The Description of *Rovnoeucoila tympanomorpha* Buffington and Perkovsky, a New Genus and Species of Fossil Eucoiline, with Observations on the Asynchronous Evolution of Diglyphosematini (Hymenoptera: Figitidae: Eucoilinae)

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THE DESCRIPTION OF *ROVNOEUCOILA TYMPANOMORPHA* BUFFINGTON AND PERKOVSKY, A NEW GENUS AND SPECIES OF FOSSIL EUCOILINE, WITH OBSERVATIONS ON THE ASYNCHRONOUS EVOLUTION OF DIGLYPHOSEMATINI (HYMENOPTERA: FIGITIDAE: EUCOILINAE)

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Abstract.—A **new genus and species** of Eucoilinae, ***Rovnoeucoila tympanomorpha* Buffington and Perkovsky**, from a Rovno Amber inclusion, is described. This new taxon differs from extant eucoilines by having a clearly segmented metasoma and singular flagellomere morphology in the antenna. The new taxon is included in the re-analysis of a fossil calibrated, relaxed molecular clock divergence date analysis of Figitidae. The new analysis infers a mean crown group age for Eucoilinae at approximately 10 million years younger than previously estimated. The age of the eucoiline tribe Diglyphosematini does not change substantially in our new dating analysis, and is much younger when compared to the age of its host lineage Agromyzidae (Diptera); we discuss potential implications of this situation within the context of natural enemy selection in biological control projects that include parasitoid Hymenoptera.

Key Words: Rovno amber, amber inclusion, divergence estimation, parasitoid

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The Cynipoidea (Hymenoptera) are a moderately diverse lineage of parasitoid Hymenoptera (summarized in Buffington et al. 2012). Members are largely parasitoids of other endopterygote insect larvae (Table 3 in Buffington et al. 2012), however, the cynipid gallwasps are dominated by phytophagous species that induce often spectacular galls on Fagacae, and to a lesser extant, Rosaceae and Asteraceae (Liljeblad et al. 2008, Buffington et al. 2012). Recent advances in higher-level Hymenoptera phylogenetics firmly places Cynipoidea within

Proctotrupomorpha, and sister-group to Platygastroidea (Castro and Dowton, 2006, Heraty et al. 2011, Sharkey et al. 2012); this relationship clearly indicates that phytophagy in Cynipidae is secondarily derived with respect to Symphyta.

Precious few fossil cynipoids have been described. The most comprehensive treatment has been Liu et al. (2007) in which several new genera and species were described, and the same paper brought together all described fossil cynipoids into an updated morphology-based phylogeny. The phylogeny presented in Liu et al. (2007), which included numerous fossil taxa, was largely consistent with the other higher-level phylogenies of Ronquist (1999), Buffington et al. (2007), and Buffington et al. (2012), but their taxon sampling was limited to mimic the dataset coverage in Ronquist (1999). The analyses of Buffington et al. (2007, 2012) placed a much greater premium on thoroughly sampling Figitidae; in none of these latter studies was Ibaliidae recovered as sister-group to Liopteridae (as posited by Ronquist, 1999), but was consistently recovered as sister-group to Liopteridae+(Cynipidae+Figitidae) (Buffington et al. 2007) or unresolved (Buffington et al. 2012). The internal relationships within Cynipoidea presented by Heraty et al. (2011) and Sharkey et al. (2012) are largely inconsistent with the more 'cynipoid-centric' studies of Ronquist (1999), Buffington et al. (2007, 2012), and Liljeblad et al. (2008), and therefore should be treated with caution.

During the first thirteen years of study on the Rovno amber fauna, the southern coeval of the famous Baltic amber (Perkovsky et al. 2010, Perkovsky and Rasnitsyn, 2013 and references therein), 187 new arthropod species and 36 new genera have been recovered (Perkovsky and Rasnitsyn 2013, Melnitsky and Ivanov 2013, Azar et al. 2013, Herczek et al. 2013, and Melnitsky and Ivanov 2013. Over 36,000 inclusions from more than 300 arthropod families are recorded from the Rovno amber by now; most of these species are listed by Perkovsky et al. (2010), but the list keeps growing each year (Perkovsky, pers. obs.).

The composition of Arthropoda is similar but not identical between the Rovno and Baltic amber faunas (Perkovsky 2011, 2013). Within the assemblage of species directly connected with the amber forest, those found in both Baltic and Rovno faunas dominate, while those found in Rovno, but not in Baltic amber, are represented mainly by unique, singleton specimens (Dlussky and Rasnitsyn 2009). In contrast, for aquatic groups, the better-explored caddisflies (Trichoptera) of the Rovno assemblage have only 18% of species in common with the Baltic amber, suggesting the Rovno amber records a unique fauna (Melnitsky, pers. comm.). Data on the Hymenoptera of the Rovno amber is summarized by Perkovsky and Rasnitsyn (2013).

Fossil animals have always informed science as to what lineages once existed on earth, and helped us understand crown-group and stem-group lineages (Darwin 1859, Simpson 1944). Recently, methods have been developed to calibrate Bayesian, molecular-based chronograms using fossil evidence to infer the ages of lineages within a phylogeny (Drummond et al. 2006, Yang and Rannala 2005, Drummond et al. 2012). Herewith, we describe ***Rovnoeucoila tympanomorpha*, n. gen., n. sp**, and include this fossil in a re-analysis of the dataset of Figitidae divergence dates presented by Buffington et al. (2012). We attempt to summarize how adding fossils to earlier divergence estimation analyses can affect our current understanding of a group, as well as addressing how

divergence estimates can be applied to fields such as biological control in agricultural systems.

Materials and Methods

The piece of amber with the new eucoiline (1g after primary treatment) was obtained from the quarry "Pugach" in Klesov, that belongs to the factory "Ukramber" (Rovno); the specimen was cut and polished by A. P. Vlaskin (SIZK). Imaging of the type-specimen was achieved using techniques described in Buffington and van Noort (2009); digital painting of the specimen was executed by Taina Litwak (Systematic Entomology Laboratory, USDA) using techniques described in Buffington and Condon (2013). The specimen is deposited in the Schmalhausen Institute of Zoology Rovno Amber Collection. Descriptive format follows that of Buffington (2010) and references therein.

Divergence date estimation analytical methodology follows that described in Buffington et al. (2012). That study employed five fossil calibrated nodes, and in the current study we added a sixth calibration prior ('C-6'), based on *Rovnoeucoila tympanomorpha* at the node subtending the clade containing the most recent common ancestor of (Eucoilini+Kleidotomini+Ganaspini). Because there may be some uncertainty regarding the age of Rovno amber inclusions (Perkovsky et al. 2007), we ran two analyses using different offset values (i.e., hard minimum age bounds) for this calibration, one using 42 mya and another using 35 mya. We assigned to the new calibration a lognormal prior distribution with a log(mean) = 2.57 and log(standard deviation) = 0.5; these settings correspond to a median age of 55 Ma and 95% upper quartile of 72 mya when using an offset value of 42, and a median of 48 mya and 95% upper

quartile of 65 mya when using an offset value of 35. Inferred divergence dates can be sensitive to which node is selected for fossil calibration (Rutschmann et al. 2007, Ho and Phillips 2009, Brady 2011). Thus, we conducted two alternative analyses in which we shifted the node calibrated by *Rovnoeucoila tympanomorpha* to be either one or two nodes deeper into the tree. This alteration changed inferred dates of major lineages by only 0–3 Ma, so we conclude that our results are not overly sensitive to the precise nodal position of our new fossil calibration.

Divergence dating analyses were conducted using BEAST v.1.7.5 (Drummond et al. 2012) under an uncorrelated lognormal relaxed clock model (Drummond et al. 2006). Runs lasted 200 million generations with a 40M burnin removed. Convergence was verified using ESS scores and consistency among multiple runs, and the results from two independent runs were combined for final age estimates.

Systematic Paleontology

Rovnoeucoila Buffington and Perkovsky, new genus

urn:lsid:zoobank.org:act:3451230D-51C0-4C47-8F34-1292A4984102

Type species: ***Rovnoeucoila tympanomorpha*** Buffington and Perkovsky, new species

Diagnosis.—Distinguished from nearly all Eucoilinae by the presence of freely articulating terga 2-4 on the metasoma (Fig. 2D); in all extant and some extinct eucoiline taxa, the metasoma is dominated by a syntergum that has resulted from the fusion of T2-T4; distinguished from the fossils *Anteucoila*, *Jerseucoila*, and *Syneucoila* by the very distinctive, drum-shaped scutellar plate (Fig. 1C and

2D); reduced and not readily visible in lateral view in these other taxa. Additional distinguishing features include: extraordinarily thin legs with elongate coxae (Fig. 1B and 2D); calcar of foreleg scythe-like, bifurcate at the tip (Fig. 2D); female flagellomeres 4–11 acorn shaped, narrow at base, broad anteriorly, with very distinct rhinaria (Fig. 1F and 2B).

Description.—As in species description (below).

Etymology.—A combination of the name of Lagerstätte, from which the inclusion originates (Rovno amber), and the type-genus of Eucoilinae, *Eucoila* Westwood.

Rovnoeucoila tympanomorpha Buffington and Perkovsky, new species

urn:lsid:zoobank.org:act:E64D540C-2CEA-43C7-A890-0BFB003EDFEC

Figures 1–2

Diagnosis.—As for the genus.

Description.—Adult female, ca. 1.5 mm; head, mesosoma and metasoma dark red to brown; scape, pedicel, and all legs much lighter, possibly yellow; flagellomeres dark brown apically.

Head: Glabrous with a few scattered setae along vertex (Fig. 2A). Ventral 1/4 of lower face without admedial clypeal furrows converging toward clypeus (Fig. 2B). Orbital furrows absent (Fig. 2B). Torulus with distinct ventral lip (Fig. 2B). Malar sulcus not visible (Fig. 2B). Malar space smooth. Genal carina absent, gena gently rounded, smooth (Fig. 2C).

Antennae: Female: 11 flagellomeres, filiform, F1 2.5x longer than wide; F2 about as long as wide; F3 slightly longer than wide, distinctly wider apically; F4-F11 much larger, each flagellomere slightly longer than wide, acorn-shaped, distinctly broader apically compared to

base, forming an elongate clava, covered in distinct rhinaria (Figs. 1F and 2B).

Pronotum: Pronotal plate small, barely visible, apparently glabrous; dorsal margin rounded; pronotal fovea not visible (Fig. 2C). Lateral pronotal carina absent. Lateral part of pronotum smooth and glabrous (Figs. 1C and 2D). When viewed laterally, pronotum protudes anteriorly very distinctly, almost boat-shaped, with pronotal plate at anterior end.

Mesoscutum: Entirely smooth, notauli absent, nearly glabrous save for few stout, elongate setae along parapsides; parapsidal ridges distinct, demarcated by obvious change in angle from central part of mesoscutum (Fig. 2C). Parascutal impression broad, apparently linear along longtidunal axis of mesosoma (Fig. 1C).

Mesopectus: Upper part and lower part of mesopleuron glabrous, apparently smooth (Fig. 2D). Mesopleural triangle and mesopleural carina not visible. Lower part of mesopleuron bordered by prominent precoxal carina (Fig. 1C).

Mesoscutellum: Scutellar fovea apparently present but impossible to diagnose (Fig. 2C). Scutellar plate relatively large, distinctly elevated, drum-shaped in lateral view (Fig. 1C), perfectly flat when viewed in profile; midpit not visible; rim of plate apparently translucent (Fig. 2C). Dorsal surface of scutellar disk apparently rugose, but difficult to see, rounded laterally and posteriorly (Fig. 2E). Lateral bars present, apparently smooth, length/depth impossible to diagnose. Axillula not visible.

Metapectal-Propodeal Complex: Metapectus entirely glabrous (Figs. 1E and 2D). Spiracular groove not visible. Posterior margin of metapectus with distinct ridge bounded by carina (Fig. 1C). Metapleural ridge not visible; entire metapleuron apparently flat. Anterior impressions of metepimeron, metepisternum,
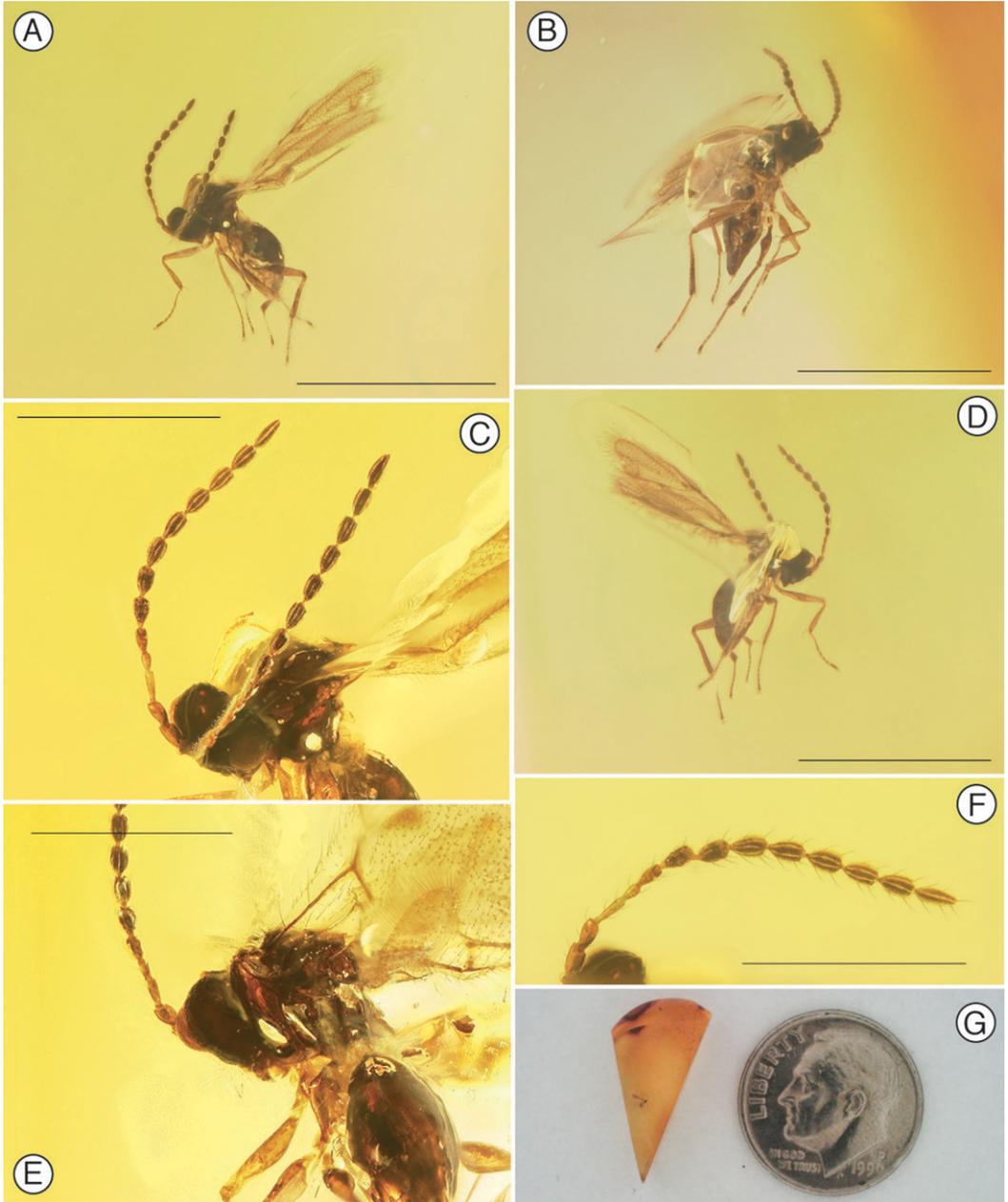
Fig. 1. Light microscope images of *Rovnoeucoila tympanomorpha*, new genus and species. 1A: antero-lateral habitus. 1B: ventral habitus. 1C: antennae, head and mesosoma, antero-dorsal view. 1D: postero-dorsal habitus. 1F: right antenna. 1E: head, mesosoma and metasoma, poster-dorsal view. 1G: scale photo of the amber inclusion adjacent to a United States dime.

anteroventral cavity not visible. Lateral aspect of propodeum covered in dense, foamy setae (Fig. 1E). Lateral propodeal carinae robust, apparently parallel, auxiliary propodeal carinae not visible (Fig. 1E); space between lateral propodeal carinae mostly glabrous with apparently small patches of foamy setae (Fig. 1E).
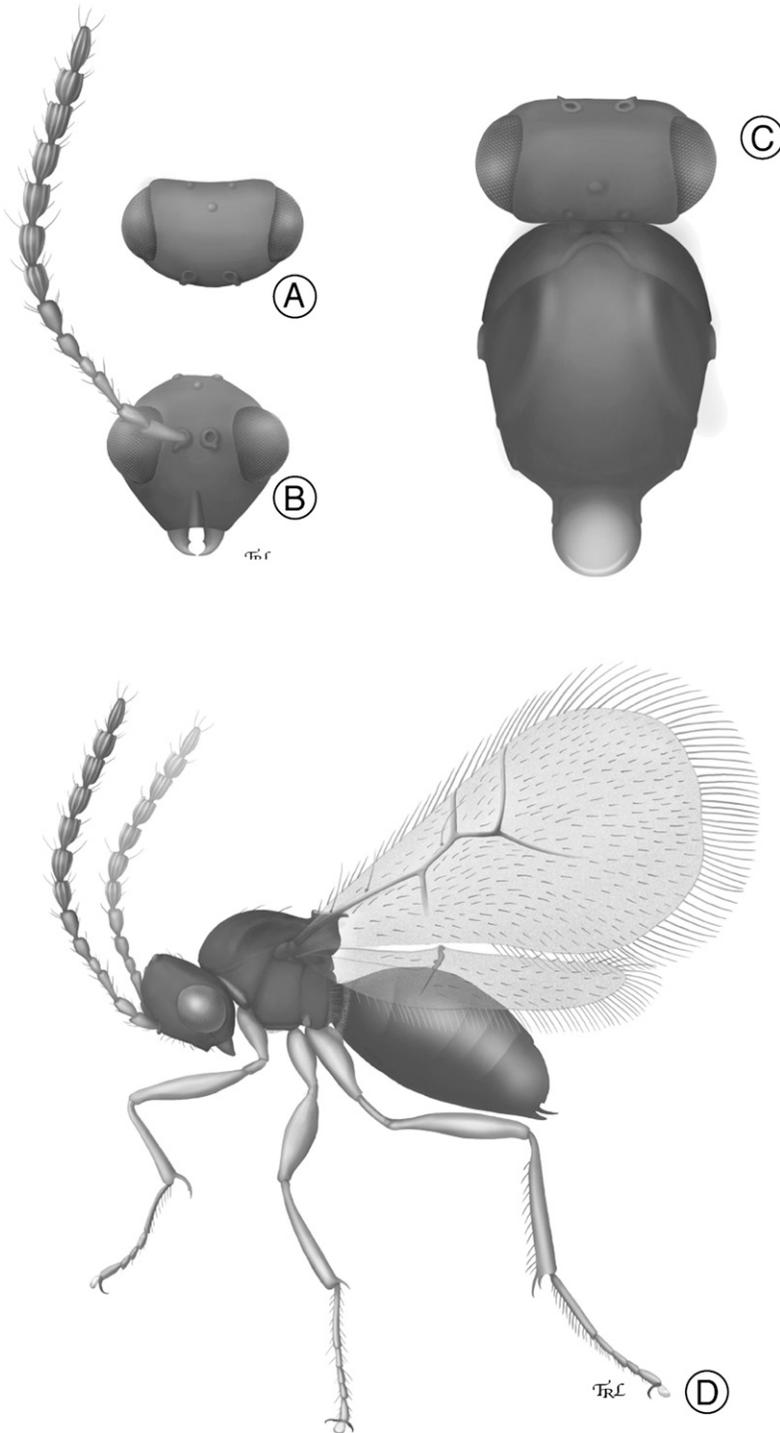
Fig. 2.   Digital paintings interpreting the Rovno amber inclusion of *Rovnoeucoila tympanomorpha*, new genus and species. Fig 2A: head, dorsal view. Fig. 2B: head, anterior view. Fig. 2C: head and mesosoma, dorsal view. Fig. 2D: lateral habitus.

Nucha obscured by foamy setae (Fig. 1E).

Wings: Hyaline, densely setose over entire surface, with distinct apical fringe; R_1 complete, reaching anterior margin of wing; marginal cell slightly longer than deep, apparently open on anterior margin (Fig. 2D). Wings rather large relative to body size of wasp (Fig. 1A).

Legs: All legs extremely elongate, delicate (Fig. 1A). Fore and mid coxa sub-equal in size, hind coxa twice the size of either fore or mid coxa; all coxae elongate (Fig. 2D). Fore and mid coxa setal patterns impossible to diagnose; hind coxa with apparent patch of foamy setae (Fig. 1E). Femora, tibiae, tarsomeres apparently glabrous, but this could be preservation artifact. Length of hind tarsomere 1 equal to the combined length of remaining hind tarsomeres (Fig. 2D). Elongate, thread-like calcar present on foretibia, apically forked (Fig. 2D).

Metasoma: Distinctly longer, more massive than mesosoma (Fig. 2D). Crenulate ring not visible. Base of syntergum with foamy setae present, apparently encompassing entire base of T2, no other setae visible; remainder of metasoma glabrous (Fig. 1C and 1E). Micropunctures not visible. All metasomal terga apparently free, syntergum absent (Figs. 1E and 2D); terga all directed posteriorly, typical eucoiline post-syntergal downturn of metasoma absent (Fig. 1D).

Type material.—Female holotype, Schmalhausen Institute of Zoology Rovno Amber Collection, K-6946.

Etymology.—The species name is derived by the Latin for drum, *tympano*, and shape, *morph*, in reference to drum-shaped scutellar plate.

Results and Discussion

Affinities of Rovnoeucoila *with extant Eucoilinae:* This new taxon may be considered a member of two extant tribes of Eucoilinae, *vis* Ganaspini and Trichoplastini. In both tribes, females frequently possess very distinct rhinaria among the claval segments of the antenna (Fig. 1F). However, this feature is generally more common and well developed in Neotropical Ganaspini (Buffington, pers. obsv.). The presence of 'foamy setae' (as interpreted here) (Fig. 1E), as well as thin, elongate legs (Figs. 1D and 2F), are both common features of many species of Trichoplastini, suggesting *Rovnoeucoila* may be an extinct species of that tribe. Without a clear view of the scutellum or mesopleuron, we are reticent to place this genus in any particular tribe. Due to this uncertainty, we placed this taxon's calibration point at the most recent common ancestor of Eucoilini+Kleidotomini+Ganaspini as analyzed in Buffington et al. (2012).

One striking feature of *Rovnoeucoila* is the presence of what appears to be a segmented metasoma (Figs. 1E and 2D). In all known extant and extinct female species of Eucoilinae, the metasoma is partially composed of a syntergum, uniting T3-T5 (Ronquist, 1999, Fontal-Cazalla et al. 2002, Buffington et al. 2007). If *Rovnoeucoila* does indeed possess a segmented metasoma, then the hypothesis of the presence of a syntergum as a locally informative synapomoprhy of Eucoilinae (Fontal-Cazalla et al. 2002) is certainly called into question. It should be pointed out, however, that the presence of synterga throughout Cynipoidea has appeared on at least three different lineages: Synergini (Cynipidae), Pycnostigminae, and Eucoilinae. Together, these groups do not form a monophyletic group; in fact, the monophyly of Synergini itself has recently come into question (Nylander et al. 2004, van Noort et al. 2007). Altogether, the presence of synterga has clearly evolved independently in several lineages, and

Rovnoeucoila may represent a extinct lineage of eucoilines that lacked a syntergum. However, it should be pointed out that eucoilines that have spent extended periods of time in yellow-pan traps in warm conditions tend to exhibit 'lines of weakness' in the metasoma that are consistent with distinct sclerites in other Figitidae (Buffington, pers. obs.). Ergo, the interpretation here that the metasoma is fully segmented may be result of preservation and not a true character state.

Implications of divergence estimation on agriculture

Monophagous parasitoids and their hosts are tightly linked through a strong antagonistic relationship, since these parasitoids impose a significant mortality risk for their host (Godfray and Schimada, 1999, Fleury et al. 2009). Put more plainly, a parasitoid *must* complete its life cycle for its genes to pass on, at the cost of the life of the host; the host, on the other hand, must avoid parasitization (among other life-threatening forces) for its own genes to be passed to the next generation. The host (the pest) and the parasitoid (the biological control agent) have co-evolved with each other, each engaging in their own evolutionary arms race, at once avoiding parasitization, while at the same time, evolving new mechanisms for exploiting the host. This intimate relationship is at the basis of successful biological control programs using monophagous parasitoids.

Within the host-parasitoid system involving leaf-miners in *Liriomyza* (Diptera: Agromyzidae), polyphagous parasitoids can be common, but are most frequently eulophids (Chalcidoidea; Johnson and Hara 1987). The evolutionary underpinnings that result in such life-history strategies within Figitidae that allow for multiple hosts to be exploited is typically harnessed by non-uniform 'success' in one host species versus another (Lee et al. 2009). Put another way, due to the immune response of the host, not all progeny from a single parasitoid species across a wide range of hosts actually survives, and this, ultimately, drives specialization. Among Cynipoidea, the age of various entomophagous lineages typically track the age of their host (Buffington et al. 2012), suggesting these host-parasitoid relationships are millions of years old, allowing time for the refinement of specialization.

Over the past few years, the advancement of methods for analyzing molecular data to estimate the age of lineages has provided the basis for examining the ages of hosts and parasitoids in ways unheard of in the recent past. And access to these data begs the question: if one had the choice between parasitoid species with a long *versus* short evolutionary history, with respect to the host in question, which species (singular or plural) would be the most effective bio-control agent? Considering the costs associated with the exploration for parasitoids, quarantine procedures, host range testing, and ultimately rearing, possibly possessing some *a priori* knowledge of the evolutionary history of the subjects under consideration may be a great advantage.

In the case under consideration in this paper, the first author was contacted in 2007 by a researcher in Texas investigating parasitoids associated with *Liriomyza huidobrensis*, the serpentine leaf-miner, on peppers. Among the figitids the first author identified, two individual tribes were included: Diglyphosematini (represented by *Sinatra pacifica* (Beardsley), *Banacuniculus utilis* (Beardsley) and *Ganaspidium pusillae* Weld) and Zaeucoilini (*Agrostocynips*

Table 1. Revised divergence estimates for Cynipoidea. Analyses follow those in Buffington et al. (2012) with the inclusion of a new calibration point based on *Rovnoeucoila tympanomorpha*, new genus and species. The first column summarizes the results of Buffington et al. (2012) for major lineages; the second column is the present analysis, with the minimum age of the node calibrated by *Rovnoeucoila* is set to 35 mya; the second column is the present analysis, with the minimum age of the node calibrated by *Rovnoeucoila* is set to 42 mya.

Clade	Buffington et al. 2012	With new fossil, Baltic amber min age 42	With new fossil, Baltic amber min age 35
Eucoilinae	80 (61–100)	71 (52–85)	67 (51–86)
Figitinae+Aspicerinae	88 (84–96)	89 (84–95)	88 (84–95)
Cynipidae	54 (45–67)	55 (46–67)	48 (38–60)
Diglyphosematini	31 (9–60)	29 (8–51)	28 (8–51)
Zaeucoilini	59 (35–82)	51 (31–71)	50 (30–70)
Eucoilini+Kleidotomini+Ganaspini	73 (53–92)	63 (47–80)	62 (46–80)

robusta (Ashmead)). Dominating the samples was the presence of *B. utilis*, though no quantification was made. At the time, this variety of parasitoids associated with single host fly on a single crop system seemed little more than curious.

The present analysis mirrors the results reported in table 2 from Buffington et al. (2012) in that the crown-group zaeucoilines and diglyphosematines diverged at very different times (ca. 50 mya for Zaeucoilini, 30 mya for Diglyphosematini, Table 1). The estimated age for Agromyzidae, the family *Liriomyza* belongs to, was recently re-estimated to be 82 mya (B. Wiegmann, pers. comm.). Buffington et al. (2012) concluded that the association of the stem-group age of zaeucoilines and diglyphosematines tracked tightly the emergence of agromyzids, even though the age estimate for agromyzids was somewhat younger than that of the eucoilines that attack them (reported as 60 mya in Buffington et al. 2012), suggesting both lineages attacked alternate hosts prior to colonization of Agromyzidae.

Buffington et al. (2012) failed to highlight, what we believe, is quite an important pattern emerging from these data: the Diglyphosematini and Zaeucoilini both attack Agromyzidae; however, the crown-group age of Diglyphosematini is far younger than that of either Zaeucoilini (Buffington et al. 2012; present study) or Agromyzidae (Wiegmann et al. 2011). Are these data simply incomplete (poor taxon and character sampling)? A typical answer to these questions would be to acquire more data and re-address this question, although we point out that the addition of our new fossil did not substantially alter the previously inferred date for Diglyphosematini (Table 1). In the meantime, we find it an interesting exercise to consider synchronous *vs.* asynchronous evolution in monophagous parasitoids with respect to the hosts they attack, and, the impact these data can have on the choice of biological control agents. In the example given above, perhaps the obvious choice would be to use *Banacuniculus utilis*, since it overwhelmed the samples. But what if there was sampling bias that precluded the collection of other species, possibly members of Zaeucoilini? Would one not expect to find more Zaeucoilini in these samples, given their evolutionary history with respect to Agromyzidae? Or, perhaps, the rather novel evolutionary association of the Diglyphosematini provide these wasps with a lethal weapon their host has not yet had time to respond to?

We envision at least two biological control scenarios based on these data. In the first, we choose the lineage with the longer historical association (older lineage), dubbed here the 'trench war' hypothesis. In this situation, we assume, due to the age of the association, the fidelity of the parasitoid to the host would be high, resulting in possibly greater efficiency in controlling the targeted host, as well as a reduced chance of host-switching to a non-target host. In the second scenario, dubbed here as the 'wild card' hypothesis, the more novel host association (younger lineage) may lead to greater lethality, since the host has not had enough evolutionary time to adapt against its new adversary. However, this potentially improved lethality may come at the cost of host fidelity, resulting in a higher chance of a host-switch post release. Given the example presented here, South Texas is where Neotropical and Nearctic species of Figitidae meet (Buffington, pers. obsv.), resulting in the presence of native species of both Zeucoilini and Diglyphosematini; for the protection of commercial peppers, perhaps the presence of both lineages is ideal.

Future directions

For systematic entomology, amber inclusions can be considered small time machines. While not all inclusions are perfect, the degree of preservation is frequently astounding. Simply put, more cynipoids from amber need to be discovered, illustrated and described. Not only will these species and genera allow us insight into the diversity of morphotypes from the past, but also allow us to place additional lower bounds on the ages of extant species and genera. Coupling these specimens with ever improving methods of divergence estimation will provide the necessary vistas for ascertaining the evolutionary history of more lineages of cynipoids, and hopefully, reduce the size of the confidence intervals for nodes of interest. Finally, it would be interesting to investigate the various ages of parasitoids that have been used for biological control, and compare these species by relative efficiency of controlling the target pest, frequency of host switching, and establishment rates. The data that emerge from such a study could have a profound effect on how parasitoids are investigated for pest control.

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