, the index is equal to $P-m_i-m_j$, where P is the parsimony score for the most parsimonious tree for the alignment containing characters i and j and m_i and m_j are the minimum possible parsimony score for characters i and j, respectively. A pair of compatible characters has a dissimilarity of 0.
>
> Parsimony score: the sum of implied character changes along a given tree topology.
>
> Retention index: defined as $(M-s)/(M-m)$, where M and m are, respectively, the maximum and the minimum possible parsimony scores, and s the actual parsimony score of the character on the tree.

Alignment Procedure

18S and 28S rRNA sequences were aligned separately with RNAsalsa software (Stocsits et al. 2009). The prealignment for RNAsalsa was conducted with the E-INS-i algorithm of MAFFT, using default settings (Katoh et al. 2002, 2005). As structure constraints, we employed the nuclear 18S and 28S structure models of *Anopheles albimanus* and *Apis mellifera*, respectively, both retrieved from the European Ribosomal Database. The stringency settings for adoption of secondary structures in different

TABLE 1. Taxa used for the morphological and molecular analysis including the respective data sources

Taxon	Species	Data source				Species
		Morphology	GenBank sequence no.			
			18S	28S	H3	
Archaeognatha	*Machilis germanica*	Blanke et al. (2012)	AY521826	AY521735	AY521695	*Machilis* sp.
Zygentoma	*Thermobia domestica*	Blanke et al. (2012)	AY338726	AY338683	AY338644	*Thermobia* sp.
Ephemeroptera	*Siphlonurus lacustris*	Blanke et al. (2012)	AY749863	AY749958	AY749718	*Siphlonurus* sp.
	Oniscigaster wakefieldi	Staniczek (2001)	AY749893	AY750016 + AY750017	GQ118330	*Oniscigaster distans*
	Heptagenia sulphurea	Blanke et al. (2012)	AY749837	AY749917	AY749699	*Epeorus longimanus*
	Ephemera danica	Blanke et al. (2012)	GQ118267	GQ118302	GQ118323	*Ephemera simulans*
Odonata	*Lestes virens*	Blanke et al. (2012)	EU055185	EU055283	EU055476	*Archilestes grandis*
	Onychogomphus forcipatus	Blanke et al. (2012)	EU055142	EU055237	EU055433	*Gomphus* sp.
	Epiophlebia superstes	Blanke et al. (2012)	EU055226	EU055324	EU055518	*Epiophlebia superstes*
Plecoptera	*Perla marginata*	Blanke et al. (2012)	EF622709	EF622866	EF622584	*Antarctoperla* sp.
	Nemoura cinerea	Moulins (1968)	EF622738	EF622894	GU066929	*Nemoura cinerea*
Grylloblattodea	*Grylloblatta campodeiformis*	Walker (1931)	DQ457275	DQ457312	DQ457377	*Grylloblatta chirurgica*
	Galloisiana yuasai	Wipfler et al. (2011)	DQ457282	DQ457319	DQ457384	*Galloisiana nipponensis*
Mantophasmatodea	*Karoophasma* sp.	Baum et al. (2007)	–	EU414719	GU066922	*Mantophasma zephyra*
	Austrophasma sp.	Wipfler et al. (2011)	AY521862	AY521791	AY521712	*Sclerophasma paresisensis*
Blattodea	*Periplaneta americana*	Wipfler et al. (2011)	EU253777	FJ806523	–	*Cryptocercus* sp.
Mantodea	*Hymenopus coronatus*	Wipfler et al. (2012)	AY491161	AY491220	AY491334	*Hymenopus coronatus*
Phasmatodea	*Timema christinae*	Tilgner et al. (1999)	AF423806	AY125302	AY125246	*Timema knulli*
	Agathemera crassa	Wipfler et al. (2011)	Z97561	AY125326	AY125269	*Agathemera crassa*
	Megacrania batesii	Friedemann et al. (2012)	AY121154	AY125294	AY125238	*Eurycantha insularis*
	Phyllium siccidifolium	Friedemann et al. (2012)	AY121161	AY125301	AY125245	*Phyllium bioculatum*
	Sipyloidea sipylus	Friedemann et al. (2012)	AY121181	AY125321	FJ474246	*Sipyloidea sipylus*
Embioptera	*Embia ramburi*	Rähle (1970)	AY338693	AY338650	AY338617	*Notoligotoma* sp.
Dermaptera	*Labidura riparia*	Kadam (1961)	Z97594	EU426876	GU066905	*Forficula auricularia*
Orthoptera	*Locusta migratoria*	Albrecht (1953)	AY121145	AY125285	AY125230	*Stenopelmatus fuscus*
Zoraptera	*Zorotypus hubbardi*	Beutel and Weide (2005)	AY521890	AY521823	AY521734	*Zorotypus hubbardi*
Psocoptera	*Stenopsocus stigmaticus*	Badonnel (1934)	AY630492	–	GU569338	*Stenopsocus nigricellus*
Hymenoptera	*Macroxyela* sp.	Beutel and Vilhelmsen (2007)	EF012907	EF013035	EF518794	*Pheidole clydei*

Note: Original data from Wipfler (2011), Wipfler (2012), Friedemann (2012), Blanke et al. (2012), Beutel and Weide (2005), and Beutel and Vilhelmsen (2007) was available to the authors.

alignment steps were relaxed (0.51), as we wanted to retain as much structure information as possible. Histone H3 was aligned with MAFFT choosing the G-INS-i algorithm (Katoh et al. 2005). Subsequent masking of the alignments was done with Aliscore v.0.2 (Misof and Misof 2009) which identifies putative ambiguously aligned regions in multiple sequence alignments using a sliding window approach. For gap treatment (g), window size (ws) and random pairwise comparisons (pc), the following settings were used: g = ambiguous characters, ws = 6 positions, pc = 4× number of taxa. Aliscore is currently not able to detect base pairings. In the case of 18S and 28S rRNA sequences, positions which are part of the consensus structure of the RNAsalsa alignments were considered as structurally conserved and were retained as paired positions in the data set. The complete molecular data set comprised 4258 sites, of which the 18S partition accounted for 1854 sites, the 28S partition for 2041 sites, and the Histone H3 partition for 363 sites.

Tree Reconstruction of the Morphological Data

The morphological data were analyzed using maximum parsimony, Bayesian inference, and maximum likelihood (ML).

Parsimony analyses and Bremer/bootstrap support calculations of the morphological data were performed with TNT (Goloboff et al. 2008) using 1000 heuristic searches starting with random addition of taxa (TBR branch swapping).

Bayesian inference of the morphological data was conducted using MrBayes v3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The MK model was applied, with among-character rate variation modeled with gamma-distributed rates across characters with 4 discrete rate categories. Priors were set adopting the default settings of MrBayes v3.2 (all state frequencies [change rates] set equal, all topologies with equal probabilities, unconstrained branch length). Two parallel analyses were run with random starting trees and 4 Metropolis coupled Markov chains (MCMC) for 1 000 000 generations. Every 100th generation was sampled to yield a posterior probability distribution of 10 000 trees. After discarding the first 1000 trees of each run as burn-in trees, a 50% majority-rule consensus tree was calculated from the sampled trees of both runs. Convergence diagnostics implemented in MrBayes, potential scale reduction factors (PSRFs), and average standard deviation of split frequencies were used as guidelines for assessing convergence. In the Bayesian analysis (BA), the average standard deviation of split frequencies had a final value of 0.0046 and the PSRF approached 1 for all parameters. The MKV model was applied in the ML analysis of the morphological data using RAxML v7.0.3 (Stamatakis 2006), with all model parameters estimated from the data, and rate heterogeneity across characters modeled using the gamma-model of Yang (1994) with 4 discrete categories. Support was estimated with 1000 bootstrap replicates with identical tree-search settings.

Tree Reconstruction Based on Molecular Data

For molecular tree inference, the concatenated data set was divided into 4 partitions: (1) 18S+28S loops, (2) 18S+28S stems, (3) first+second codon position of Histone H3, and (4) third codon position of Histone H3. The consensus structures of the RNAsalsa alignments were used to define paired and unpaired partitions of 18S and 28S, respectively. According to the results of the Akaike Information Criterion in MrModeltest v2.3 (Nylander 2004), the GTR+Γ+I model was selected as the best model of nucleotide substitution for partition (1)+(2)+(3). The GTR+Γ model was chosen for partition (4). Based on the selected models, a BA was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), using 2 parallel runs each with 4 simultaneous Markov chains (1 cold and 3 heated) for 10 000 000 generations. Trees were sampled every 100th generation. Excluding the first 25 000 trees of each run as burn-in, a 50% majority-rule consensus tree with posterior probabilities was constructed from the remaining 150 002 trees.

Tracer v1.4.1 (Rambaut and Drummond 2008) was used to determine the burn-in and to check convergence of parameter estimates by inspecting effective sample size (ESS) values and traces of the MCMC samples. The average standard deviation of split frequencies had a final value of 0.003, the PSRF approached 1 for all parameters, the ESS value of each parameter exceeded the recommended threshold of 200, and the traces of corresponding parameters in independent runs converged to the same optimum.

The ML analysis of the molecular data was conducted with RAxML v7.3.2 (Stamatakis 2006). The data set was partitioned into (1) 18S+28S loops, (2) 18S+28S stems, (3) first+second codon position of Histone H3, and (4) third codon position of Histone 3. The consensus structures of the RNAsalsa alignments were used to define paired and unpaired partitions. The GTR+Γ+I model was used for all 4 partitions. Node support for the best-scoring ML tree was evaluated with 1000 rapid bootstrap replicates (Stamatakis et al. 2008). ML analyses were computed on HPC Linux clusters at the Regionales Rechenzentrum Köln (RRZK) using Cologne High-Efficient Operating Platform for Science (CHEOPS). Support values are given in parentheses in the following order: (RaxML bootstrap value [BS]/Bayes posterior probability [PP]/Bremer support [BR]/parsimony bootstrap [PB]). As Bremer support values are still frequently shown in morphology-based phylogenetic studies, we decided to present them here despite of inherent problems pointed out by DeBry (2001). For the molecular tree, node support is given in the following order: BS/PP. "X" indicates no support.

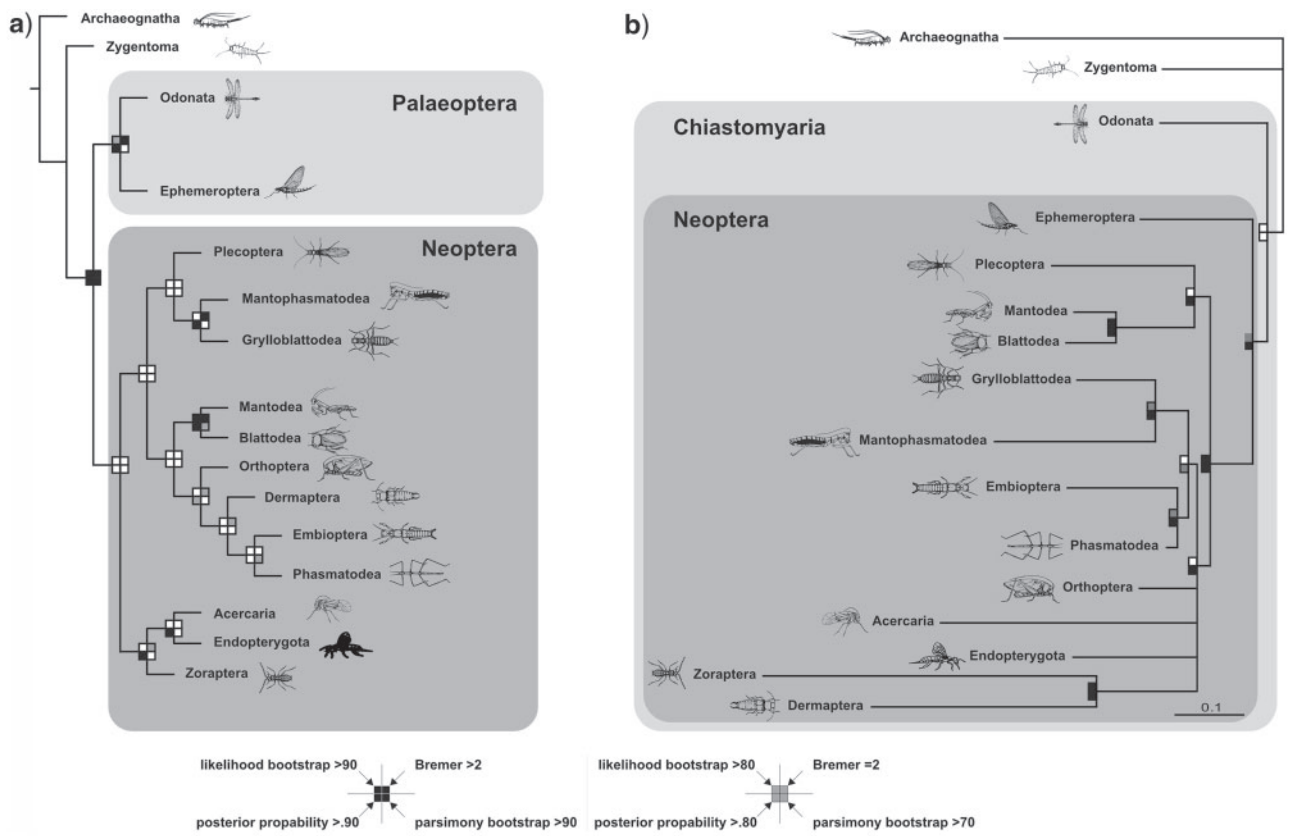

FIGURE 3. Tree inference from analysis of the morphological and molecular data. a) Consensus tree of the morphological data analyzed with Bayesian inference, ML, maximum parsimony, and parsimony bootstrapping. Tree topology derived from the parsimony analysis. b) Consensus tree from the analysis of the molecular data using Bayesian inference and ML (Bremer support and parsimony bootstrap were not calculated). Branch lengths and tree topology are derived from the Bayes analysis. White squares indicate support below the respective boundary values indicated below the trees. The underlying morphological data can be found at doi:10.5061/dryad.1q3b6 in online Supplementary Material 1. Detailed trees for each reconstruction method are available at doi:10.5061/dryad.1q3b6 in online Supplementary Material 2, and online Supplementary Material 3.

CONFLICT BETWEEN MOLECULAR DATA AND MORPHOLOGY

Morphological data (Fig. 3a) provide consistent support (BS .83/PP .94/BR 3/PB 59) for a clade Palaeoptera (Ephemeroptera+Odonata) whereas the molecular approach (Fig. 3b) partly yields Chiastomyaria (Ephemeroptera+Neoptera; BS X/PP .99). The monophyly of Neoptera is weakly supported in the morphology-based analysis of the cephalic data (BS 59/PP X/BR 1/PB 32), and the BA of the molecular data (BS X/PP .74). An obvious explanation is that the evolutionary diagnostic changes are thoracic and wing joint characters, which are not included in our data.

Some of the unorthodox results of the molecular analysis can be explained by the limited taxon sampling. However, for the specific analytical approach applied here, an identical or at least very similar taxon sampling was required. The purpose of the molecular analysis was not to provide a reliable tree of Neoptera, but to provide a reference tree for the earliest pterygote branching events. Focusing on the Palaeoptera problem, Chiastomyaria partly supported by molecular evidence is a hypothesis frequently encountered (Kjer 2004; Misof et al. 2007; Simon et al. 2009; von Reumont et al. 2009).

Identifying Morphological Characters with the Highest Incompatibility with the Molecular Results

In the workflow of Holland et al.'s (2010) analysis (Fig. 2), morphological characters are identified that agree least with the molecular trees by calculating their excess indices. These characters are further analyzed by calculating their pairwise excess indices. The basic idea of this formalized approach is to subsequently identify cliques of characters that are more compatible with each other than to either the molecular or the morphological trees. If this is the case, at least some of these cliques may represent instances of concerted convergence and thus violate the assumption of character independence. The inclusion or treatment of these characters in tree reconstruction then has to be reconsidered.

As a starting hypothesis, we assume that Chiastomyaria are a natural clade. Based on the

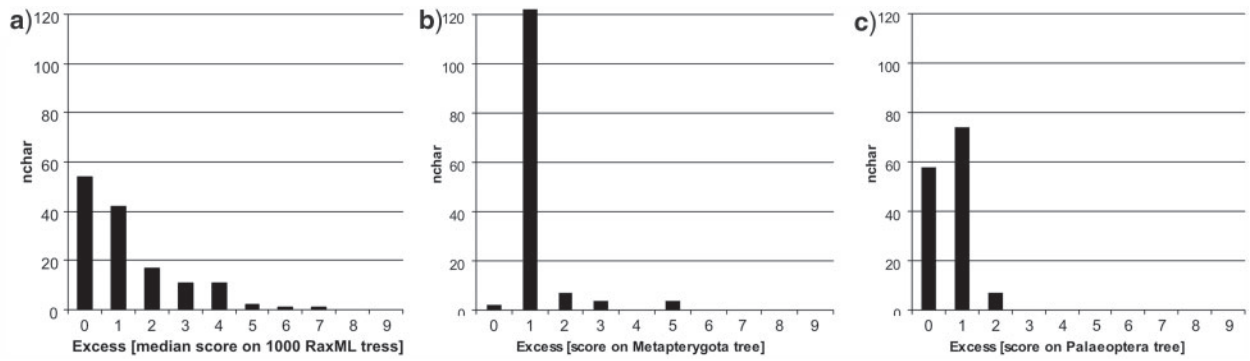

FIGURE 4. Frequency histograms of the median excess indices for the 139 characters derived by Blanke et al. (2012) on a) the set of 1000 RaxML trees sampled from the molecular analysis; b) an artificial metapterygotean tree; and c) an artificial palaeopteran tree.

molecular tree, we identified the morphological characters responsible for the incongruence between the molecular and morphological trees (Holland et al. 2010). First, we recorded the fit of the morphological characters to the trees derived from the molecular data. We took a random sample of 1000 trees from the RaxML bootstrap analysis of the molecular data and calculated the excess index as a measure of fit for each morphological character on these trees (Fig. 4a). We also tested the excess distribution on the alternative hypotheses (Fig. 4b,c) by changing only the sister group relationship between Ephemeroptera and Odonata (Fig. 4b = Metapterygota; Fig. 4c = Palaeoptera). The rest of the tree was left unchanged, that is, identical to the molecular tree reconstruction. The excess index of a particular character is defined as the number of extra state changes above the minimum number possible (which is the number of character states −1) (Holland et al. 2010). Thus, a character with 2 states (0 and 1) and 5 state changes on a given tree has an excess of 4 (5 − 1). The median excess index is derived from the excess index of each character calculated for all bootstrap trees. The median excess index is thus a measure of the average fit for each single character over all molecular trees. High-excess values indicate a poor fit.

The excess indices derived from the molecular data (Fig. 4a) show an exponential decrease. This implies that most of the characters fit the bootstrap trees quite well (peaks 0 and 1), while some characters with higher excess indices do not match the branching pattern implied by the molecular data. Basically, calculation of the excess indices already allows identification of characters with a poor fit to the molecular trees. However, this procedure alone is not sufficient for an exploration of possible character interdependencies, that is, a higher compatibility with each other than to either the molecular or the morphological trees.

The excess distribution of the characters can be used as a decision basis for choosing cut-off values so that specific groups of characters can be analyzed further. In contrast to Holland et al.'s (2010) study, we decided to proceed with all morphological characters (see also Fig. 2), since characters important for the estimation of the basal pterygote splits have a good fit on both the molecular trees (excess index of 0–1; Fig. 4a) and on theoretical alternative trees supporting Metapterygota (Fig. 4b) or Palaeoptera (Fig. 4c). Excess frequencies in both cases show maximum peaks at either 0 or 1 indicating that most of the characters have a good fit on the respective hypotheses. For example, the anterior ball-and-socket joint of the mandible has an excess index of zero under the Metapterygota hypothesis, and an excess of 1 under either the Palaeoptera or Chiastomyaria hypothesis.

Analysing Incongruent Groups of Characters

To identify mutually compatible morphological characters, we calculated their dissimilarity as pairwise excess indices. We then plotted the dissimilarity values on the matrix representation of characters and ordered them according to the median retention index the characters have on the 1000 RAxML bootstrap trees ("Dissimilarity matrix"; Fig. 5). The matrix shows that there are several character groups that are highly compatible to each other but have a rather poor fit on the molecular bootstrap trees (see arrows in Fig. 5). Furthermore, as could be expected, several characters with a good fit to the trees are also highly compatible to each other.

Identification of Character Cliques

We next selected cliques of mutually compatible characters by performing a cluster analysis (UPGMA in Paup Version 4.0b10) of the dissimilarity matrix. The rationale behind this was that sets of mutually compatible characters represent instances of potentially concerted convergence. The analysis yielded 2 larger cliques of characters (Fig. 6). We ran separate parsimony analyses with these 2 cliques (size 48 and 26 characters) in TNT using 1000 heuristic searches with random addition of taxa and TBR branch swapping (Fig. 7a,b). Separate analyses of the characters in cliques 1 and 2 both yielded incongruent results to those obtained with both the molecular data and the entire morphological character set. In fact, these trees are incompatible with

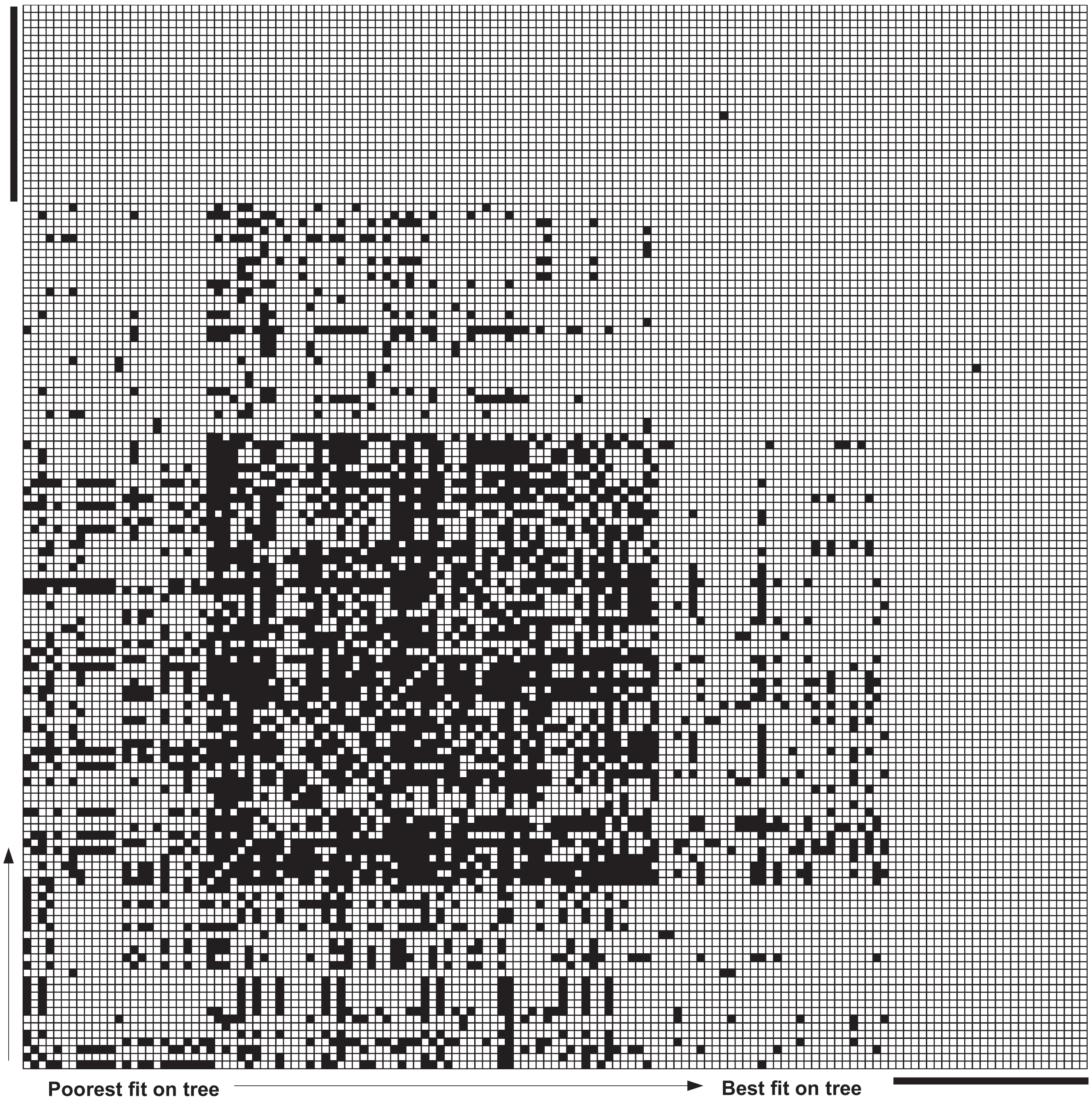

FIGURE 5. Excess index matrix showing the pairwise excess indices for each character pair of the morphological data matrix sorted according to their fit (retention index) on the 1000 RaxML trees of the molecular data (vertical and horizontal arrows). White dots show compatible pairs of characters. Black dots indicate incompatible pairs of characters. The black bars indicate parsimony uninformative characters (apomorphies). A detailed pairwise excess matrix is available at doi:10.5061/dryad.1q3b6 in online Supplementary Material 4.

classical and generally accepted concepts such as the monophyly of Pterygota, Holometabola, Odonata, and Ephemeroptera. We thus conclude that the characters in these 2 cliques represent instances of concerted convergence. If we take this into account, the amount of convergence in the remaining characters (65 characters; excluding cliques 1 and 2) should be substantially lower. A tree calculated from the remaining characters (henceforth referred to as character set 3) is compatible with the Palaeoptera hypothesis, the monophyly of Odonata, Ephemeroptera, Xenonomia (Grylloblattodea + Mantophasmatodea), and Phasmatodea. The second major clade shows a sister group relationship between Zoraptera and Acercaria+Holometabola and Plecoptera as sister to this assemblage.

Clique Composition

Cliques 1 and 2 account for 53% of the original characters (clique 1=35% and clique 2=18%). We further analyzed the character composition concerning

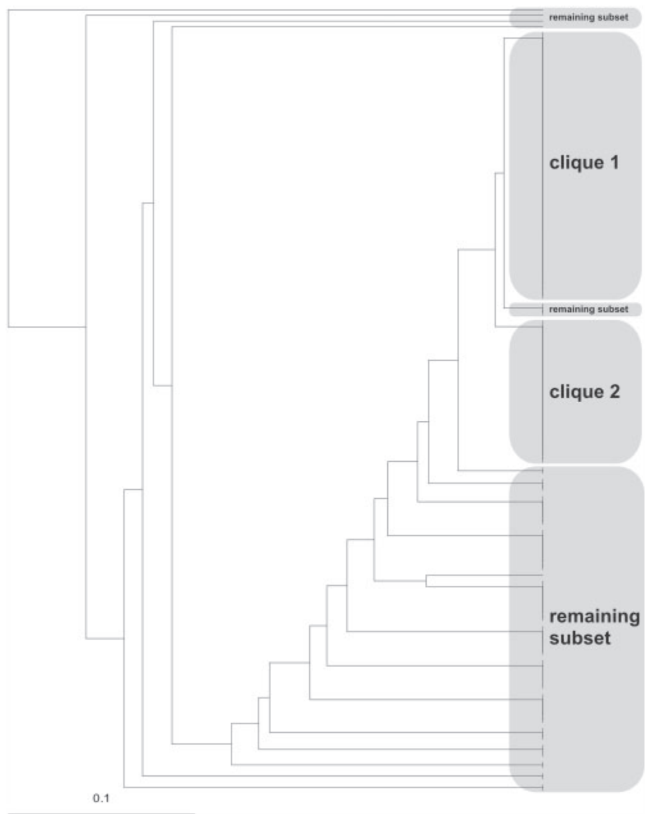

FIGURE 6. UPGMA clustering of the pairwise excess index matrix calculated in PAUP. Clustered characters are indicated by a vertical terminal line. The 2 cliques and the remaining subset of characters are indicated with gray boxes. For a detailed tree with all characters mapped see doi:10.5061/dryad.1q3b6 in online Supplementary Material 5.

morphological units in cliques 1 and 2 and character set 3 (Fig. 6). The morphological data matrix was divided into character groups representing mouthparts (labrum, mandibles, maxillae, and labium), head capsule, tentorium, antennae, and hypopharynx/pharynx. Finally, the percentage of characters in each character group in both of the cliques and character set 3 was calculated (Fig. 8).

Clique 1 contains a high number of head capsule characters (25%) whereas mandibular characters are underrepresented (2%). In contrast, mandibular characters group together in clique 2 (19%), which also contains more tentorial characters (19%). Only 2 characters of the head capsule (8%) are contained in this clique.

The remaining characters (set 3) contain more hypopharyngeal/pharnygeal and antennal characters relative to the complete data set. Again head capsule characters are underrepresented (8%).

DISCUSSION

Our study demonstrates that at least 2 sets of cephalic characters—cliques 1 and 2—are apparently

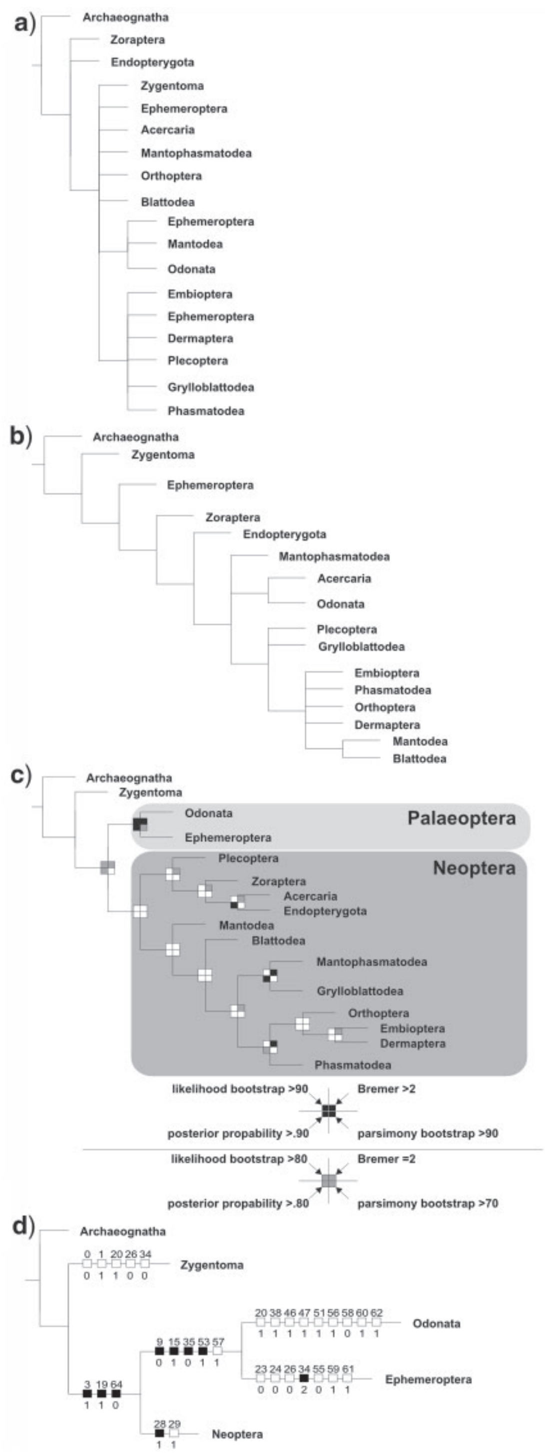

FIGURE 7. Phylogenies calculated from the 2 cliques of characters (a and b) and from the remaining character subset (c) of the morphological data matrix. a) Strict consensus of 25 trees; 48 characters; tree length=56; RI=88; CI=89. b) Strict consensus of 16 trees; 26 characters; tree length=38; RI=93; CI=86. c) The single most parsimonious tree derived from parsimony analysis; 65 characters; tree length=192; RI=71; CI=45. The support values are mapped on the parsimony tree. d) Detail of tree c showing the specific characters for each node focused on the Palaeoptera problem. Details for each clique and the remaining character set can be found at doi:10.5061/dryad.1q3b6 in online Supplementary Material 5. Trees for each reconstruction method used in Figure 7c are available at doi:10.5061/dryad.1q3b6 in online Supplementary Material 6.

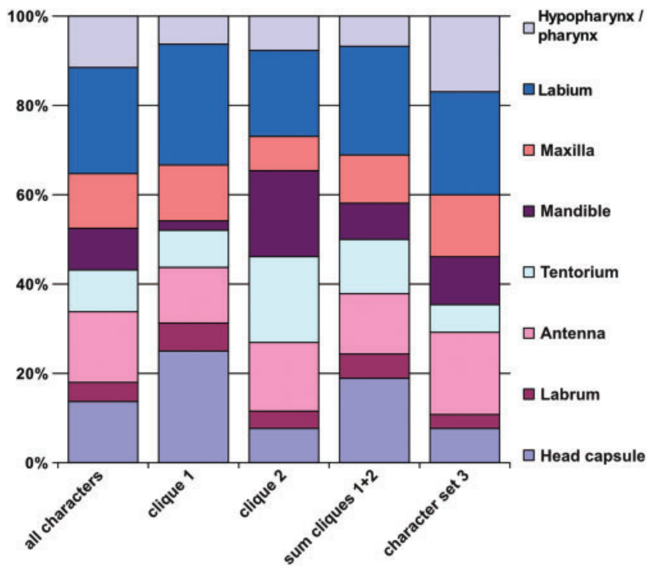

FIGURE 8. Distribution of characters in the complete data matrix (leftmost bar) and in each of the derived character cliques (bars 2–5) and in the remaining character set (bar on the right margin).

affected by concerted convergence and are therefore potentially biasing tree inference. Trees derived from the remaining morphological characters support the clade Palaeoptera, whereas the molecular data partly support the Chiastomyaria concept, an incongruence that will be evaluated in the following.

It is well known that molecular data are not free of homoplasy. Phylogenetically independent shifts in base composition can be considered as cases of concerted convergence. Holland et al. (2010) used a tree based on molecular data as a null hypothesis to identify candidate morphological characters with a high-excess index on the molecular trees. We also tested the morphological data against the molecular trees (Fig. 4), but took a different approach afterwards by including all morphological data into the subsequent analyses. This was necessary as the characters relevant in the context of the Palaeoptera problem (subgena [8], anteclypeus [17], antennal configuration [27], antennal circulatory organs [38], mandibular [66,67,69], and lacinial structure [83]) fit well on the molecular trees. These characters change only once or twice (depending on the underlying tree) at the basal-most pterygote node. This is fundamentally different to the situation described in Holland et al. (2010), where the relationships of 9 groups of water birds were explored. Characters in the Holland et al. (2010) study had higher excess values than those we evaluated here. We also tested the exclusion of characters that fit well on the molecular trees (those with an excess of 0 or 1), but this eroded the signal for the deep pterygote nodes completely (see online Supplementary Material 7).

Moreover, by retaining all morphological characters we rule out the selection of high-excess characters based on a questionable molecular hypothesis; selecting only high-excess characters could heavily influence clique formation and clique composition.

Based on the present analysis, the mutually compatible characters of cliques 1 and 2 are indicative of concerted convergence. Convergence is a well-known and frequent pattern in insect evolution (Grimaldi 2001; Carapelli et al. 2007). Concerted convergence—the congruent evolution of entire character groups in relatively distantly related taxa—can give rise to biased inference and/or inflated tree support, ultimately resulting in misleading phylogenies (Sanderson and Doyle 1992; Patterson and Givnish 2002; Givnish et al. 2006).

The detection of cliques of characters is straightforward but the interpretation of concerted convergence is a decision based on additional information.

In our case, we showed that character cliques 1 and 2 support highly implausible relationships and represent biased subsets of the total character matrix. For example, analysis of clique 1 resulted in a comb-like tree with Zoraptera and Holometabola as the first split after Archaeognatha. Clique 2 shows some more plausible relationships with Zygentoma as sister group to Pterygota and monophyletic Ephemeroptera as sister group to the clade Neoptera(=Chiastomyaria). However, clique 2 characters support implausible relationships inside Neoptera, for instance, Zoraptera as sister group to all other Neoptera, and Odonata as sister to Acercaria deeply nested inside Neoptera. All other resulting relationships within Neoptera are morphologically equally implausible and not encountered in any literature sources.

Based on these results, we interpret that the signal within both cliques is affected by concerted convergence. Consequently, these characters should be down weighted or omitted in future tree reconstructions.

Several additional conclusions follow from this result. First, the dissimilarity score of Holland et al. (2010) indeed helped to identify patterns of concerted convergence. Second, character set 3 potentially represents a data set with a better signal-to-noise ratio in the morphological data. These characters as well as the characters of both cliques should be carefully investigated to assess their potential phylogenetic signal.

Clique composition (Fig. 8) shows that especially characters of the head capsule, tentorium, and mandible are prone to concerted convergence. Characters of the head capsule are mainly related to ridges or sutures (37% of the characters in the complete matrix) and the general shape of the head (42%). All characters related to ridges and sutures (6–11) appear in the cliques (character 9 in clique 2, the rest in clique 1). Head shape characters (1,5,12) cluster also in clique 1. The phylogenetic value of ridges and sutures has been a matter of controversy (Strenger 1952; Kristensen 1981; Klass and Eulitz 2007; Beutel et al. 2008). Apparently, their possible correlation with the general head shape is still not well understood. (Staniczek 2000, 2001) assumed that the presence of the subgenal ridge is a synapomorphy of Metapterygota. In conjunction with the formation of a subgenal ridge, he considered a lateral shift and broadening of the anterior tentorial arms as

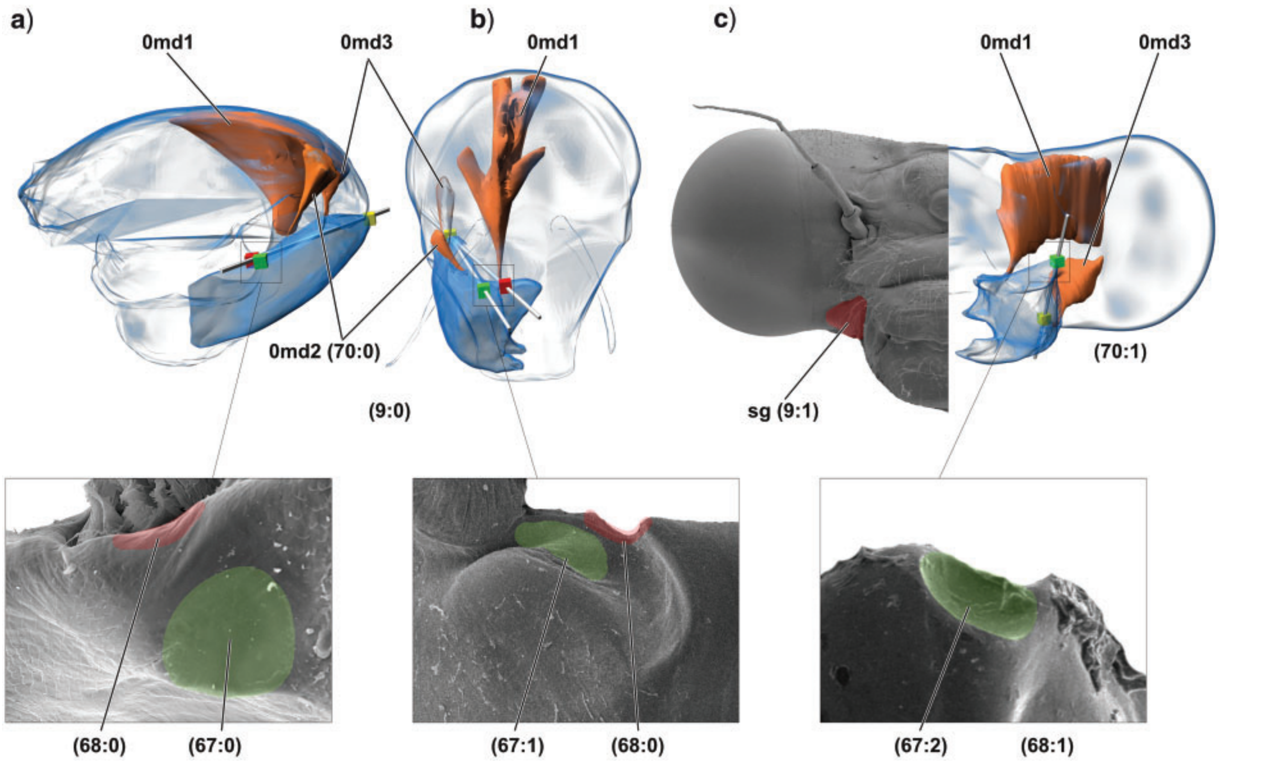

FIGURE 9. 3D reconstructions and SEM micrographs of a part of the problematic head characters which clustered in clique 2. Character numbers and states in brackets, heads, and mouthparts in frontal view. Red, green, and yellow cubes indicate the location of the mandibular articulation complexes, gray bars the assumed rotation axis of the mandible. a) *Tricholepidion gertschi* (Zygentoma); b) *Siphlonurus lacustris* (Ephemeroptera); c) *Lestes virens* (Odonata). The corresponding character states for Neoptera are identical to the situation in Odonata. Character 9: Subgenal ridge (sg): (0) absent; (1) present; Character 67: anterior mandibular joint: (0) cuticular hardening on the mandibular depression; (1) channel-joint (2) ball-and-socket joint; Character 68: anterolateral part of the anterior mandibular articulation (paratentorial joint): (0) present; (1) absent; Character 70: Musculus craniomandibularis externus anterior (0md2): (0) present; (1) absent. 0md1: Musculus craniomandibularis internus; 0md3: Musculus craniomandibularis externus.

further synapomorphies and as responses to enhanced forces resulting from reduced degrees of freedom at the mandibular base (anterior articulation modified as ball-and-socket joint in Odonata+Neoptera). However, if the Palaeoptera hypothesis is correct, the subgenal ridge (9), the anterior ball-and-socket joint (67), and the tentorial modifications are independent developments of Odonata and Neoptera. This scenario also implies the independent reduction of the paratentorial joint (68) and the Musculus craniomandibularis externus anterior (70), which are both present in Ephemeroptera (Fig. 9). All these characters are represented in the morphological data matrix (9,67,68,70) and they group together in clique 2. Based on the present analysis, it appears highly advisable to treat the 4 characters as 1 (or to exclude 3 of them) to prevent a hidden weighting of structural transformations associated with the evolution of the anterior mandibular articulation. Likewise, the fusion of the anterior and posterior tentorium (48, set 3) and the reduction of all intratentorial muscles (57–60; clique 2) are closely correlated. This set of muscles should therefore be treated similarly as one character in future analyses. The tentorial fusion already accounts for the entire complex of structural modifications.

In this study, we use a character matrix which is based on widely accepted and established homology hypotheses. However, the concerted convergence approach applied here may also point toward nonhomology of characters thereby exposing putatively homologous character states as nonhomologous. The application of Holland et al.'s (2010) convergence assessment on the Palaeoptera problem, however, is not completely unproblematic. It has been shown that exclusion of characters obviously related to each other may not remove all the homoplasy involved (Worthy and Lee 2008). Formal convergence assessments also do not release investigators from the task of working out primary homology hypotheses for each morphological character. No automated procedure can determine if, for instance, the lacinia mobilis is homologous across Crustacea, Myriapoda, and Hexapoda (Richter et al. 2002). Thus, the principal responsibilities of evolutionary morphologists regarding character identification and homology assessment remain untouched by the concerted convergence approach. Nevertheless, the analytical framework tested here is a useful step toward downweighting (or removing) convergent characters using a formal procedure.

The corroboration of Palaeoptera by our convergence assessment does not settle the deep-rooted problem of basal splits in Pterygota. The data set contains only cephalic characters, and the taxon sampling is limited. However, it is now evident that in future studies addressing this issue, attention should be paid to the evolutionary dependence of characters of the head capsule and mandibles. Character systems that seem to be less problematic are those related to the antennae, labrum, maxillae, labium, hypopharynx, and pharynx. For a better understanding of character evolution related to the early pterygote splits, it will also be necessary to obtain more detailed and well-documented data for the 2 key taxa, Zygentoma, and Archaeognatha.

Supplementary Material

Supplementary material, including data files and/or online-only appendices, can be found in the Dryad data repository at http://datadryad.org, doi:10.5061/dryad.1q3b6.

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