Title

The evolutionary history of Trichoptera (Insecta): A case of successful adaptation to life in freshwater

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Short title

The evolutionary history of Trichoptera

Abstract. The insect order Trichoptera (caddisflies) forms the second most species-rich monophyletic group of animals in freshwater. So far, several attempts have been made to elucidate its evolutionary history with both morphological and molecular data. However, none have attempted to analyze the time frame for its diversification. The order is divided into three suborders, Annulipalpia, Integripalpia and "Spicipalpia". Historically, the most problematic taxon to place within the order is "Spicipalpia", whose larvae do not build traditional cases or filtering nets like the majority of the caddisflies. They have previously been proposed to be the sister group of all other Trichoptera or more advanced within the order, with equivocal monophyly and with different interordinal placements among various studies. In order to resolve the evolutionary history of the caddisflies as well as timing their diversification, we utilized fragments of three nuclear (carbamoylphosphate synthase, isocitrate dehydrogenase and RNA polymerase II) and one mitochondrial (Cytochrome oxidase I) protein coding genes, with 16 fossil trichopteran taxa used for time calibration. The "spicipalpian" families are recovered as ancestral to all other caddisflies, though paraphyletic. We recover stable relationships among most families and superfamilies, resolving many not previously recognized phylogenetic affinities among extant families. The origin of Trichoptera is estimated to be around 234 millions of years ago, i.e. Middle - Late Triassic.

Introduction

Adaptation to a life in freshwater from previously marine or terrestrial animal lineages has occurred numerous times (Balian *et al.*, 2007) and involved one or more life-stages (Lévêque *et al.*, 2005). Due to factors like tectonic events, sedimentation and climate variations affecting water volume, chemistry and temperature (Carpenter *et al.*, 1992; Kundzewics *et al.*, 2008; Heino *et al.*, 2009), freshwater bodies are unstable environments seen in a geological time perspective. Being successful and diversifying under such conditions requires effective adaptation to the various freshwater habitats and available food resources, as well as sufficient dispersal abilities (Wiggins, 2004). Time-calibrated phylogenetic hypotheses are valuable tools for research on the evolution and processes affecting the aquatic biodiversity. Such studies have been performed on some groups of freshwater organisms, e.g. aquatic Coleoptera (Hunt *et al.*, 2007), Amphibia (Zhang *et al.*, 2005), Chironomidae (Cranston *et al.*, 2012), but are still lacking for the majority of other aquatic groups. The insect order Trichoptera (caddisflies) constitutes the second largest extant monophyletic animal group in freshwater, only surpassed by Culicomorpha/Psychodomorpha (Diptera) (Fig. 1). Several hypotheses of the phylogenetic history of Trichoptera have been published, but none with a time frame for its evolutionary history inferred by analytical methods, although a few hypotheses have been suggested based on existing fossil data (Ivanov & Sukatsheva, 2002; Wiggins, 2004; Grimaldi & Engel, 2005).

The aquatic Trichoptera and the terrestrial Lepidoptera (butterflies and moths) together constitute the superorder Amphiesmenoptera, a sister group relationship well supported by both morphological and molecular data (Kristensen, 1999; Whiting, 2002; Beutel *et al.*, 2011). With 14,300 described extant species in 49 families and 688 genera (Holzenthal *et al.*, 2011), they form the seventh largest order of all insects. The group is recorded from all biogeographical regions and sub-regions with the exception of the Antarctic (de Moor & Ivanov, 2007). Their larvae inhabit a large range of freshwater habitats, from various lentic and lotic systems to temporary pools. A few species are adapted to coastal marine waters. For exploitation of this diversity of environments, the larvae exhibit a wide variety of life history strategies and morphological adaptations; many which may be attributed to a diverse and often complex usage of silk produced from the labial glands, a shared amphiesmenopteran trait (Mackay & Wiggins, 1979; Kristensen, 1997).

The order is traditionally divided into three suborders based on morphology and behaviour of adults and immature stages (Wiggins, 2004). (I) Annulipalpia have larvae that spin silken retreats and filtering nets to aid feeding on fine organic particles, periphytes or small animals found around or caught in their nets. These larvae are mainly found in running waters or in wave zones of lakes. (II) Integripalpia have larvae that construct portable, predominantly tubular cases, which enables them to produce a water current inside the shelter by undulating the body for higher gas exchange efficiency (Wiggins, 2004), as well as giving protection against predators. They are usually feeding on decomposing plant material, algae, fungi and plants, and a few are predators. The group is abundant in both running and still waters. (III) ”Spicipalpia”, or the closed-cocoon-making caddisflies, are generally regarded as non-monophyletic (Fig. 2). This group includes families whose larvae are either predators or grazers. Predatory, free-living larvae may spin silken security lines along the substrate to avoid being

dragged away by the current. Epiphyte grazing larvae belong to families whose larvae construct cases in a non-tubular manner, e.g. saddle or purse-shaped. They are mostly found in running waters, but the family Hydroptilidae is also very abundant in lentic waters.

The ”spicipalpian” immatures produce closed, rigid semipermeable cocoons free from the pupal shelter, whereas the larvae of the other two suborders produce permeable cocoons that are woven into the pupal shelter. These and other morphological characters, as well as molecular data have led to various competing hypotheses for the relationships within Trichoptera (Fig. 2). Even though it seems clear that Integripalpia and Annulipalpia constitute separate and strongly supported monophyletic lineages, the positions of the ”spicipalpian” families have been debated. Some authors proposed ”Spicipalpia” as a paraphyletic grade leading to Integripalpia or as a monophyletic sister group to Integripalpia (Ross, 1967; Kjer *et al.*, 2002; Holzenthal *et al.*, 2007) (Fig. 2a), others considered them as a sister to Annulipalpia (Weaver & Morse, 1986) (Fig. 2b). They have also been suggested to form different groups along the lineages leading to the other two suborders (Frania & Wiggins, 1997; Ivanov, 1997, 2002) (Fig. 2c). Furthermore, ”Spicipalpia” have also been considered the monophyletic sister group to Integripalpia + Annulipalpia (Wiggins & Wichard, 1989; Wiggins, 2004) (Fig. 2d).

Most of the earlier hypotheses relied on morphological and life-history characters (Morse, 1997), but recent attempts for reconstructing the evolutionary history of the Trichoptera have used molecular and morphological data in combination (Kjer *et al.*, 2001, 2002; Holzenthal *et al.*, 2007). These recent hypotheses corroborate the hypothesis detailed in Figure 2a, but the resolution obtained for deeper divergences are based almost solely on the nuclear rRNA subset of the data (Holzenthal *et al.*, 2007).

The evolution of major lineages of Trichoptera in a temporal context using molecular data and fossils in combination has not been studied previously. The fossil record of caddisflies is extensive with 608 extinct species as well as 7 families and 85 genera being entirely comprised of fossils. Older fossils, i.e. from the Early Jurassic and Triassic, are found to be very difficult to correctly place into existing crown groups, and possibly represent ancestral lineages to Trichoptera, Lepidoptera or Amphiesmenoptera. Several fossils originally described as Trichoptera were subsequently found to belong to other orders (Kristensen, 1997; Ansorge, 2002; Vladimir D Ivanov & Sukatsheva, 2002; Grimaldi & Engel, 2005), while *Nebritus willistoni* Melander was originally described as a fly, before being recently placed *insertae sedis* in Trichoptera by Hauser and Irwin (Hauser & Irwin, 2005). The oldest fossil that is identified as a caddisfly with an acceptable degree of certainty is *Liadotaulius maior* (Handlirsch, 1906) dated to Early Jurassic (Late Lias, 185–180 millions of years ago (Ma)), and is considered the ancestor to all extant Trichoptera (Ansorge, 2002). In comparison, the oldest fossil designated the sister group Lepidoptera is *Archaeolepis mane* Whalley, 1985 dated to Early Jurassic (Early Lias, 190-200 Ma) (Grimaldi & Engel, 2005; de Jong, 2007). This demonstrates that the common ancestor of Amphiesmenoptera diverged into the lineages leading to Trichoptera and Lepidoptera earlier than 190 Ma. In a molecular dating study across Holometabola by Wiegmann *et al.* (2009) the Lepidoptera-Trichoptera split was recovered at around 230 Ma. The oldest fossils of larval cases appear in Early Jurassic strata in Transbaikalia, and are believed to represent the family Hydroptilidae or the extinct family Vitimotauliidae. Fossils from Mid-Jurassic layers in

Mongolia are attributed to Baissoferidae, an extinct Integripalpian family (Ivanov & Sukatsheva, 2002). Other old fossils classified as caddisflies are dated to Middle and Late Jurassic, as well as to Early Cretaceous. Many of which have been placed into extinct families (*e.g.* Vitimotauliidae, Baissoferidae, Dysoneuridae), whereas others are placed in extant families. Among the latter are some represented only by wing fragments, while others consist of poorly preserved larvae and cases with uncertain taxonomical placement. Fossil species from these periods have been placed in the extant families Rhyacophilidae, Philopotamidae, Lepidostomatidae, Calamoceratidae, Plectrotarsidae and Helicophidae. The caddisflies in this period are regarded to have diversified into the major extant groups and represent many of the families recognized today (Ivanov & Sukatsheva, 2002; Grimaldi & Engel, 2005).

In order to test previous hypotheses on the evolutionary history of Trichoptera and higher groups within the order, we used fragments of three nuclear protein-coding genes: 850 bp of carbamoylphosphate synthase (CPSase of CAD), 711-720 bp of isocitrate dehydrogenase (IDH) and 772 bp of RNA polymerase II (POL); and 658 bp of the mitochondrial cytochrome oxidase I (COI). These genes have previously, in different combinations, been used for inferring phylogenies for family level or above within Trichoptera, Lepidoptera, Hymenoptera and Diptera as well as among insect orders (Moulton & Wiegmann, 2004; Danforth *et al.*, 2006; Wiegmann *et al.*, 2009; McKenna & Farrell, 2010; Mutanen *et al.*, 2010; Winterton *et al.*, 2010; Malm & Johanson, 2011; Johanson *et al.*, 2012). We selected protein-coding genes in order to minimize subjectivity in homologization, that may pose a problem when using morphological characters and during alignment of e.g. ribosomal genes. We conducted analyses of the different codon positions for each gene, in order to determine their respective positive or negative impact on the derived phylogenetic hypotheses, as well as their usefulness in recovering branching events at various levels within Trichoptera. We present a fossil-calibrated, timed phylogenetic hypothesis of the order and infer its evolutionary history based on this hypothesis.

Material & Methods

The total data set comprises 147 species, including 10 outgroup species that cover the early lineages within Lepidoptera. The ingroup comprises all families except Rossianidae, and 70 of the 79 recognized subfamilies. Voucher information and GenBank sequence reference numbers are displayed in supplementary table S1.

Laboratory procedures for extraction, PCR and sequencing follows that of Malm & Johanson (2011), for the 142 taxa sequenced at the Swedish Museum of Natural History (NHRS), and Wahlberg & Wheat (2008) for six Lepidoptera taxa taken from a previous study (Mutanen *et al.*, 2010). Sequences for COI, CAD and POL were aligned manually to correct for missing data at beginning or end of fragments. The IDH sequences, for which there was length variation up to 15 bp, were aligned by using MAFFT (Katoh *et al.*, 2005), following the E-INS-i protocol. Data tables and matrices were created with VoSeq v.1.2.4 (Peña & Malm, 2012). The total gene coverage among the included specimens was 97.3% (when counting also partial sequences). CAD sequences were lacking for four ingroup taxa, COI for one outgroup taxa, IDH for five ingroup taxa and POL for six outgroup taxa (Table S1).

To determine whether some sites were saturated or misinformative, each codon position partition from all separate gene fragments was examined for its respective Phylogenetic Informativeness (PI), substitution saturation and base frequency homogeneity. The PI values were calculated from character substitution rates according to Townsend (2007), and visualized as time-calibrated curves (PI profiles) displaying the estimated amount of phylogenetic information in a data set/partition. We used the calculations given by Townsend (2007) with and without the last step involving normalization ($PI_{original}$ and $PI_{modified}$, respectively). The normalization step is applied to attribute equivalent net information for each rate over time, and was created to hinder slower evolving sites reaching greater net informativeness than faster sites. Partitions with high PI values/curves are considered phylogenetically informative, but those with early high peaks and sequential sharp declines may indicate saturation (noise) or misleading signal within the temporal frame of the decline (Klopfstein *et al.*, 2010; Townsend & Leuenberger, 2011). PI values and curves were calculated in R (www.r-project.org/) from site rates determined in HYPHY 2.1020111108Beta (Kosakovsky-Pond *et al.*, 2005), derived from a time-calibrated tree based on the complete data set. Level and probability of substitution saturation was determined using the Xia test of saturation in DAMBE (Xia, 2001), as well as base frequency homogeneity in PAUP* (Swofford, 2003). This was calculated for four different taxon sampling schemes (4, 8, 16 and 32 taxa) and two types of inferred topologies, symmetric and asymmetric, with no fixed starting tree.

The best fitting model of sequence evolution was determined for each partition with MrModeltest v.2.3 (Nylander, 2004) using the Akaike Information Criterion (AIC) and jModeltest v. 0.1.1 (Posada, 2008) using the Bayesian Information Criterion (BIC). When proportion of invariable sites were suggested by MrModeltest as included in the best model together with among-site rate variation, the former was ignored in the used models due to the possible effect of correlation with the gamma shape parameter (Ren *et al.*, 2005). Invariant sites were excluded from estimation in jModeltest. Resulting models were implemented in the Bayesian analyses described below. Phylogenetic analyses were performed using Maximum Likelihood modeling (ML) with RAxML (Stamatakis, 2006) on RAxML BlackBox (Stamatakis *et al.*, 2008) with the GTRCAT model for bootstrap replicates (Stamatakis, 2006). Bayesian inferences (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) were calculated on the Bioportal online portal for phylogenetic (and other) analyses (www.bioportal.uio.no) (Kumar *et al.*, 2009). State frequencies, shapes, transition/transversion rates and substitution rates, were unlinked between partitions. All BI analyses were run with four chains each in two separate runs, for a minimum of 15,000,000 generations and always well after reaching stability, as visually determined in Tracer v1.5 (Drummond & Rambaut, 2007) and AWTY (Nylander *et al.*, 2008). The analyses were performed on the complete data set as well as on a reduced data set derived from the codon position partitioning analyses described below. The final analyses were partitioned according to gene fragment. For discussion of clade support, BI Posterior Probabilities (PP) above 0.95 and ML Bootstrap Frequencies (BF) above 75% are considered as strong, and below those values as weak or unsupported.

Divergence time analyses were executed in BEAST (Drummond & Rambaut, 2007) on a fixed tree topology obtained from the BI analyses, according to a Birth-Death process with independent GTR+G models for each partition. Branch lengths were set to vary

under a relaxed clock model with an uncorrected lognormal distribution. Sixteen nodes within Trichoptera were used as calibration points for ages based on fossil data (Table 1), with uniform distributions and hard upper and lower boundaries. The lower boundaries were set according to minimum fossil age and the upper as high as not to interrupt the maximum age of the posterior distributions, and to include node ages suggested in earlier studies (Ivanov, 2002; Grimaldi & Engel, 2005). The fossil taxon representation was chosen to include as many groups as possible, and we preferred fossils that could be safely placed in extant families and genera, although a few older fossils with putative placement in extant families or genera were used but with extended calibration age span (e.g. *Dolophilodes shurabica* Sukatsheva 2004; *Baissoplectrum separatum* Ivanov 2006). Hence, most of the older (>120 Ma) fossils were excluded due to classification uncertainties. Fossils that could be attributed to extant families or genera but not to any particular split within the tree were used as calibrations for the stem of that group, i.e. the philopotamid *D. shurabica*, the brachycentrid/lepidostomatid *B. separatum*, the dipseudopsid *Phylocentropus cretaceous* Wichard & Boelling, 2000, the odontocerid *Marilia altrocki* Wichard, 1986, and the rhyacophilid *Rhyacophila antiquissima* Botosaneanu & Wichard, 1983. In order to confirm that any one calibrated node did not disproportionally affect the results, additional analyses were run with each calibration point excluded in turn. Analyses were also run without sequence data on priors alone in order to compare priors with posteriors. Additional analyses were made using various competing topologies for unresolved nodes in the BI tree, as approximation of the influence on times of divergence of the different topological resolutions. These reconstructions were made as one symmetric, with all the collapsed nodes collected into one group, and one asymmetric, where the collapsed nodes were arranged one after the other leading to the more apical groups.

Results

Data quality

The aligned datasets for the individual gene segments included for CAD 850 nucleotides (523 variable), COI 658 nucleotides (425 variable), IDH 720 nucleotides (441 variable) and POL 772 nucleotides (329 variable). The complete combined dataset consisted of 3000 nucleotide sites (1718 variable), whereas the reduced combined dataset included 1999 sites (970 variable). The alignments for both the complete and the reduced datasets are provided as supporting information (Supplementary Appendix S1-2). The GTR+I+G model by MrModeltest 2.0 and GTR+G by jModeltest were found to be the best fitting models for all genes, as was for all gene codon position partitions except the 2nd position of POL, for which F81+I+G was suggested. Phylogenetic Informativeness curves, that attempt to visualize the amount of phylogenetic information over time for a given dataset, showed high and sharp peaks younger than 50 Ma in the plot using the normalization step ($PI_{original}$, Fig. 3a) for the 3rd codon positions of all genes. In the analysis without the normalization step ($PI_{modified}$, Fig. 3b) these peaks were relatively lower and more extended towards older times. In both plots the PI curves of 1st and 2nd positions increased slowly with time until 50 Ma ($PI_{original}$) and 150 Ma ($PI_{modified}$), but did not exhibit the same narrow peaks or sharp declines as the 3rd codon positions. In the $PI_{original}$ plot the 3rd codon positions received higher PI values

than the 1st and 2nd positions at maximum age (234 Ma), whereas in the $PI_{modified}$ plot they declined below the highest non-3rd position curve at about 130-150 Ma. The high, young peak values compared to the much lower trailing values towards older ages should indicate high levels of saturation for these partitions. The 3rd codon position exhibiting the slowest decline as well as the highest PI values of older ages (>150 Ma), and thus considered the most informative for the early radiation within Trichoptera, was that of POL. The profile of the 2nd codon position of POL was close to zero during the whole time frame, though slowly increasing. Comparing the curves between the two different PI calculations, the normalization step (used in $PI_{original}$) seem to underrate slower evolving sites over time, leading to the faster 3rd codon partitions exhibiting greater PI values even at the oldest parts of the tree. This may be true for the net informativeness, but when saturation and potential misleading information is taken into account it makes little use for comparison among parititions. The $PI_{modified}$ plot on the other hand, without being normalized, make comparison among partitions easier, since the slower evolving partitions at some point surpass the faster in informativeness. This should indicate when the need to remove the faster positions may be impending. Thus, curve shapes of the $PI_{modified}$ plot (Fig. 3b) indicate that most 3rd codon positions (except POL) may be uninformative or even misleading at ages older than 130-150 Ma, during a period when the earlier branching events within the order might have taken place (Kristensen 1997; Wiggins 2004; Grimaldi & Engel 2005).

According to the Xia tests of saturation, all 3rd codon positions were close to saturation or significantly saturated for both asymmetric and symmetric tree shapes, irrespective of taxon sample sizes (Table 2). The 3rd codon position of POL was significantly informative for asymmetric trees regardless of taxon sample sizes, and was thus considered to be the most informative of all the 3rd coding positions in this data set. This corroborates the results from the PI analysis. The 2nd position of POL was considered uninformative due to the high rate of conservativeness, with only 17 out of 257 characters being variable. All partitions of the 1st codon positions were found to be unsaturated. The base frequency homogeneity test gave significant values for biased base composition for all 3rd codon positions ($P>0.99$), whereas all 1st and 2nd positions were significant nonbiased ($P<0.0001$).

Based on this data quality assessment, two separate data sets were analysed. The full data set included all codon positions for all genes, whereas the reduced data set excluded the 3rd codon positions of CAD, COI and IDH, and the 2nd codon positions of POL.

Full data set

The BI, but not the ML analyses return strong support for the monophyly of Trichoptera (PP=1; BF<50%), and reveals Rhyacophilidae and Hydrobiosidae as the two basalmost families (Supplementary Fig. S1). Glossosomatidae and Hydroptilidae together form a monophyletic sister group to Annulipalpia, though support values are only moderate (PP<0.90; BF<60%) for the basal branches involving ”Spicipalpia”. Annulipalpia and Integripalpia are monophyletic with PP’s of 1 and 1, and BF’s of 74% and 75%, respectively. Within Annulipalpia, there is strong support (PP=1; BF=82%) for Hydropsychidae as a sister family to the other families, and Philopotamoidea forms the

sister group to the families in Psychomyioidea *sensu* Ivanov 2002, with strong support (PP=1; BF=94%). Integripalpia includes a polyphyletic Leptoceroidea; with Leptoceridae forming the sister group to all other Integripalpia families. Both Plenitentoria (e.g. Phryganopsychidae-Limnephilidae) (PP=1; BF=100%) and Sericostomatoidea (PP=1; BF=72%) are recovered as monophyletic. We recover non-monophyly of the families Psychomyiidae, Helicophidae, Odontoceridae and Anomalopsychidae. Psychomyiidae forms a paraphyletic grade leading to Xiphocentronidae, Helicophidae is paraphyletic and includes Barbarochtonidae, and the latter two families are both rendered as polyphyletic: Odontoceridae with *Barynema* Banks, 1939 as sister taxon to Philorheitridae, and *Marilia* Mueller, 1880 nested within Leptoceroidea; and Anomalopsychidae where *Contulma* Flint, 1969 forms a sister taxon to Helicopsychidae, and *Anomalopsyche* Flint, 1967 forms the sister taxon to Antipodoecidae. All other families are each strongly supported as monophyletic (PP>99; BF>79%).

Reduced data set

The BI analyses of the reduced and most informative data set (CAD $1^{st}+2^{nd}$, COI $1^{st}+2^{nd}$, IDH $1^{st}+2^{nd}$, POL $1^{st}+3^{rd}$) (Fig. 4) strongly support a monophyletic Trichoptera (PP=1; BF=66), with the free-living ”spicipalpian” families ordered as a paraphyletic grade, leading to a bifurcation between Annulipalpia and Integripalpia (PP=0.93; BF<50). The BI PP values for the basal branches involving ”Spicipalpia” are moderately strong (PP>0.90), revealing less uncertainty for those branching events compared to the analysis of the complete data set, whereas these are not supported by a majority of the ML bootstrap trees. Except for the better-supported resolution of ”Spicipalpia” and the paraphyletic (though collapsed) Leptoceroidea, the superfamilies and families have almost identical topology with that of the full data set. Non-similar topologies are predominantly within Plenitentoria or among the families recovered as non-monophyletic in the complete data set. In this analysis all families are monophyletic (including Psychomyiidae, Odontoceridae and Anomalopsychidae), except Lepidostomatidae which included Brachycentridae; and Helicophidae which includes Hydrosalpingidae and Barbarochtonidae. All monophyletic families were strongly supported by both BI and ML (PP>0.97; BF=85%), except for Psychomyiidae (PP=0.84; BF=54%), Odontoceridae (PP=67; BF<50%), Helicopsychidae (PP=1; BF=64%) and Anomalopsychidae (PP=0.97; BF=59%).

Divergence times

The analyses of divergence times in BEAST were based on the calibrated nodes marked by a dot (•) in Fig. 4. All posterior distributions of calibrated node ages were normally distributed within the uniform boundaries, except a slight skew to younger ages for *Silo* Curtis, 1830 (Goeridae) and *Agraylea* Curtis, 1834(Hydroptilidae). Comparison between all 16 analyses with individual calibrations excluded did not indicate strong time-dependence to any single fossil calibration point. However, we recovered slightly younger ages for the Lepidoptera-Trichoptera split in the analyses excluding *Agraylea* (215 Ma), *Molanna* Curtis, 1834 (223 Ma) or *Silo* (218 Ma). The mean ages for those

calibrated nodes were obtained as less than 5 million years (Myr) from their respective means in the analyses with all calibrations included. We found that the upper calibration boundaries did not affect the posterior estimates of times of divergence and thus in practice our analyses were constrained by hard minimum bounds. When comparing posteriors from analyses with sequence data included with those obtained from prior-only analyses we could not detect any notable prior-dependent impact on the analyses. The analyses of the different alternative topologies of the polytomy from the BI analyses affected the divergence times by less than ±2 Myr for the nodes not directly related to the alternative topologies. As expected, the ages of the re-arranged branches from the polytomy were more strongly affected by changing topologies (not shown).

The analyses of divergence times (Fig. 5 & Fig. S2) recovered a mean age of the split between Trichoptera and Lepidoptera at 234 Ma. The first branching event within Trichoptera, at 226 Ma, involved divergence of ancestral Rhyacophilidae from the ancestor of the remaining caddisflies. The complementary earliest divergence in Lepidoptera was that of ancestral Micropterigidae that diverged from its ancestral sister group around 204 Ma. The second major branching event in Trichoptera was the split between the remaining "Spicipalpia" and the ancestor to Annulipalpia + Integripalpia about 209 Ma. Annulipalpia and Integripalpia separated about 203 Ma. Within Annulipalpia, the Hydropsychidae separated from the ancestral sister group around 180 Ma. The latter group separates into the Philopotamoidea and the Psychomyioidea around 15-20 Myr later (163 Ma). Within Integripalpia, the first split, between Plenitentoria and Leptoceroidea + Sericostomatoidea, appeared around 160 Ma. The first Sericostomatoidea separated from the Leptoceroidea around 135 Ma.

Discussion

Data quality

After evaluation of the efficiency of the separate gene partitions, we conclude that analyses excluding most 3rd position partitions produce more consistent results than when all 3rd positions were included. The inefficiency of these partitions, apparently suffering from saturation, undermines the signal from the more conservative data partitions in the study. This saturation could be made negligible by appropriate modeling, but may even then enforce erroneous signal in the analysis (Xia *et al.*, 2003). POL had the least saturated of all 3rd codon positions, which appeared phylogenetically informative over the whole tree and was therefore retained in the reduced data set. In effect, we recovered better supported basal branches in the phylogenetic hypotheses based on the reduced data, compared to hypotheses based on all data, which could be due to the aforementioned saturation effects. However, it should be stressed that removal of data deemed to be uninformative for older divergence events, will potentially reduce or change the topological resolution of divergences among recent taxa. For instance, we find that Lepidostomatidae includes Brachycentridae in the trees based on the reduced data set (Fig. 4), while in the full data set these two families are recovered as sister taxa (Fig. S1). In addition, a few families receive lower support values in the results based on the reduced data set compared to the results from the full data set (e.g. Helicopsychidae). But the results based on the reduced data set also recover monophyly of families not recovered as such with the complete data set, i.e.

Odontoceridae, Psychomyiidae and Anomalopsychidae (96-130 million years old). In search for a well supported hypothesis for the older radiations within Trichoptera, we rely on the reduced data set, which seems most informative at suborder to family levels.

Recent phylogenetic analyses comprising taxa of similar age in Insecta have dealt with character assessment in different ways. With a Ditrysia (Lepidoptera) data set, Regier *et al.* (2009) concluded that 3rd codon positions should be carefully examined and assessed before inclusion due to saturation and compositional heterogeneity problems, which may give misleading phylogenetic signal. For the same reasons, Mutanen *et al.* (2010), using a data set covering all major lepidopteran groups, after examination removed all 3rd codon positions except those of Elongation factor I alpha (EF-1α). For taxa outside Amphiesmenoptera, Wiegmann *et al.* (2011) excluded the 3rd codon positions from the coding genes in their Diptera analyses, and Heraty *et al.* (2011) presented analyses with and without those for the coding genes in their Hymenoptera study, with somewhat different resolution between the trees. In contrast, McKenna & Farrell (2010) did not distinguish between codon positions in a study on Holometabola, except for separate partitions for the different codon positions of EF-1α.

Phylogenetic considerations

With few exceptions, our hypothesis about the evolutionary history of the suborders within Trichoptera (Fig. 5) is similar to the hypothesis by Wiggins and Wichard (1989), based on morphology and life history traits only. However, they considered ”Spicipalpia” as monophyletic, whereas we recover it as paraphyletic. Similar to their hypothesis, we recovered Annulipalpia and Integripalpia as monophyletic, with Integripalpia divided into Plenitentoria and Leptoceroidea (paraphyletic) + Sericostomatoidea. This was also obtained in previous molecular analyses by Kjer *et al.* (2001, 2002) and Holzenthal *et al.* (2007a), but in contrast to their hypotheses, the relationships among families within these groups, as well as the placement of the ”spicipalpian” families, were markedly different. Apart from better support values for a majority of the clades in our hypothesis, our analyses generated interesting differences in the relationships among several families compared to their work.

Trichoptera is recovered as monophyletic in regard to the outgroup lepidopterans, with high support from the BI analyses but relatively low from ML. This pattern is also true for some other basal branching events within the order. Such disparity in support between these two methods is not uncommon in larger phylogenetic studies (e.g. Winterton *et al.*, 2010; Heraty *et al.*, 2011). This result can be an effect of the different way these methods are performed (see e.g. Djernaes *et al.*, 2012), how they handle areas in the phylogeny with low information content (Alfaro *et al.*, 2003), but may also be correlated to clade size and level of homoplasy (Brandley *et al.*, 2009). The support values for monophyly of Trichoptera in the ML analyses seem to be affected by the sparse sampling of the outgroup, leading to events of outgroup taxa recovered in the ingroup. This is especially true for analyses of the full data set (Fig. S1). The ML support values are improved when removing the 3rd codon positions and thus most saturated sites.

Within Annulipalpia, the filter net-makers, we recover Hydropsychidae as the sister family to the remaining annulipalpian families with high support, a placement not proposed in earlier studies. In the morphological analyses by Frania & Wiggins (1997), the position of Hydropsychidae was unresolved with regard to Philopotamidae, Stenopsychidae, and Psychomyioidea, and Kjer *et al.* (2001, 2002) was able to resolve this polytomy by morphological characters only. Philopotamidae and Stenopsychidae have been regarded as very old, based on Triassic - Mid Jurassic fossils putatively attributed Philopotamidae (see Wang *et al.* 2009). This family pair is in the works by Kjer *et al.* (2001, 2002) and Holzenthal *et al*. (2007a) always recovered basal-most within Annulipalpia. Our results give no support for such a placement. As most characters exhibited by these old philopotamid fossils are apparently plesiomorphic for Annulipalpia, we did not consider these fossils as trustworthy calibrations points for the group (see also Ivanov & Sukatsheva 2002). Psychomyioidea is recovered as monophyletic with unequivocal support, and Ecnomidae and Polycentropodidae are strongly supported as sister families, similar to other molecular data based hypotheses (Kjer *et al.*, 2001, 2002; Johanson & Espeland, 2010; Johanson *et al.*, 2012).

Within Integripalpia, the portable case-makers, we find Plenitentoria (Fig. 5) as the sister group to Leptoceroidea + Sericostomatoidea. The recognized paraphyletic grade of Leptoceroidea is not completely resolved, similar to results from earlier molecular analyses where this has proven difficult (Holzenthal *et al.*, 2007; Malm & Johanson, 2011).

Within Plenitentoria we find that Brachycentridae and Lepidostomatidae, the only families where larvae construct cases being rectangular in cross-section, form a well supported (PP=1; BF=91) monophyletic group, but within which the Brachycentridae representative is found between the two Lepidostomatidae genera. However, this placement is not very strongly supported, and appears after removal of the faster evolving 3rd codon positions, which seem to be needed to clearly separate these two closely related families. Plectrotarsidae and Oeconesidae also form a monophyletic group, corroborated by a unique morphological hind wing synapomorphy, i.e. the merging of the two hind wing veins radius 1 and radius 2. Kjer *et al.* (2002) and Holzenthal *et al.* (2007) placed Plectrotarsidae as the sister group to the remaining Plenitentoria. Our results instead support the notion of Wiggins & Gall (1993) in recovering Phryganopsychidae as the sister group to the remaining Plenitentoria. According to these authors, Phryganopsychidae constitutes a "phylogenetic relict" within the case-making caddisflies, based on assessment of several supposedly plesiomorphic (relative to Integripalpia) morphological characters in the larvae, pupae and adults.

Within Sericostomatoidea we find higher resolution and support values than earlier hypotheses based on molecular characters. We recover well supported (PP>0.95; BF>60) monophylies of all families except for Anomalopsychidae (PP=0.97; BF=59) and the Australian and South American family Helicophidae, which here includes the South African monotypic families Barbarochtonidae and Hydrosalpingidae. Helicophidae was found to be monophyletic by Johanson & Keijsner (2008), but without representation of Barbarochtonidae and Hydrosalpingidae in their analyses. Nevertheless, our results indicate a close relationship among these three families, but stronger conclusions regarding these taxa awaits further, detailed analyses. A close

relationship as sister families between Calocidae and Conoesucidae was found by Johanson *et al.* (2009) and Johanson & Malm (2010), but these two families are here found in sequence leading to Helichopidae+Barbarochtonidae+Hydrosalpingidae.

The age of Trichoptera obtained in this study agrees well with the molecular dating study of Holometabola by Wiegmann *et al.* (2009), though they only included one Trichopteran specimen. It is also similar to the chronogram proposed by Grimaldi & Engel (2005), who suggested it to be about 240 Ma. However, since their phylogeny is based on the work by Kjer *et al.* (2001, 2002), the ages of the suborders and many higher groups differ from the mean ages presented here, though they are within the 95% confidence intervals. Ivanov (2002) also proposed an origin of Trichoptera in the late Triassic, but overall the proposed divergence times by Ivanov (2002) and Grimaldi & Engel (2005) for the larger groups are slightly older than their respective mean ages recovered in this study. This is possibly an effect of the inclusion of extinct families in their phylogenies whose positions we consider as highly uncertain, as well as old fossils that we did not use in our analyses due to their uncertain systematic affiliations.

Evolution and adaptation to aquatic life

The split between Lepidoptera and Trichoptera is in our analyses dated to around 234 Ma, and the earliest branching event within Trichoptera leading to an extant lineage appeared around 10 My later, when the ancestral rhyacophilids diverged from the ancestor to the rest of the Trichoptera. We find the first branching event within the extant Lepidoptera to be around 30 Myr after the Trichoptera-Lepidoptera split, when Micropterigidae diverged from the ancestor of the other Lepidoptera. The differentiation between the terrestrial moths and the aquatic caddisflies, as well as the adaptation to a life in fresh water of the latter lineage, could have been spurred by climatic events during late Triassic, when great oscillations between arid and humid climates have been proposed to have occurred – i.e. the Carnian Pluvial Event (Breda *et al.*, 2009; Roghi *et al.*, 2010).

According to Kristensen (1997) the early amphiesmenopterans possibly lived in humid forests, with soil-dwelling larvae and spore/pollen feeding adults. The larva could have produced a slightly rigid but porous pupal cocoon that allowed oxygen and carbon dioxide flux, like most Lepidoptera and other insect orders have today (Kristensen, 1997; Wiggins, 2004). Adaptation to a life in freshwater by early trichopterans could have been driven by avoidance of terrestrial predators, but would have to include a closing of the larval spiracles connected to the tracheal gas exchange system, to permit diffusion of oxygen over the body surface (Wiggins, 2004). The pupal cocoon subsequently evolved to become fully closed, and had gas exchange with surrounding water through diffusion (Fig. 5). The semi-permeable closed cocoon found among the earliest caddisflies apparently worked well for life in oxygen rich lotic environments, which possibly represents the environment in which the caddisflies first invaded water (Wiggins & Wichard, 1989; Wiggins, 2004), even though the small size of Hydroptilidae made it possible to survive more oxygen poor lentic waters as well. A modification from semipermeable to permeable cocoons in Trichoptera evolved in the ancestor to Annulipalpia + Integripalpia about 200 Ma (Fig. 5) (Wiggins & Wichard, 1989; Wiggins, 2004). The first occurrence of case and net-based constructions

appeared around 180 Ma, when Hydroptilidae and Glossosomatidae diverged (both building simple cases in last instar phases). This was followed by the diversification in the net-spinners (Annulipalpia) shortly after. This evolutionary scenario fits well with findings of Siberian larval cases, attributed to the family Hydroptilidae, dated to Early Jurassic. The silken nets spun by annulipalpian larvae degrade very quickly and have consequently not been found preserved as fossils. Construction of portable cases in the Integripalpia could have lagged behind the others, and based on our results this behaviour first appeared sometime between 200-160 Ma. This age span corresponds well with records of fossil tubular cases from the Mongolian Mid Jurassic deposits. Construction of portable cases by the ancestor of this group possibly decreased the predation pressure and made it possible for the larvae and pupae to produce a water movement over the body inside the case and cocoon by undulating body movements, increasing oxygen uptake (Wiggins, 2004). These two factors may have contributed to the group being able to exploit new habitats, such as oxygen poor lentic waters. Exploitations of new habitats may have spurred a very rapid diversification around 150 Ma, which could explain the difficulties in resolving the phylogenetic pattern within Leptoceroidea (Fig. 5).

In summary, the timing of the diversification within Trichoptera inferred from our analyses provides a framework for further studies within the order. Our phylogenetic hypothesis strengthens the knowledge about the relationships among and within the suborders and superfamilies of Trichoptera, as well as resurrects earlier notions about the placement and arrangement of the "spicipalpian" families, and also about the origin of Trichoptera. Future work using more data will hopefully help to resolve outstanding questions and refine our knowledge on the timeframe of diversification in caddisflies.

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Table 1. Information about the calibration points used for the divergence-time analyses, with calibrated tree segment, calibration ages, fossil taxa and fossil description reference.

Calibrated tree segment	Calibration age (Ma), lower-upper boundaries	Fossil taxa	Reference
Divergence point between *Agraylea* and (*Hydroptila,* (*Itytrichia, Neotrichia*))	90-180	*Agraylea cretaria*	(Botosaneanu, 1995)
Divergence point between *Costatrichia* and *Alisotrichia*	20-140	*Alisotrichia arizela*	(Wells & Wichard, 1989)
Divergence point between *Baraeodes* and (*Ernodes*, (*Baraea*, *Baraeomiya)*)	40-120	*Baraeodes pectinatus*	(Ulmer, 1912)
Stem to (*Lepidostoma*, (*Micrasema*, *Theliopsyche*)	60-160	*Baissoplectrum separatum*	(Ivanov, 2006)
Divergece point between *Diplectrona* and (*Hydropsyche*, *Asmicridea*)	40-170	*Diplectrona minima; Hydropsyche viduata*	(Ulmer, 1912)
Stem to (*Pseudoneureclipsis*, (*Protodipseudopsis*, *Dipseudopsis*)	90-160	*Phylocentropus cretaceous*	(Wichard & Boelling, 2000)
Divergence point between *Ganonema* and *Anisocentropus*	40-120	*Ganonema regulare*	(Ulmer, 1912)
Divergence point between *Helicopsyche angusta* and *Helicopsyche albescens*	40-120	*Helicopsyche confluens*	(Ulmer, 1912)
Divergence point between *Lype and Psychomyia*	20-180	*Lype recta*	(Mey, 1988)
Stem to *Marilia*	20-180	*Marilia altrocki*	(Wichard, 1986)
Divergence point between *Molannodes* and *Molanna*	40-130	*Molanna crassicornis; Molanna indubius*	(Ulmer, 1912)
Stem to (*Chimarra*, (*Cryptobiosella*, *Hydrobiosella*)	90-275	*Dolophilodes shurabica*	(Sukatsheva & Rasnitsyn, 2004)
Stem to *Rhyacophila*	60-275	*Rhyacophila antiquissima*	(Botosaneanu & Wichard, 1983)
Divergence point between *Silo* and *Silonella*	20-60	*Silo brevicornis*	(Ulmer, 1912)
Divergence point between *Stenopsyche* and *Stenopsychodes*	40-140	*Stenopsyche imitata*	(Ulmer, 1912)
Divergence point between *Triplectides* and (*Athripsodes*, *Oecetis*)	40-160	*Triplectides rudis*	(Ulmer, 1912)

Table 2. Results from the Xia test of saturation in DAMBE for the different codon position partitions of each gene, with partition names (gene + codon position), number of operational taxonomic units (nOTU) used, indexes of substitution saturation (I_{ss}), the critical I_{ss} values for symmetrical and asymmetrical trees, along with their respective P-values. Coloration of values follow the interpretation guide.

Partition	nOTUs	Iss	ISS.cSym	P	Iss.cAssym	P	Partition	nOTUs	ISS	ISS.cSym	P	Iss.cAssym	P
CAD 1st	4	0.247	0.794	0.0000	0.824	0.0000	IDH 1st	4	0.238	0.777	0.0000	0.767	0.0000
	8	0.246	0.776	0.0000	0.711	0.0000		8	0.237	0.734	0.0000	0.637	0.0000
	16	0.244	0.597	0.0000	0.470	0.0007		16	0.248	0.643	0.0000	0.455	0.0000
	32	0.247	0.767	0.0000	0.520	0.0001		32	0.252	0.691	0.0000	0.376	0.0066
CAD 2nd	4	0.183	0.795	0.0000	0.825	0.0000	IDH 2nd	4	0.203	0.777	0.0000	0.770	0.0000
	8	0.182	0.777	0.0000	0.713	0.0000		8	0.193	0.736	0.0000	0.640	0.0000
	16	0.190	0.597	0.0000	0.471	0.0004		16	0.203	0.639	0.0000	0.454	0.0000
	32	0.191	0.769	0.0000	0.523	0.0000		32	0.209	0.693	0.0000	0.382	0.0004
CAD 3rd	4	0.645	0.781	0.0020	0.790	0.0010	IDH 3rd	4	0.669	0.794	0.0073	0.822	0.0011
	8	0.644	0.749	0.0160	0.666	0.6097		8	0.657	0.775	0.0093	0.709	0.2414
	16	0.639	0.618	0.6274	0.456	0.0000		16	0.653	0.598	0.2200	0.469	0.0001
	32	0.643	0.719	0.0786	0.432	0.0000		32	0.651	0.765	0.0115	0.516	0.0028
COI 1st	4	0.410	0.786	0.0000	0.802	0.0000	POL 1st	4	0.267	1.232	0.0057	1.549	0.0017
	8	0.402	0.758	0.0000	0.682	0.0000		8	0.260	1.517	0.0031	1.769	0.0014
	16	0.401	0.609	0.0005	0.460	0.3121		16	0.272	0.604	0.2443	1.149	0.0175
	32	0.404	0.736	0.0000	0.463	0.3001		32	0.279	2.005	0.0010	2.630	0.0002
COI 2nd	4	0.293	0.785	0.0000	0.800	0.0000	POL 2nd	4	0.600	1.260	0.2307	1.590	0.1576
	8	0.283	0.757	0.0000	0.679	0.0000		8	0.530	1.561	0.0893	1.831	0.0709
	16	0.294	0.610	0.0004	0.459	0.0547		16	0.556	0.610	0.8329	1.193	0.1960
	32	0.298	0.733	0.0000	0.458	0.0667		32	0.576	2.079	0.0796	2.754	0.0551
COI 3rd	4	0.711	0.785	0.0908	0.801	0.0408	POL 3rd	4	0.617	1.616	0.0000	2.113	0.0000
	8	0.738	0.757	0.6425	0.681	0.1619		8	0.612	2.134	0.0000	2.612	0.0000
	16	0.740	0.610	0.0008	0.459	0.0000		16	0.593	0.711	0.2209	1.760	0.0000
	32	0.738	0.735	0.9274	0.460	0.0000		32	0.592	3.026	0.0000	4.335	0.0000

Interpretation of results:

	Significant Difference	
	Yes	No
Iss<Iss.c	Little saturation	Substantial saturation
Iss>Iss.c	Useless sequences	Very poor for phylogenetics

Figure Captions

Figure 1. Bar chart showing the number of described species from the thirteen largest monophyletic, aquatic animal groups. [1] Rueda (2008), Ferrington (2007), Currie & Adler (2008), Wagner *et al.* (2007), Borkent (2012). World species of biting midges (Diptera: Ceratopogonidae). pdf available from URL: http://www.inhs.illinois.edu/research/FLYTREE/Borkent.html. [Accessed: 2012-03-22] [2] Holzenthal *et al.* 2011 [3] Crump (2009) [4] Sabatino *et al.* (2008) [5] Kalkman *et al.* (2008) [6] Hunt *et al.* (2007) [7] Fochetti and Figueroa (2008) [8] Lévêque *et al.* (2008) [9] Ferraris (2007) [10] Barber-James *et al.* (2007) [11] Hunt *et al.* (2007).

Figure 2. Earlier proposed hypotheses of the relationships among the three trichopteran suborders, modified from: a) Ross (1967); b) Weaver & Morse (1986); c) Frania & Wiggins (1997) and Ivanov (1997); d) Wiggins & Wichard (1989).

Figure 3. Phylogenetic Informativeness (PI) plots for all data partitions, derived from the phylogeny based on the complete data set. a) $PI_{original}$ b) $PI_{modified}$. These two analyses differ in the use of a normalization step for the informativeness values in $PI_{original}$, that is lacking in $PI_{modified}$. The X-axes denotes time from 240 Ma to present (0), while the Y-axes denote Phylogentic Informativeness.

Figure 4. Phylogenetic hypothesis derived from BI analyses based on the reduced, "most informative" data set, where all 3rd codon partitions were excluded except for that of POL. Values displayed above branches correspond to Bayesian posterior probabilities and below branches to ML Bootstrap frequencies. Nodes marked with a black circle (•) are used for divergence-time estimations (Fig. 5).

Figure 5. Condensed (to monophyletic families) chronogram resulting from time of divergence analysis in BEAST, based on the reduced, "most informative" data set and the topology from the BI analysis of this data set, displayed in Figure 4. Node values correspond to mean times and node bars for 95% confidence interval of node times. Dashed lines display arbitrarily resolved branches, that were collapsed in the original input tree. Colored branches represent the three suborders: "Spicipalpia" (yellow), Annulipalpia (red) and Integripalpia (blue). Drawings in the right margin show generalized pupal cocoons in dorsolateral cross-section, with surrounding stone structures and water flow (blue arrows), while drawings in the right margin show typical larval cases from respective families.

Supplementary information

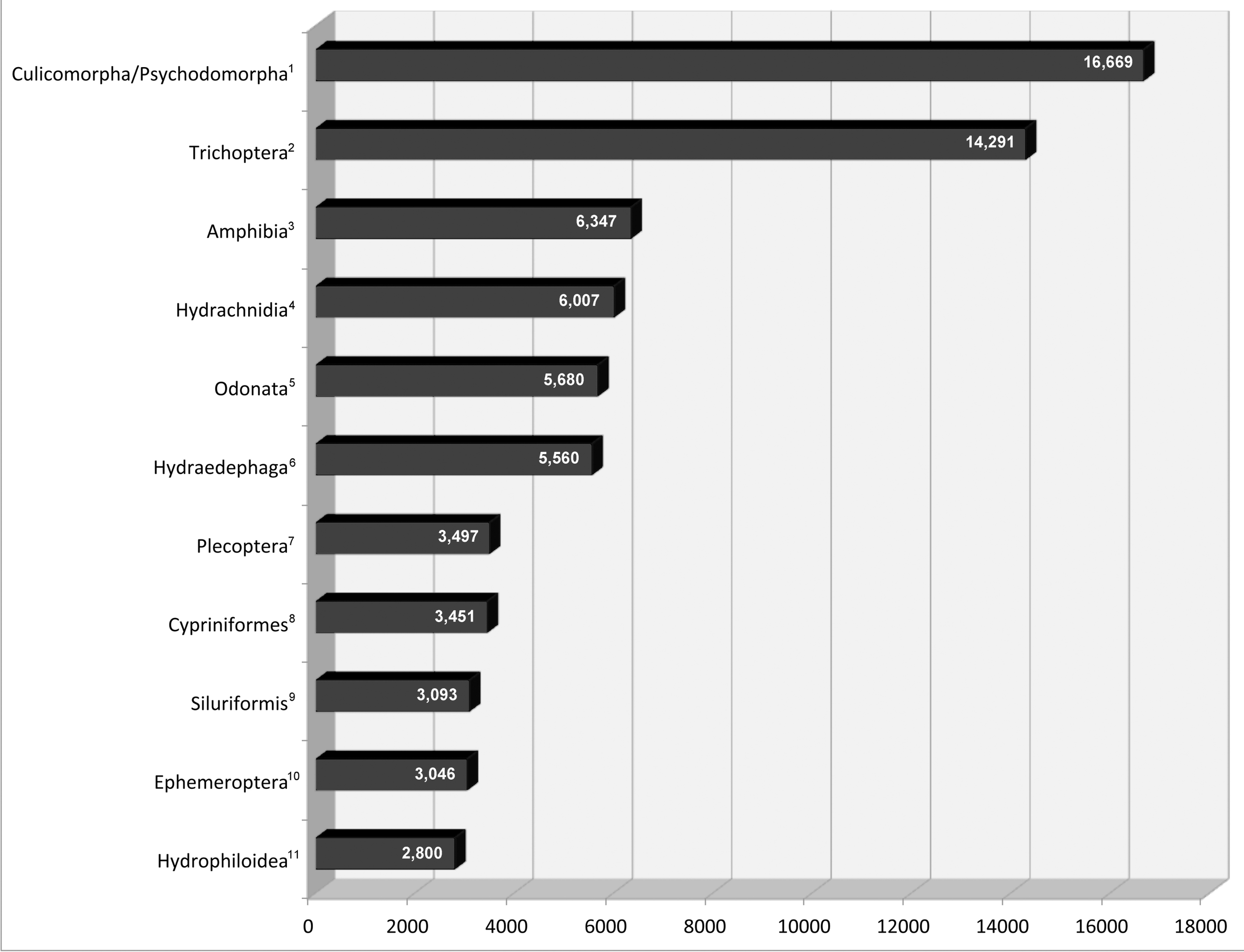
Table S1. List of species and respective families included in the analysis. DNA voucher codes without asterix (*) refer to unique tissue voucher codes at the Entomology Department, Swedish Museum of Natural History (NHRS). DNA voucher codes with asterix refer to unique voucher codes at University of Oulu, Finland and University of Turku, Finland. Each gene sequence fragment is given with length of fragment and GenBank accession number. Dashes represent missing sequences.

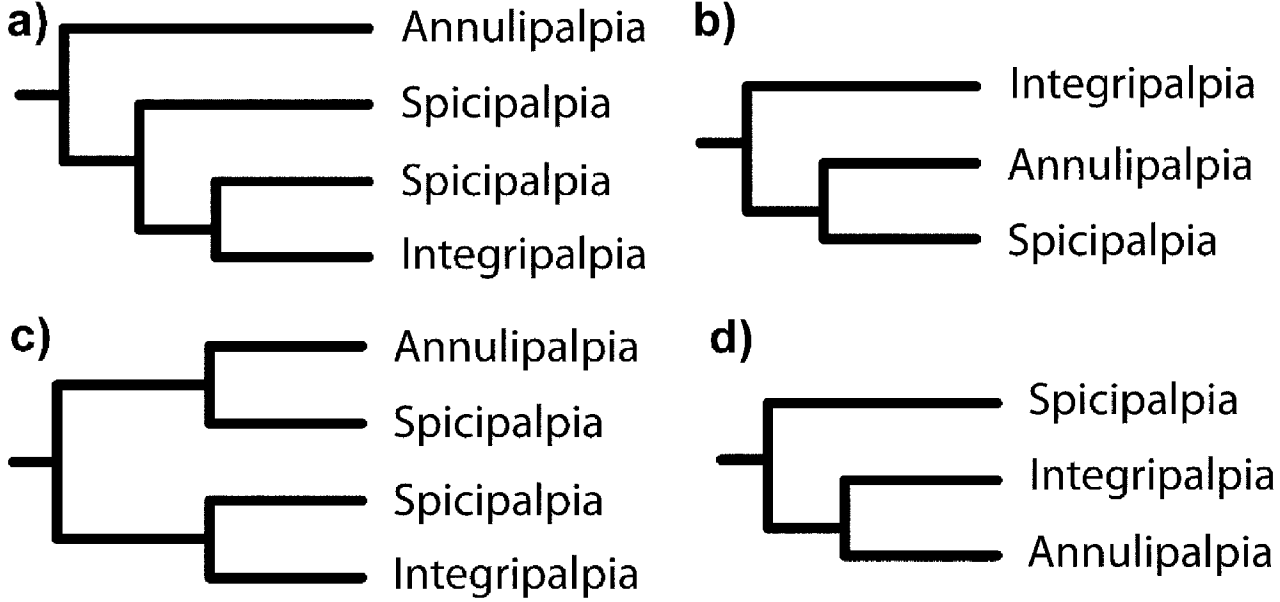
Figure S1. Phylogenetic hypothesis derived from Bayesian Inference (BI) analyses based on the complete data set. Values displayed above branches correspond to BI posterior probabilities and below branches to Maximum Likelihood (ML) bootstrap frequencies.

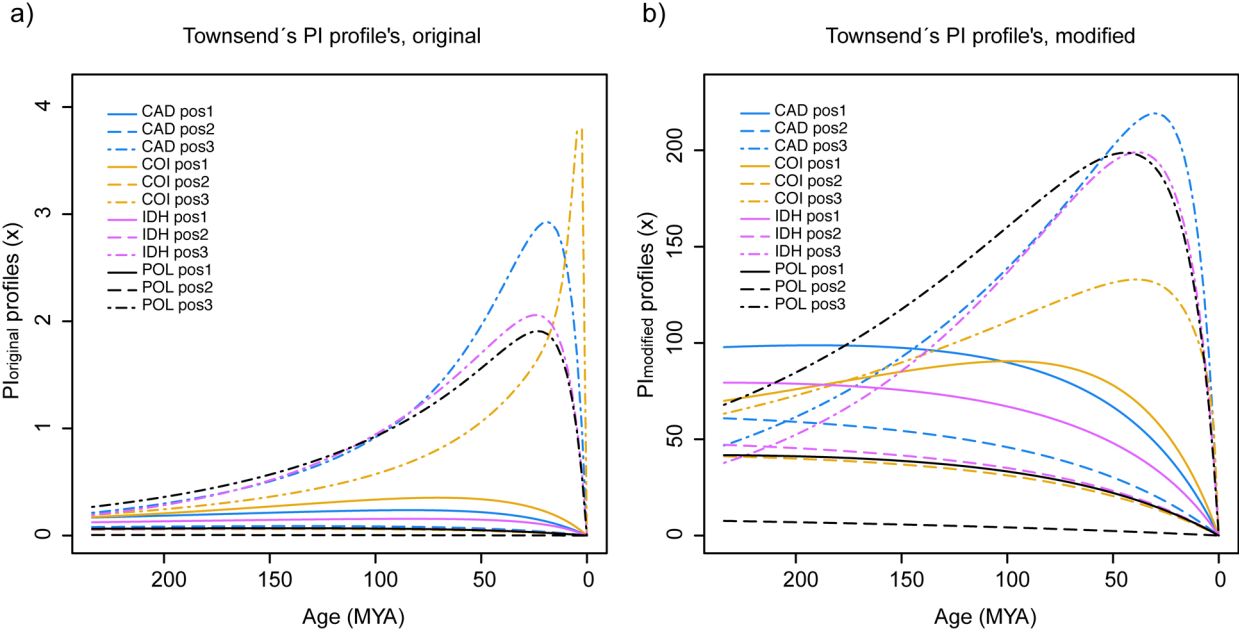
Figure S2. Chronogram resulting from time of divergence analysis in BEAST, based on the reduced, "most informative" data set and the topology from the BI analysis of this data set, displayed in Figure 4. Node values correspond to mean times and node bars for 95% confidence interval of node times.Appendix S1. Alignment in Nexus format of the complete data set.

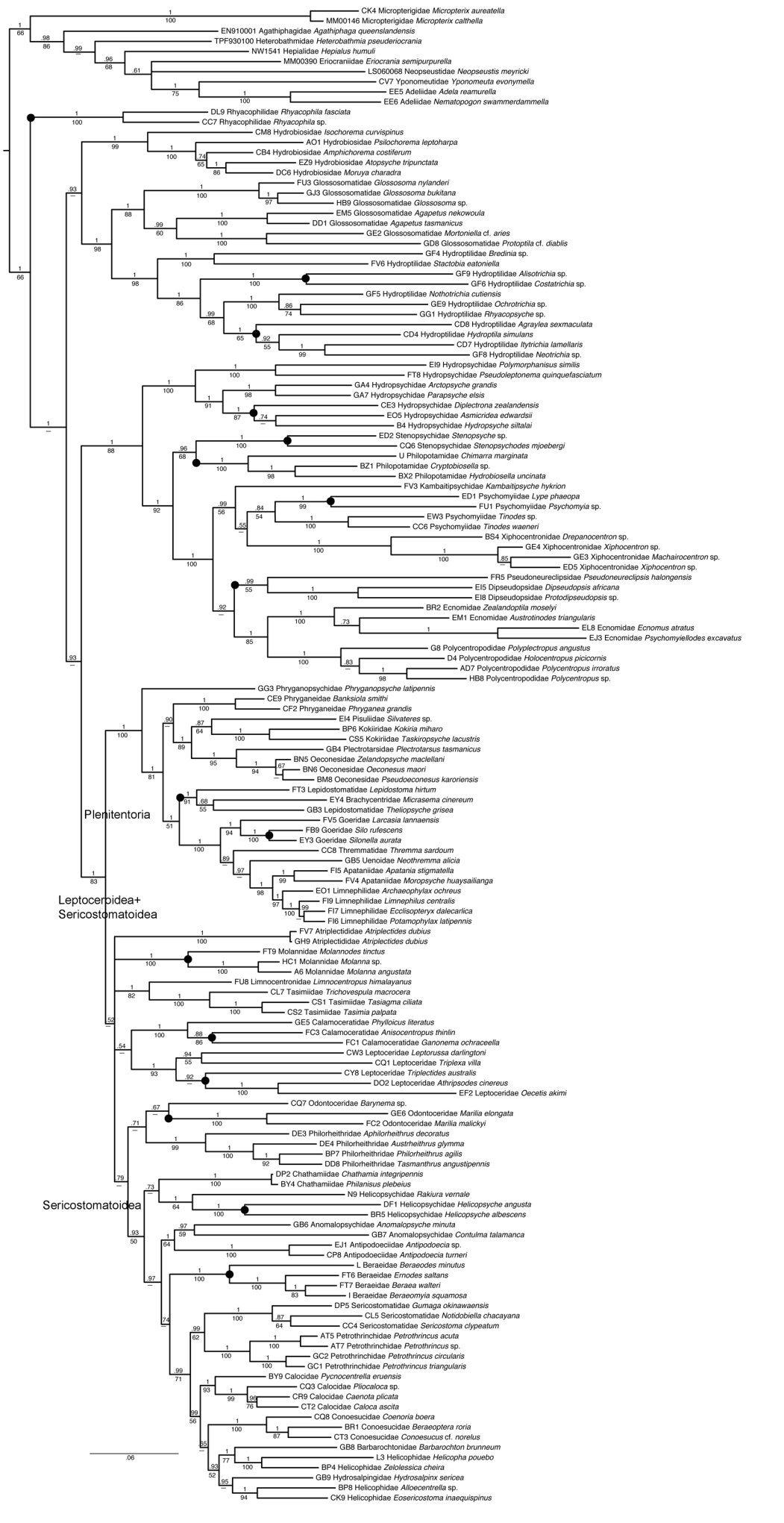
Appendix S1. Alignment in Nexus format of the full data set.

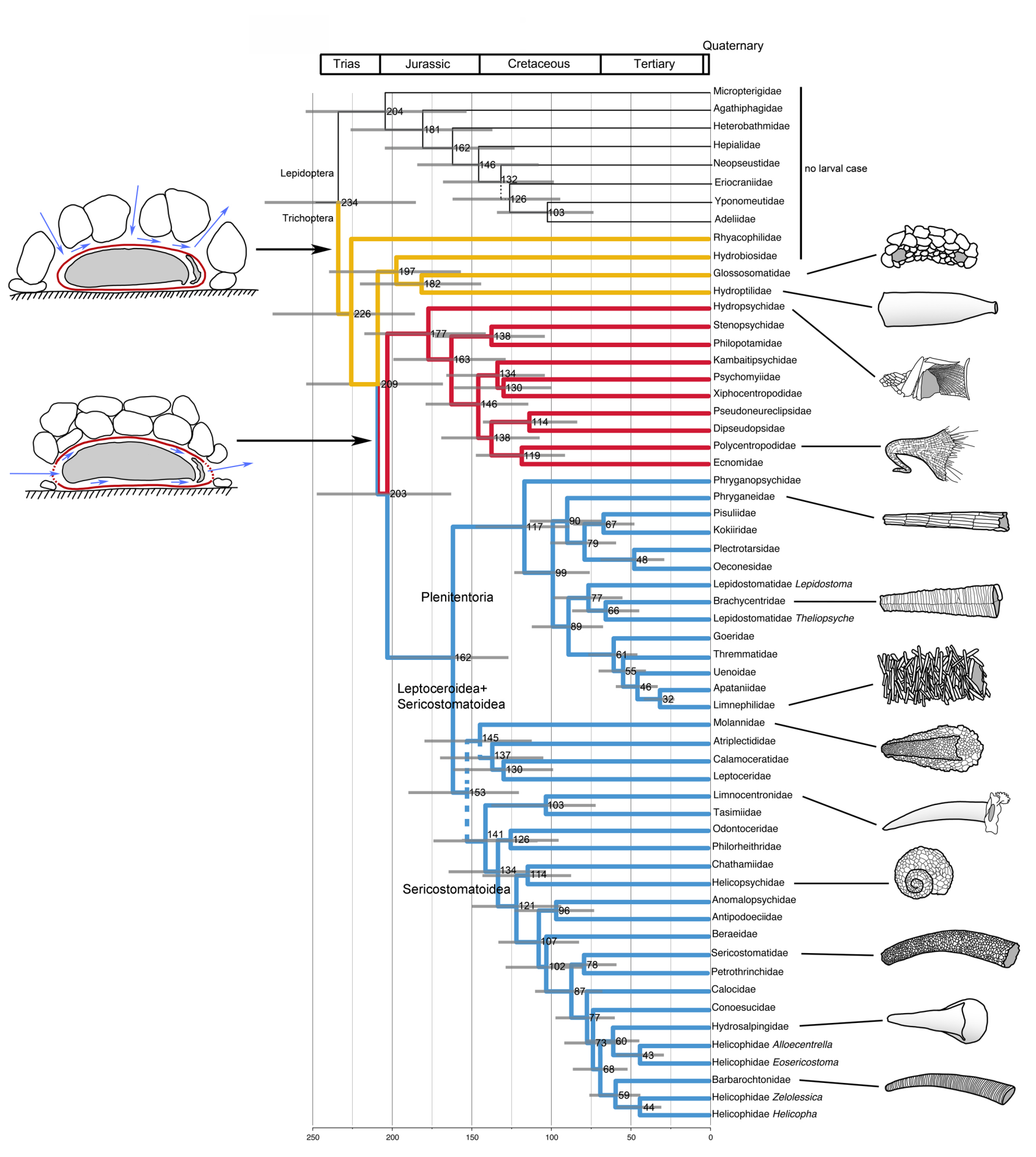
Appendix S2. Alignment in Nexus format of the reduced data set.


Culicomorpha/Psychodomorpha[1]
16,669
Trichoptera[2]
14,291
Amphibia[3]
6,347
Hydrachnidia[4]
6,007
Odonata[5]
5,680
Hydraedephaga[6]
5,560
Plecoptera[7]
3,497
Cypriniformes[8]
3,451
Siluriformis[9]
3,093
Ephemeroptera[10]
3,046
Hydrophiloidea[11]
2,800
0
2000
4000
6000
8000
10000
12000
14000
16000
18000


a)
Annulipalpia
Spicipalpia
Spicipalpia
Integripalpia
b)
Integripalpia
Annulipalpia
Spicipalpia
c)
Annulipalpia
Spicipalpia
Spicipalpia
Integripalpia
d)
Spicipalpia
Integripalpia
Annulipalpia


a)
Townsend´s PI profile's, original
CAD pos1
CAD pos2
CAD pos3
COI pos1
COI pos2
COI pos3
IDH pos1
IDH pos2
IDH pos3
POL pos1
POL pos2
POL pos3
PIoriginal profiles (x)
0
1
2
3
4
200
150
100
50
0
Age (MYA)
b)
Townsend´s PI profile's, modified
CAD pos1
CAD pos2
CAD pos3
COI pos1
COI pos2
COI pos3
IDH pos1
IDH pos2
IDH pos3
POL pos1
POL pos2
POL pos3
PImodified profiles (x)
0
50
100
150
200
200
150
100
50
0
Age (MYA)


CK4 Micropterigidae Micropterix aureatella
MM00146 Micropterigidae Micropterix calthella
EN910001 Agathiphagidae Agathiphaga queenslandensis
TPF930100 Heterobathmiidae Heterobathmia pseuderiocrania
NW1541 Hepialidae Hepialus humuli
MM00390 Eriocraniidae Eriocrania semipurpurella
LS060068 Neopseustidae Neopseustis meyricki
CV7 Yponomeutidae Yponomeuta evonymella
EE5 Adeliidae Adela reamurella
EE6 Adeliidae Nematopogon swammerdammella
DL9 Rhyacophilidae Rhyacophila fasciata
CC7 Rhyacophilidae Rhyacophila sp.
CM8 Hydrobiosidae Isochorema curvispinus
AO1 Hydrobiosidae Psilochorema leptoharpa
CB4 Hydrobiosidae Amphichorema costiferum
EZ9 Hydrobiosidae Atopsyche tripunctata
DC6 Hydrobiosidae Moruya charadra
FU3 Glossosomatidae Glossosoma nylanderi
GJ3 Glossosomatidae Glossosoma bukitana
HB9 Glossosomatidae Glossosoma sp.
EM5 Glossosomatidae Agapetus nekowoula
DD1 Glossosomatidae Agapetus tasmanicus
GE2 Glossosomatidae Mortoniella cf. aries
GD8 Glossosomatidae Protoptila cf. diablis
GF4 Hydroptilidae Bredinia sp.
FV6 Hydroptilidae Stactobia eatoniella
GF9 Hydroptilidae Alisotrichia sp.
GF6 Hydroptilidae Costatrichia sp.
GF5 Hydroptilidae Nothotrichia cutiensis
GE9 Hydroptilidae Ochrotrichia sp.
GG1 Hydroptilidae Rhyacopsyche sp.
CD8 Hydroptilidae Agraylea sexmaculata
CD4 Hydroptilidae Hydroptila simulans
CD7 Hydroptilidae Ityrtrichia lamellaris
GF8 Hydroptilidae Neotrichia sp.
EI9 Hydropsychidae Polymorphanisus similis
FT8 Hydropsychidae Pseudoleptonema quinquefasciatum
GA4 Hydropsychidae Arctopsyche grandis
GA7 Hydropsychidae Parapsyche elsis
CE3 Hydropsychidae Diplectrona zealandensis
EO5 Hydropsychidae Asmicridea edwardsii
B4 Hydropsychidae Hydropsyche siltalai
ED2 Stenopsychidae Stenopsyche sp.
CQ6 Stenopsychidae Stenopsychodes mjoebergi
U Philopotamidae Chimarra marginata
BZ1 Philopotamidae Cryptobiosella sp.
BX2 Philopotamidae Hydrobiosella uncinata
FV3 Kambaitipsychidae Kambaitipsyche hykrion
ED1 Psychomyiidae Lype phaeopa
FU1 Psychomyiidae Psychomyia sp.
EW3 Psychomyiidae Tinodes sp.
CC6 Psychomyiidae Tinodes waeneri
BS4 Xiphocentronidae Drepanocentron sp.
GE4 Xiphocentronidae Xiphocentron sp.
GE3 Xiphocentronidae Machairocentron sp.
ED5 Xiphocentronidae Xiphocentron sp.
FR5 Pseudoneureclipsidae Pseudoneureclipsis halongensis
EI5 Dipseudopsidae Dipseudopsis africana
EI8 Dipseudopsidae Protodipseudopsis sp.
BR2 Ecnomidae Zealandoptila moselyi
EM1 Ecnomidae Austrotinodes triangularis
EL8 Ecnomidae Ecnomus atratus
EJ3 Ecnomidae Psychomyiellodes excavatus
G8 Polycentropodidae Polyplectropus angustus
D4 Polycentropodidae Holocentropus picicornis
AD7 Polycentropodidae Polycentropus irroratus
HB8 Polycentropodidae Polycentropus sp.
GG3 Phryganopsychidae Phryganopsyche latipennis
CE9 Phryganeidae Banksiola smithi
CF2 Phryganeidae Phryganea grandis
EI4 Pisuliidae Silvateres sp.
BP6 Kokiiridae Kokiria miharo
CS5 Kokiiridae Taskiropsyche lacustris
GB4 Plectrotarsidae Plectrotarsus tasmanicus
BN5 Oeconesidae Zelandopsyche maclellani
BN6 Oeconesidae Oeconesus maori
BM8 Oeconesidae Pseudoeconesus karoriensis
FT3 Lepidostomatidae Lepidostoma hirtum
EY4 Brachycentridae Micrasema cinereum
GB3 Lepidostomatidae Theliopsyche grisea
FV5 Goeridae Larcasia lannaensis
FB9 Goeridae Silo rufescens
EY3 Goeridae Silonella aurata
CC8 Thrammatidae Thremma sardoum
GB5 Uenoidae Neothremma alicia
FI5 Apataniidae Apatania stigmatella
FV4 Apataniidae Moropsyche huaysailianga
EO1 Limnephilidae Archaeophylax ochreus
FI9 Limnephilidae Limnephilus centralis
FI7 Limnephilidae Ecclisopteryx dalecarlica
FI6 Limnephilidae Potamophylax latipennis
FV7 Atriplectididae Atriplectides dubius
GH9 Atriplectididae Atriplectides dubius
FT9 Molannidae Molannodes tinctus
HC1 Molannidae Molanna sp.
A6 Molannidae Molanna angustata
FU8 Limnocentropidae Limnocentropus himalayanus
CL7 Tasimiidae Trichovespula macrocera
CS1 Tasimiidae Tasiagma ciliata
CS2 Tasimiidae Tasimia palpata
GE5 Calamoceratidae Phylloicus literatus
FC3 Calamoceratidae Anisocentropus thinlin
FC1 Calamoceratidae Ganonema ochraceella
CW3 Leptoceridae Leptorussa darlingtoni
CQ1 Leptoceridae Triplexa villa
CY8 Leptoceridae Triplectides australis
DO2 Leptoceridae Athripsodes cinereus
EF2 Leptoceridae Oecetis akimi
CQ7 Odontoceridae Barynema sp.
GE6 Odontoceridae Marilia elongata
FC2 Odontoceridae Marilia malickyi
DE3 Philorheithridae Aphilorheithrus decoratus
DE4 Philorheithridae Austrheithrus glymma
BP7 Philorheithridae Philorheithrus agilis
DD8 Philorheithridae Tasmanthrus angustipennis
DP2 Chathamiidae Chathamia integripennis
BY4 Chathamiidae Philanisus plebeius
N9 Helicopsychidae Rakiura vernale
DF1 Helicopsychidae Helicopsyche angusta
BR5 Helicopsychidae Helicopsyche albescens
GB6 Anomalopsychidae Anomalopsyche minuta
GB7 Anomalopsychidae Contulma talamanca
EJ1 Antipodoeciidae Antipodoecia sp.
CP8 Antipodoeciidae Antipodoecia turneri
L Beraeidae Beraeodes minutus
FT6 Beraeidae Ernodes saltans
FT7 Beraeidae Beraea walteri
I Beraeidae Beraeomyia squamosa
DP5 Sericostomatidae Gumaga okinawaensis
CL5 Sericostomatidae Notidobiella chacayana
CC4 Sericostomatidae Sericostoma clypeatum
AT5 Petrothrinchidae Petrothrincus acuta
AT7 Petrothrinchidae Petrothrincus sp.
GC2 Petrothrinchidae Petrothrincus circularis
GC1 Petrothrinchidae Petrothrincus triangularis
BY9 Calocidae Pycnocentrella eruensis
CQ3 Calocidae Pliocaloca sp.
CR9 Calocidae Caenota plicata
CT2 Calocidae Caloca ascita
CQ8 Conoesucidae Coenoria boera
BR1 Conoesucidae Beraeoptera roria
CT3 Conoesucidae Conoesucus cf. norelus
GB8 Barbarochtonidae Barbarochton brunneum
L3 Helicophidae Helicopha pouebo
BP4 Helicophidae Zelolessica cheira
GB9 Hydrosalpingidae Hydrosalpinx sericea
BP8 Helicophidae Alloecentrella sp.
CK9 Helicophidae Eosericostoma inaequispinus
Plenitentoria
Leptoceridea+
Sericostomatoidea
Sericostomatoidea
.06


Quaternary
Trias
Jurassic
Cretaceous
Tertiary
Micropterigidae
Agathiphagidae
Heterobathmidae
Hepialidae
Neopseustidae
Eriocraniidae
Yponomeutidae
Adeliidae
Rhyacophilidae
Hydrobiosidae
Glossosomatidae
Hydroptilidae
Hydropsychidae
Stenopsychidae
Philopotamidae
Kambaitipsychidae
Psychomyiidae
Xiphocentropodidae
Pseudoneureclipsidae
Dipseudopsidae
Polycentropodidae
Ecnomidae
Phryganopsychidae
Phryganeidae
Pisuliidae
Kokiiridae
Plectrotarsidae
Oeconesidae
Lepidostomatidae Lepidostoma
Brachycentridae
Lepidostomatidae Theliopsyche
Goeridae
Thremmatidae
Uenoidae
Apataniidae
Limnephilidae
Molannidae
Atriplectididae
Calamoceratidae
Leptoceridae
Limnocentronidae
Tasimiidae
Odontoceridae
Philorheithridae
Chathamiidae
Helicopsychidae
Anomalopsychidae
Antipodoeciidae
Beraeidae
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Petrothrinchidae
Calocidae
Conoesucidae
Hydrosalpingidae
Helicophidae Alloecentrella
Helicophidae Eosericostoma
Barbarochtonidae
Helicophidae Zelolessica
Helicophidae Helicopha
no larval case
Lepidoptera
Trichoptera
Plenitentoria
Leptoceroidea+
Sericostomatoidea
Sericostomatoidea
204
181
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