Evidence for an Earliest Late Carboniferous Divergence Time and the Early Larval Ecology and Diversification of Major Holometabola Lineages

EVIDENCE FOR AN EARLIEST LATE CARBONIFEROUS DIVERGENCE TIME AND THE EARLY LARVAL ECOLOGY AND DIVERSIFICATION OF MAJOR HOLOMETABOLA LINEAGES

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Abstract.—Four fossil taxa of earliest Holometabola recently were identified based on a variety of determinative evidence and assigned to a stem-group or a basal lineage within a modern order. One consequence of these new discoveries is that the divergence date between the Holometabola and its sister clade has been set earlier than previously thought on the basis of fossils or molecular phylogenetic evidence. This new date provides a minimal calibration date pegged to the Early Carboniferous–Late Carboniferous boundary, and approximates an absolute date of 318 million years. The four taxa provide new insights into the life habits, particularly feeding habits, of early holometabolan larvae. Additionally, they require an explanation of why there is an 80 million year lag between the origin and the taxonomic dominance of this profound developmental innovation in early terrestrial ecosystems. An earlier lead time is likely present for these earliest holometabolan fossils, which can be extrapolated conservatively to the Late Mississippian, 328 to 318 million years ago. Consequently, a focused investigation on earlier, Late Mississippian strata to search for ancestral holometabolan insects is imperative.

INTRODUCTION

Perhaps the most significant issue in the study of fossil insects has been the origin of the Holometabola. The Holometabola, also known as the Endopterygota, are characterized by complete metamorphosis in which a larva hatches from an egg, undergoes several discrete growth increments, eventually becomes a pupa, typically the resting stage, and ends as an emergent adult. Considerable attention has been devoted toward understanding three features of the currently highly speciose Holometabola. First has been the timing of the origin of the Holometabola during the late Paleozoic (Hennig, 1969; Kukalová-Peck, 1997; Gaunt and Miles, 2002; Nel et al., 2007; Wiegmann et al., 2009). Second, there have been many attempts to identify taxa that comprised the earliest holometabolans, either from morphological or evolutionary developmental traits of the modern taxa or from phylogenetic inference based on fossil occurrences (Hornschemeyer, 2002; Whiting, 2004; Nel et al., 2007; Béthoux, 2008; Wiegmann et al., 2009). Third, considerable effort has been devoted toward understanding the processes that resulted in the endopterygote

condition, from both an intrinsic, evolutionary developmental perspective that accounts for the origin of the larval and pupal stages, and from the regional or global environmental conditions during the late Paleozoic that would have selected for holometabolous traits (Lameere, 1908; Hinton, 1963; Sehnal et al., 1996; Truman and Riddiford, 1999; Nel et al., 2007). These three features circumscribe the principle issues defining the early history of the Holometabola, and until recently, there has been minimal progress on all three fronts. However, recent concerted efforts have narrowed the compass of possibilities for addressing these issues and, in some cases, have provided surprising results in determining when the Holometabola initially diversified, the groups or clades that constituted the earliest Holometabola, and the selective advantage of the endopterygote condition over other insect developmental patterns under late Paleozoic and Triassic environmental conditions.

The distinctive developmental trajectory that defines the Holometabola is contrasted with the hemimetabolous condition. Under hemimetabolous development, an egg directly hatches into a series of discrete nymphal stages that largely resemble and give rise to the adult stage, which also can be compared to the ametabolous

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condition characterized by minimal developmental change. Insects with holometabolous development currently are the most diverse clade of multicellular organisms, consisting of approximately 766,100 described living species (Davis et al., 2010), although the total number is probably a several-fold multiple of that figure (Kristensen, 1999). The Holometabola presently constitute about 82.7 percent of all insects, and probably have been the dominant clade of insects since the mid Triassic. When considered as a group, the Holometabola has invaded every terrestrial and freshwater habitat and have made considerable inroads along marginal marine–land and marine–air interfaces (Wheeler, 1990; Mayhew, 2007). Collectively, they have an amazingly broad dietary repertoire, and include taxa that range from detritivores and other decomposers, microvores, fungivores to especially herbivores, but also are consumers of animals as predators, parasitoids and parasites and inhabit unusual habitats such as oil seeps, playa lake brines, and hot springs (Wheeler, 1990; Kristensen, 1999; Labandeira, 1999, 2002).

One of the key features of the holometabolous condition is the developmental and ecological separation of their life cycle into a feeding stage, the larva, which typically bear working mouthparts for the bulk processing food but lack capabilities for mating and flight, versus the reproductive stage, the adult, which is endowed with locomotory organs of functional wings and legs, and genitalia that efficiently complete reproduction. The distinctive larva undoubtedly originated from a hemimetabolous mode of development once in the history of insects, albeit in one of two ways (Truman and Riddiford, 1999). The nymphal replacement hypothesis (Berlese, 1913; Imms, 1937) proposes that the new larval instar emerging from the insect egg essentially became a feeding embryo and progressively overtook the feeding responsibilities of successive nymphal instars, such that nymphal instars were reduced to a single instar that became the pupa in holometabolous development. Alternatively, the nymphal equivalency hypothesis indicates that complete metamorphosis originated by equivalency between nymphal and larval instars, with the pupal stage arising as a new, inserted, resting stage as the morphological disparity between larva and adult widened (Poyarkoff, 1914; Hinton, 1963). Although the nymphal equivalency hypothesis is more widely supported and accounts for larval structures such as abdominal prologs as subsequently derived structures (Sehnal et al., 1996), the nymphal replacement hypothesis better explains origin of complete metamorphosis as a modification of embryonic stages (Truman and Riddiford, 1999). The consequence of these profound, developmental changes was that holometaboly immediately provided larval-feeding and adult-reproductive adult instars access to very different habitats that often were deployed in a seasonal or other spatiotemporally exclusive manner—thus allowing survival of both during mutually unfavorable conditions.

The principal goal of this contribution is to provide divergence times from recent evidence that document the presence of holometabolous taxa during the Late Carboniferous Period, herein equivalent to the Pennsylvanian Subperiod (Ogg et al., 2008). Four recent reports indicate that structurally varied larval and adult Holometabola, representing four distinctive taxa, were present during Pennsylvanian times. A related objective is to place these taxa in a relevant temporal context and to indicate the early larval ecology of the Holometabola. Last, a discussion ensues regarding the selection pressures affecting these earliest known taxa during an interval of major environmental change.

METHODS

Several reports have mentioned Pennsylvanian age Holometabola. Four taxa from these reports are accepted (Fig. 1) and are positioned on a recent, well-supported cladogram (Wiegmann et al., 2009) as proximal as possible to their inferred insect order or stem-group (Fig. 2), based on characters and taxonomic assignments from the respective descriptions. Accordingly, the earliest occurrences of many holometabolous clades had to be moved downward, earlier in time, to accommodate not only long-known Early Permian fossils, but also the four earlier holometabolous occurrences mentioned herein (also see Davis et al., 2010). The latter four taxa are reillustrated (Fig. 1), and the likelihood of a correct assignment is discussed. Implicit in this procedure was acknowledgment that varied types of evidence can point to the presence of early Holometabola in the fossil record, including compelling trace-fossil and body-fossil data, information on larval and adult stages, and uncertainties regarding placement of

taxa in a preferred but frequently less than ideal clade or stem-group. As well, other claims of Pennsylvanian age or earlier fossils of Holometabola are reviewed and evaluated.

RESULTS

Of the reports that provide reasonable evidence for the existence of Pennsylvanian Holometabola, all were assigned conservatively to either basal or basalmost segments of modern orders, or to stem groups subtending modern orders (Fig. 1). These occurrences originated from three Euramerican localities: Mazon Creek, yielding *Adiphlebia lacoana* Scudder 1885 (Béthoux, 2008) and "*Srokalarva berthei*," informally described by Kukalová-Peck and Shear (1990), both complete body fossils from the late Moscovian-age Carbondale Formation in the north-central Illinois Basin; Berryville and Calhoun, producing *Pteridotorichnos stipitopteri* Labandeira and Phillips 2002 galls from the Kasimovian-age Calhoun Coal of the Mattoon Formation in the southern Illinois Basin; and Pas-de-Calais, providing *Westphalomerope maryvonneae* Nel, Roques, Nel, Prokop and Steyer 2007 wings from the Bashkirian-age Vicoigne Series strata of northeastern France. These occurrences, summarized in Table 1, collectively represent an interval of approximately 15 million years, accounting for most of the Pennsylvanian Subperiod. In addition, placement of these fossils on a cladogram (Wiegmann et al., 2009) represent about half of the major Holometabola lineages and their stem-groups presumed to be present during the Pennsylvanian, namely the Antliophora (*Srokalarva*), Mecopteroidea (*Westphalomerope*), early Coleoptera (*Adiphlebia*), and early Hymenoptera or its stem-group (*Pteridotorichnos*). Additional fossils dated to the Pennsylvanian and Late Mississippian age may supply many of the previously missing lineages present during the earliest diversification phase of the Holometabola, and provide a divergence-time estimate of Late Mississippian, equivalent to the Serpukhovian Stage ranging from approximately 328 to 318 million years ago. There is precedent for finding plant-insect associations in Late Mississippian sedimentary strata, which may reveal the presence of insect taxa that were not originally deposited or have not yet been found. For example, the existence of distinctive external foliage feeding damage on leaves has revealed the probable presence of an orthopteroid insect (Iannuzzi and

Table 1. Documentation for a Divergence-Time Estimate from the Earliest Holometabola Insect Fossils.

| Taxon and figure	Clade assignment	Age and formation	Locality	Fossil details	References
Adiphlebia lacoana Scudder 1885 **(Fig. 2A)**	Coleoptera	Middle Pennsylvanian, late Moscovian Stage; Carbondale Formation	Mazon Creek, north-central Illinois Basin, Illinois, USA	Body fossils; adult insect in a siderite nodule (five specimens)	Scudder 1885; Béthoux 2008
"*Srokalarva berthei*", but Kukalová-Peck never formally described this fossil **(Fig. 2G)**	Antliophora stem-group	Middle Pennsylvanian, late Moscovian Stage; Carbondale Formation	Mazon Creek, north-central Illinois, USA	Body fossil; larval insect in a siderite nodule (one specimen)	Shear & Kukalová Peck 1990; Kukalová-Peck 1997; Labandeira & Santiago-Blay 2002
Pteridotorichnos stipitopteri Labandeira and Phillips 1996 **(Fig. 2D–F)**	?Hymenoptera	Late Pennsylvanian, Kasimovian Stage Mattoon Formation	Berryville and Calhoun, Illinois Basin, south eastern Illinois, USA	Trace fossils; galls in *Stipitopteris* rachises of *Psaronius chasei* Morgan (about 35 specimens)	Lesnikowska 1990; Labandeira & Phillips 1996, 2002
Westphalomerope maryvonneae Nel, Roques, Nel, Prokop and Steyer 2007 **(Fig. 2B, C)**	Mecopteroidea	Early Pennsylvanian, Bashkirian Stage ("Veine Maroc, Vicoigne Series")	Pas-de-Calais, Bruay-la-Brussière, northern France	Body fossil; wing impression	Nel et al. 2007
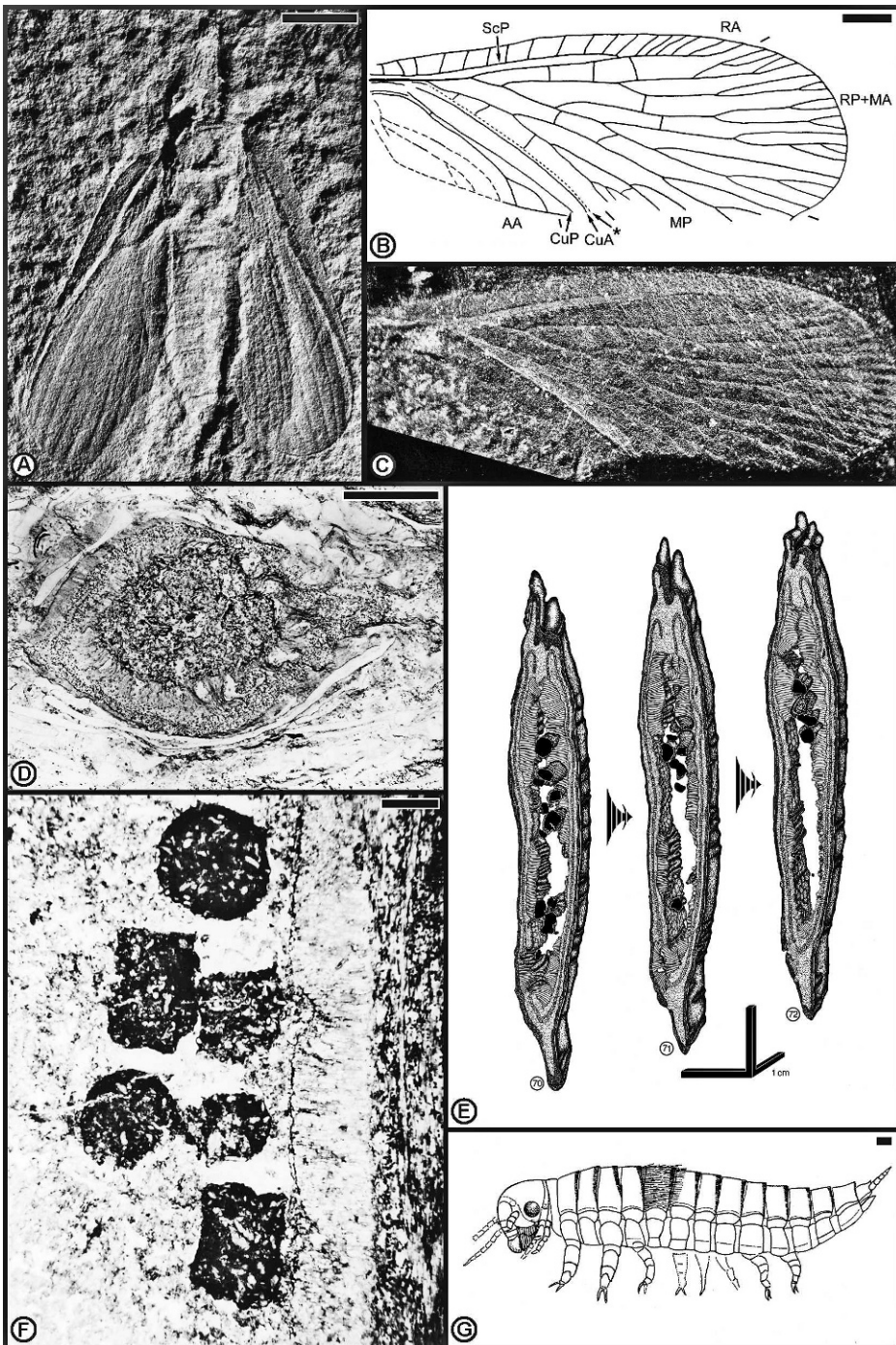
Fig. 1. The earliest examples of Holometabola in the fossil record. **A.** *Adiphlebia lacoana* Scudder 1885, an early member of the Coleoptera; from the Middle Pennsylvanian of Mazon Creek, U.S.A. Reprinted with permission from Béthoux (2008). Specimen USNM 38140; scale bar = 0.5 mm. **B.** Drawing of *Westphalomerope maryvonneae* Nel, Roques, Nel, Prokop and Steyer 2007, an early member of the Mecopteroidea, from the Early Pennsylvanian of Pas-de-Calais, France. Reprinted with permission; from Nel, et al. (2007). Specimen MNHN-Lp-R.55181; scale bar =

Labandeira, 2008), even though it precedes relevant body-fossil record by several million years (Labandeira, 2008).

Prior to discovery of the four occurrences mentioned above, there were three reports of holometabolous insects present in Devonian and Pennsylvanian strata, all based on interpretations of wing venation. A crucial evaluation of those records earlier than the Permian is important to place the presence of the four accepted Pennsylvanian Holometabola in proper context. These earlier, presumptive occurrences were mentioned by Hennig in his 1969 book, *Die Stammesgeschichte der Insekten*, wherein the methodology of modern phylogenetic systematics was presented. Commenting on the origin of the Holometabola, Hennig indicated that the clade originated deep in pre-Carboniferous time, a date that evidently was informed by discovery of *Eopteridium striatum* Rohdendorf 1961, erroneously interpreted as a derived insect wing (Crowson, 1970) instead of a crustacean telson (Schram, 1980). Because of this misidentification, Hennig (1969) indicated that the endopterygote condition originated as early as perhaps the Devonian, deemed a significant underestimate by one reviewer of the book (Crowson, 1970), but now plausible given recent molecular phylogenies that are converging on a Mississippian date (Gaunt and Miles, 2002; Wiegmann et al., 2009; Davis et al., 2010). It now seems that the interval of the initial holometabolan radiation occupied the entirety of the Pennsylvanian and extended upward to the mid Early Permian with the earliest occurrences of several modern orders (Kukalová-Peck and Willmann, 1990; Novokshonov, 2004; Beckemeyer and Hall, 2007; Davis et al., 2010; Minet et al., 2010). Hennig accepted the Holometabola as a monophyletic group, and provided

evidence that supported many of the groupings recognized by earlier (Carmean et al., 1992; Pashley et al., 1993; Chalwatzis et al., 1996) and more recent (Whiting, 2002, 2004; Beutel and Pohl, 2006; Wiegmann et al., 2009) molecular phylogenies, especially the clades Neuropteroidea, Coleopteroidea (possibly including the Strepsiptera), Hymenoptera, Mecopteroidea, Antliophora (possibly including the Siphonaptera), and Amphiesmenoptera.

Among candidate taxa representing early, Pennsylvanian occurrences of holometaboly, *Metropator* and *Fatjanoptera* are probably most notable (Hennig, 1969; Štys and Soldán, 1980). The wings of these two taxa were considered to have synapomorphies of the Holometabola, although eventually these taxa were removed from the Holometabola for different reasons—*Metropator* because its affinities where shifted to a nonholometabolous group, and *Fatjanoptera* because of a change in date from Upper Carboniferous to Lower Permian based on subsequent stratigraphic correlations. *Metropator pusillus* Handlirsch 1906 is of Early Pennsylvanian age (Namurian Stage) and originates from the Altamont Coal Mine of the Lower Pottsville Group in central Pennsylvania. This fossil wing was initially placed in the Mecoptera by Tillyard (1926), and subsequently assigned to the poorly known order Miomoptera by Carpenter (1965), which has an occurrence ranging from the Middle Pennsylvanian to the Late Permian. The affinities of the Miomoptera have been controversial, once thought to be holometabolous in origin (Martynov, 1938) but now considered either a stemgroup of the Holometabola (Kukalová-Peck, 1991) or more distantly related to the Holometabola (Grimaldi and Engel, 2005; Beutel and Pohl, 2006). Most likely the Miomoptera is a nonholo-

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0.5 mm. **C.** Photographic image of entire wing specimen at same scale as drawing in (B). **D.** Photographic image of a section of the stem gall of *Pteridotorichnos stipitopteri* Labandeira and Phillips in the *Stipitopteris* rachis of *Psaronius chasei* Morgan (Marattiales: Psaroniaceae), showing galled inner parenchyma and proliferation of nutritive tissue. Berryville collection of the Calhoun Coal; specimen USNM–BV32–Mtop; Scale bar = 10 mm. **E.** Three-dimensional reconstruction of an obliquely sectioned galled petiole of *P. stipitopteri* representing 70 successive acetate peels of tissue and cellular anatomy; specimen UIUC–31272, from the same provenance as (E) above; solid bars in 3-D. The scale each represents 10 mm. **F.** Detail of inner histological detail, including nutritive tissue, mandibulate bite marks, barrel-shaped coprolites and insect frass of a *P. stipitopter*i gall from same provenance as (D) above; specimen UIUC–30823–Dbot, peel 25; scale bar = 1 mm. **G.** Reconstruction of *Srokalarva berthei* by J. Kukalová-Peck, from the Middle Pennsylvanian of Mazon Creek, U.S.A. Reprinted with permission from Kukalová-Peck (1997). Specimen MCP–322; scale bar = 1 mm.
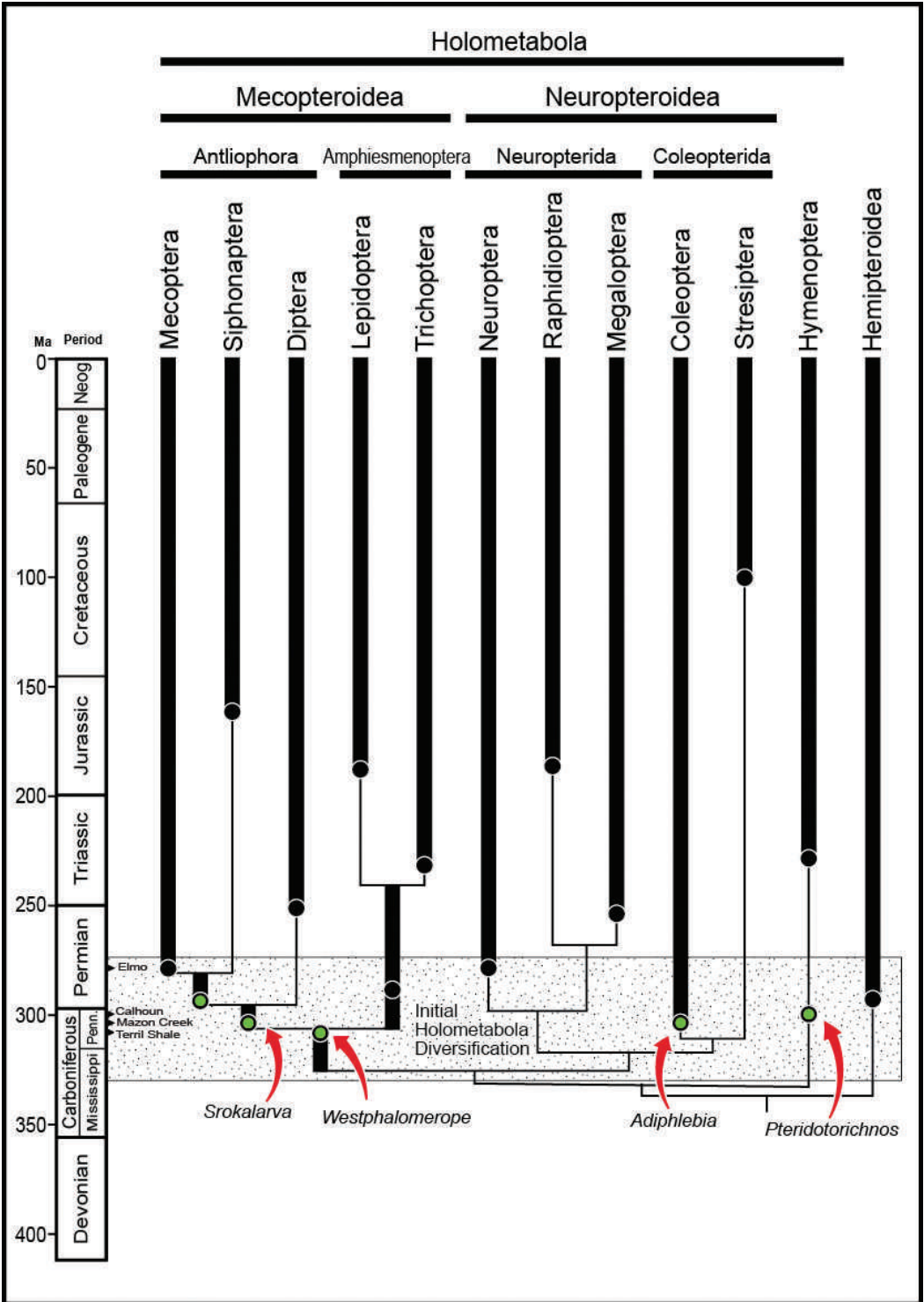
Fig. 2.   Placement of fossil evidence for the earliest Holometabola within a phylogenetic context. Geologic time line at left is after Ogg, et al. (2008); note that the Mississippian is equivalent to the Early Carboniferous and

metabolan lineage, as it lacks any diagnostic apomorphies that would allow referral to the Holometabola (Kristensen, 1999; Nel et al., 2007).

Fatjanoptera mnemonica Martynova 1961 is an insect wing assigned to the Raphidioidea and found in the Burgukli Series of the Tugunska Basin, from the Krasnoyarsk Region of Russia (Crowson et al., 1967). The age of the Lower Burgukli Series was considered to be of Late Carboniferous (Pennsylvanian) age by Martynova (1961), but subsequently was determined to be of Early Permian age, most recently by Kotlyar and colleagues (2006). Even though this specimen now is Early Permian in age, the assignment of *F. mnemonica* to the Raphidioidea is suspect, as the next youngest reliable assignment is mid Mesozoic (Engel, 2002). This extended ghost range for the Raphidioidea between *F. mnemonica* and the earliest widely accepted snakefly from the mid Mesozoic (Engel, 2002) thus is a second reason for doubting the early holometabolous nature of this specimen.

DISCUSSION

There are three issues resulting from the geochronologic extension of fossil Holometabola to the Pennsylvanian–Mississippian Subperiod boundary or even earlier. First is whether there are alternative cladistic relationships of major holometabolous clades that would affect the conclusions presented herein. Second, direct and indirect data reveal the life histories of three distinct larval types present during Pennsylvanian time, and thus provide a context for larval life-history modes on various hypotheses regarding the developmental patterns of earliest Holometabola. Third, is the significance, if any, of the prolonged time lag between the origin of holometabolan insects and their eventual dominance in terrestrial and aquatic ecosystems.

Relevance of alternative cladistic relationships to the timing of Holometabola origins

There are alternative cladistic arrangements of major clades within the Holometabola, although almost all recent analyses recognize three major superclades (Fig. 2; also see Kristensen, 1999). The first superclade is a mecopteroid assemblage (Mecopteroidea), comprised of the Amphiesmenoptera, consisting of the well supported Lepidoptera and Trichoptera; plus the Antliophora, consisting of the internally less well supported Mecoptera, Siphonaptera and Diptera (Kristensen, 1999). The second superclade is a neuropteroid assemblage (Neuropteroidea), comprised of the Neuropterida, consisting of the Raphidioptera, Megaloptera and Neuroptera, which internally has variable support; plus the Coleopterida, comprising the Coleoptera and Strepsiptera (Wiegmann et al., 2009). The Hymenoptera is a third superclade that is positioned either at the base of the Holometabola, and thus sister-group to all other clades (Savard et al. 2005), or alternatively sister to the Mecopteroidea (Hennig, 1969; Kristensen, 1999). The position of the Strepsiptera has been variably affiliated with the Diptera (Whiting, 2002), although a relationship with or even within the Coleoptera recently has been the preferred hypothesis (Crowson, 1981; Wheeler et al., 2001; Wiegmann et al., 2009). An unresolved issue has been the relationships of three clades of the Neuropterida (Kristensen, 1999), but more contentious are the relationships within the Antliophora that has provided more fundamental problems, such as whether the Siphonaptera is embedded within the Mecoptera (Whiting, 2002; Beutel and Pohl, 2006), and the possible paraphyly of the Nannochoristidae, traditionally included as the basalmost clade in the Mecoptera (Friedrich and Beutel, 2009, but see Wiegmann et al., 2009). At the two levels of

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Pennsylvanian equivalent to the Late Carboniferous. Earliest reliable occurrences of taxa (solid dots, followed by a thick black line) are after various sources mentioned in the text; major localities for the initial diversification of the Holometabola are: Elmo, Kansas, the "insect bed" of the Wellington Formation from the Artinskian Stage of the Early Permian; Calhoun, the Calhoun Coal Member of the Mattoon Formation, from the Kasimovian Stage of the Late Pennsylvanian; Mazon Creek of the Francis Creek Shale Member of the Carbondale Formation, from the Moscovian Stage of the Middle Pennsylvanian; and the Terril Shale at Pas-de-Calais, Bruay-la-Bussière, France, from the Bashkirian Stage of the Early Pennsylvanian. The horizontal stippled bar at bottom represents the initial diversification and the earliest fossil occurrences of holometabolan insects in the fossil record.

highest rank (Fig. 2), clades of the Holometabola are relatively stable; by contrast, it is some ordinal-rank groupings that have unstable inter-relationships. In summary, these uncertainties have minimal impact on the timing of clade emergence during the Pennsylvanian presented in Fig. 2, a consequence attributable to (1), the taxonomically coarse-grained nature of holometabolous lineages present during the Pennsylvanian; and (2), the geochronological stability of supraordinal ranks within the Holometabola that extend to the earliest Pennsylvanian.

Importance of the larval stage for early holometabolan taxonomic diversity

By the end of the Pennsylvanian Subperiod apparently there was establishment of three major types of holometabolous larvae, given evidence from adult and larval body fossils as well as a distinctive plant-insect association. The presence of "*Srokalarva berthei*", an adult body fossil, indicates that an eruciform, externally feeding caterpillar was actively locomotory (Kukalová-Peck, 1997) and probably had a phytophagous diet. Attribution of this fossil to an eruciform larva is more parsimonious than the suggestion of an adult myriapod (Kristensen, 1999; Nel et al., 2007), especially as the abdominal leglets of *S. berthei* appear to be functional and structurally different from the three pairs of thoracic legs. Supporting this interpretation is Novokshonov's (2004) description of an early Permian eruciform larva with legs. The presence of leglets in *S. berthei*, perhaps functioning similar to the crotchets of Lepidoptera, is attributable to an expression pattern of the *Distal-less* gene that regulates early developmental expression of abdominal appendages throughout basal clades of Holometabola (Labandeira and Santiago-Blay, 2002), similar to abdominal structures found in extant scorpionfly and sawfly caterpillars. In addition, the presence of adult *Westphalomerope maryvonneae*, assigned to the Mecopteroidea stem group, provides a complimentary, albeit distantly related, adult stage for the *S. berthei* larva.

In a parallel development, *Pteridotorichnos stipitopteri* provides an independent line of evidence for a different type of eruciform larva, legless and endophytic in feeding habits. This interpretation is based on highly distinctive galls of the inner rachis parenchyma from marattialean tree-fern fronds. This assignment is based on a broad spectrum of exquisitely permineralized pristine and damaged tissue anatomy, demon-strating a typical holometabolous life style (Labandeira and Phillips, 1996, 2002). While this interpretation has been challenged by Grimaldi and Engel (2005) as being perhaps attributable to mites, repeated by others (Nel et al., 2007), there are significant factual problems with asserting a mite as a causal agent, as mites are an order-of-magnitude smaller in size, even during the Pennsylvanian, and all of the internal features of the gall, including barrel-shaped coprolites, man-dibulate bite marks, exit hole plugs, and nutritive tissue formation in the host plant strongly suggest a holometabolan galler (Labandeira and Phillips, 1996, 2002). In addition, while this type of feeding damage was not referred to any particular holometabolan clade, it is closest to symphytan Hymenoptera in overall features, an informal similarity buttressed by recent phylogenetic anal-yses indicating that the Hymenoptera is the earliest derived lineage within the Holometabola (Schlumeister et al., 2002; Schlumeister, 2003; Savard et al., 2005; Wiegmann et al., 2009), supported by morphological studies of adults (Vilhelmsen, 1997a, b), from which can be inferred an endophytic larva resembling *P. stipitopteri* during the late Pennsylvanian.

The third larval type is indicated by *Adiphlebia lacoana*, representing the earliest and phylogenet-ically basalmost occurrence of the Coleoptera, suggesting that its larva was a campodeiform predator, based on phylogenetic inferences (Ku-kalová-Peck and Lawrence, 2004), and extant archostematan beetles having a campodeiform larval stage (Crowson, 1981). Hennig (1969) provided a context for his views regarding the origin of the Holometabola by stating that the ancestral larva was active, predatory and campo-deiform. This attribution was inconsistent with a much older hypothesis that the ancestral endop-terygote larva was a feeder on internal plant tissues, as suggested a century ago by Lameere (1899, 1908, 1917) and more recently by Malyshev (1968). The proposal suggested here indicates that the primordial holometabolous larva, now based on a variety of fossil evidence, rapidly differenti-ated evolutionarily into three forms: (1), an externally feeding, actively walking, eruciform caterpillar; (2), an endophytic, phytophagous and largely legless eruciform grub; and (3), a

predatory, campodeiform larva. As a consequence, the origin of the endopterygote condition was associated with both the partitioning of plant tissues as well as active predation.

The Lag between the origin of the Holometabola and its eventual dominance

A considerable amount of literature has been devoted toward understanding the global or regional conditions during the late Paleozoic that may have favored a life cycle whereby a feeding stage is temporally separated from a corresponding reproductive stage by an extended resting stage undergoing diapause (Roscher and Schneider, 2006). Such a temporal separation presumes that selection is operating at the level of the individual and species, and that resource availability would vary annually, upon which a fitness advantage would accrue. This setting would occur in highly seasonal environments based on temperature, water availability or some other local climatically related feature. Accordingly, variation in food, mating-site availability, and other essential resources would negatively impact insects that would have continual, year-round requirements. Such a development would lead to taxonomic turnover through extinction of nonholometabolous species and origination of new holometabolous lineages.

It is clear any potential benefits of holometaboly that would have resulted in large-scale diversification patterns were not realized during the Pennsylvanian or Permian, but rather were delayed to the Middle to Late Triassic (Labandeira, 2005). It is significant that there was about an 80 million-year lag between the origin of holometaboly and its eventual global implementation, significantly after major climatic perturbations during the earlier Permian (DiMichele et al., 2010) and the multiple environmental crises towards the end of the period (Erwin, 2006). This delay (Nel et al., 2007; Béthoux, 2008) is attributable to an exponential diversification process whereby long lead times of comparatively low diversity are an extended prelude to a much later, sudden, and noticeably increased burst of diversification (Labandeira, 2005). For the Holometabola, this is best explained by the presence of sufficient climatic oscillation in the Pangaean equatorial belt such that fitness benefits gradually would accrue to holometabolous clades through

heightened interspecies competition and through creation of underutilized or otherwise ecologically transformed niches during the Permian (Roscher and Schneider, 2006; DiMichele et al., 2010). Initial entry of holometabolous clades thus would have been achieved by selective forces encountered at the species level through gradual replacement of nonholometabolous taxa disadvantaged by Permian climatic oscillations. But their permanent establishment would have been assured only after the emptying of ecological space after the end-Permian event. It is this profound, post-Permian ecological transformation that provided a sufficient number of available niches that allowed permanent establishment of several major clades with complete metamorphosis (Yang, 2001; Mayhew, 2002, 2007). This suggests that holometaboly was initially important in species-by-species replacement of nonholometabolous taxa resulting from Permian climatic vicissitude that only later was implemented during the Triassic when a different process of niche reorganization allowed occupation. The profound, end-Permian event or series of events (Erwin, 2006), led to the rapid diversification of holometaboly in freshwater and terrestrial ecosystems.

There are important examples of ecologically important clades, which exhibit delayed lags in the fossil record that may differ or parallel the pattern exhibited by the Holometabola of the late Paleozoic and Triassic. Several marine invertebrate clades differ from the pattern seen in the Holometabola by showing a protracted and only modest rise in diversity during the mid Cretaceous through the Cenozoic, but not a later diversification event. This unspectacular global change and early saturation of diversity was bereft of a later diversification that would have been prompted by the creation of new habitats in wake of the end-Cretaceous extinction event (Alroy et al., 2008). Alternatively, the diversification of placental mammals that commenced during the mid Cretaceous represents a "slow phylogenetic fuse" like marine invertebrate clades, and similarly was unaffected by the end Cretaceous extinctions (Bininda-Emonds, 2007). However, unlike the diversity pattern among marine invertebrate groups, a dramatic, several-fold diversification increase was launched during the Eocene and Oligocene, at least 10 million years after the end of the Cretaceous. This latter pattern of a prolonged period of an extended but persistently low

diversity in placental mammal record, followed by major diversification, is similar to the trend exhibited by the Holometabola during the Permian and Triassic, suggesting a commonality in mechanism.

SUMMARY

There are five summary points resulting from this exploration of the origin and early history of the Holometabola. These statements reflect recent paleobiological and molecular phylogenetic developments and hopefully will serve as a template for further investigation.

1. *Earliest holometabolan fossils.* Four fossils assigned to the Holometabola have been identified throughout Early to Late Pennsylvanian (Late Carboniferous) deposits from the Illinois Basin and a site in Western Europe.
2. *Divergence time estimate for the Holometabola.* These four occurrences of the currently earliest documented Holometabola suggest an earlier divergence time of Late Mississippian (Serpukhovian Stage), from 328 to 318 million years ago.
3. *Early holometabolan larval feeding strategies and life cycles.* These discoveries indicate that the earliest holometabolan larvae represent three major feeding strategies during Pennsylvanian time. They are (1), an eruciform, externally feeding caterpillar; (2), an eruciform, internally feeding grub; and (3), a campodeiform, actively mobile predatory larva.
4. *Major temporal lag between the origin and eventual diversification of the Holometabola.* There was an approximate 80 million-year time lag between the estimated origin of holometaboly during the Late Mississippian and its eventual, dramatic diversification during the Middle to Late Triassic. This extended interval suggests that climate transformation provided an initial selective force, which was followed by occupation of available ecological niches by holometabolan lineages.
5. *A priority for future paleobiological investigations of Paleozoic Holometabola.* Considerable effort should be directed toward investigating suspect terrestrial deposits from the later Mississippian Subperiod that likely would yield fossil insect material, including a common ancestor to the multiple holometabolous lineages present during the Pennsylvanian.

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