A molecular phylogeny of Glaphyridae (Coleoptera: Scarabaeoidea): evolution of pollination and association with ‘Poppy guild’ flowers

GUIDO SABATINELLI1, JONAS EBERLE2,3, SILVIA FABRIZI2 and DIRK AHRENS2

1Department d’Entomologie, Muséum d’histoire naturelle, Geneva, Switzerland, 2Arthropoda Department, Centre of Taxonomy and Evolution, Zoologisches Forschungsmuseum Alexander Koenig Bonn, Bonn, Germany and 3Paris Lodron University, Salzburg, Austria

Abstract. Among Scarabaeoidea, pollen feeding occurs in two major lineages, pleurostict Scarabaeidae and Glaphyridae. Here we infer for the first time the phylogeny of the scarabaeoid lineage Glaphyridae (Coleoptera) based on molecular data using partial gene sequences for 28S rRNA, cytochrome oxidase I (cox1) and 16S rRNA (rrnL) for 41 species. Based on the resulting tree topology, we inferred the timing of the origin of pollination and of their coevolution with different flower host taxa, with particular focus on the prominent red-coloured ‘poppy guild’ flowers. All genera of Glaphyridae that were sampled with multiple species were recovered as monophyletic. According to this analysis, the origin of Glaphyridae was around 140 Ma, while crown group divergence was dated to have occurred c. 112 Ma. Pollen feeding originated in Glaphyridae only once and much later than in other important pollinator groups, between 97 and 67 Ma. According to the reconstruction of ancestral feeding traits, Asteraceae (Cicharoidae) were the first hosts of Glaphyridae. Presumably, a further adaptive radiation was triggered by feeding on and pollination of red flowers (poppy guild) which arose at a later stage. It occurred for the first time between 30 and 40 Ma, whereby the clades that use red Ranunculaceae (Pygopleurus spp.) are older than clades using exclusively red Papaveraceae (Eulasia spp.) (25–30 Ma). The rather young age of red Ranunculaceae would imply that Pygopleurus species only subsequently used red Ranunculus species as flower hosts, and that a broad parallel host shift probably from red Papaver spp. to red Ranunculus asiaticus has occurred rather recently.

Introduction

Beetles are the oldest pollinators (Grimaldi, 1999). Flowers, especially their pollen, provide an important food resource (Faegri & van der Pijl, 1979) and many groups have shifted to pollen feeding independently (e.g. Hunt et al., 2007; Ahrens et al., 2014). Among Scarabaeoidea, pollen feeding occurs in two major lineages, pleurostict Scarabaeidae and Glaphyridae (Scholtz & Grebennikov, 2005; Ahrens et al., 2014). Some species of Glaphyridae (genera Pygopleurus Motschulsky and Eulasia Truqui) are dominant pollinators of plants with red bowl-shaped flowers (poppy guild; Dafni et al., 1990) in the eastern Mediterranean region (Sommer, 2010) (Fig. 1). The guild of red bowl-shaped flowers includes mainly Ranunculaceae (e.g. Anemone coronaria, Ranunculus asiaticus) but also Liliaceae (e.g. Tulipa agenensis) and Papaveraceae (e.g. Papaver rhoes). Due to the strong ecological relationship between glaphyrids and pollinated poppy guild flowers, it was suggested that there was convergent evolution of red bowl-shaped flowers in the Eastern Mediterranean region (Dafni et al., 1990; Dafni & Potts, 2004), which is concordant with the area of highest diversity of the glaphyrid genera Eulasia and Pygopleurus. This idea is supported by the fact that the floral phenology (and therefore the timing of edible pollen production) of the ‘poppy guild’ species positively correlates with the visiting frequencies of the glaphyrid pollinators (Dafni...
et al., 1990). Inversely, the phenology of some Glaphyridae species coincides with the flowering period of various species of poppy guild flowers (Dafni et al., 1990).

In the eastern Mediterranean region, the poppy guild flowers blossom in early spring, as early as February in the Jordan valley, in a period when most efficient insect pollinators are not yet active. Thus, the ‘early-spring phenology’ of some species of Glaphyridae plays a very important role in pollination in the absence of other abundant insect pollinators (G. Sabatinielli, 2006–2019, personal observations, in Israel, Jordan, Syria, Lebanon, Turkey, Greece, Armenia, Iraq, Iran and Tunisia). In March–May, the phenology of Glaphyridae and red flowers overlaps in a temporal succession of plant and beetle species that supports this close plant–pollinator interaction (Dafni et al., 1990).

The strong association of *Pygopleurus* species with poppy guild flowers is enabled by the presence of red-sensitive photoreceptors in addition to green- and UV-sensitive ones that are more common among anthophilous beetles (Martinez-Harms et al., 2012). Given the importance of floral colour as a cue for Glaphyridae, the presence of a red-sensitive photoreceptor in their visual system can be understood as a determinant character involved in the recursive processes that conserve this form of plant–pollinator interaction.

But flowers are also mating sites for insects (Thornhill & Alcock, 1983; Barth, 1985). This is the case for many species of Glaphyridae, whose adults feed on floral pollen and mate on flowers (Kears et al., 2010). Several species of these beetles rely strongly on visual cues to find particular flowers, as receptive females on flowers are highly dispersed in space and to find them may be difficult for males, especially if the density of females is lower than that of flowers. Even if we assume pheromones as an essential mechanism in partner finding (Robbins et al., 2006), the chance of finding a mate may improve if both males and females orient to a subset of the flowers, and visit them preferentially. Thus, the evolutionary scenario of the pollinator–flower relationship could be two-fold.

The family Glaphyridae includes five extant genera in the Palaearctic Region (*Amphicoma* Latreille, *Anthypna* Eschscholtz, *Eulasia* Truqui, *Pygopleurus* Motschulsky, *Glaphyris* Latreille) with 193 species (Nikodým & Bezděk, 2016) and one genus in the Nearctic region (*Lichnanthe* Burmeister, 1844) with eight species (Carlson, 1980). Furthermore, 18 fossil species belonging to five genera are known from the Lower Cretaceous to the Miocene (Krell, 2000, 2006, 2007; Nikolajev, 2005; Nikolajev & Ren, 2011, 2012, 2013; Nikolajev et al., 2011; Zhao et al., 2016).

The uniqueness of the glaphyrids has been recognized for a very long time. Nevertheless, the status of the group as well as the phylogenetic position of the Glaphyridae within the Scarabaeoida has been the subject of debate (Machatschke, 1959; Zunino, 1988). The group is considered to represent ‘intermediate’ forms between ancient and modern lineages of Scarabaeoida. Browne & Scholtz (1995) found the Glaphyridae to be the monophyletic sister group of the trogid subgroup [Trogidae, Bolboceratinae (Geotrupidae) and Pleocomidae] based on characters of wing articulation (d’Hotman & Scholtz, 1990; Scholtz, 1990; Browne & Scholtz, 1995), while in the phylogenetic study of Ahrens et al. (2014) using mitochondrial and ribosomal DNA, results indicate Glaphyridae to be sister to Pleurosticti. Two Chilean genera (*Lichnia* Ericson and *Arcotodon* Burmeister) previously included in Glaphyridae ended up among Melolonthinae lineages in a phylogenetic analysis based on 18S and 28S rDNA (Smith et al., 2006) and were consequently removed from Glaphyridae; they now comprise the melolonthine tribe Lichniini Burmeister, 1844 (Hawkins, 2006).

Except for a few species, the life history of most glaphyrids is poorly known. Adults are often brightly coloured, densely setose, active diurnally, and strong flyers. Adults of many species have coloured bands of setae on the abdomen and resemble bumble bees; they are feeding on pollen but some species do not feed at all. Pollen feeders either stay on the flower surface (surface feeder) or dig deeply into the bottom of the flower (deep feeder). Larvae feed on roots or on detritus (Medvedev, 1951, 1952; Ritcher, 1966; Westcott, 1976; Carlson, 1977, 1980), with some species [*Lichnanthe vulpina* (Hentz, 1826)] considered to be pest species (Carlson, 1977, 1980; Averill & Sylvia, 1998).

Here, we investigate the phylogeny of Glaphyridae using mitochondrial and ribosomal DNA markers. Based on this first phylogenetic hypothesis of the group, using the most comprehensive sampling so far available, we aim to reconstruct the evolution of feeding behaviour of these beetles and its time frame, with particular reference to host association. We are particularly interested in what drives host choice, and whether we can identify a closer evolutionary link to the red ‘poppy guild’ flowers.

**Materials and methods**

**Taxon sampling, DNA extraction, and DNA sequencing**

Forty-five populations of 42 species of Glaphyridae were sampled from various biogeographic regions (Table 1). The analysis additionally included nine outgroup taxa. DNA was extracted from thoracic leg muscle tissue using Promega WizardSV extraction plates. Following DNA extraction, beetles were dry mounted. Vouchers are deposited at the Zoological Research Museum A. Koenig (ZFMK) in Bonn. Phylogenetic analysis was based on three markers: mitochondrial gene regions including cytochrome *c* oxidase subunit 1 (*cox1*) and 16S ribosomal RNA (*rrnL*) as well as nuclear 28S rRNA (domains D3–D6). PCR and sequencing was performed using primers Pat and Jerry for *cox1* and 16Sar and 16S B2 for *rrnL* (Simon et al., 1994); 28S rRNA was amplified using primers FF and DD (Monaghan et al., 2007). Sequencing was performed on both strands using BigDye v. 2.1 and an ABI3730 automated sequencer. Sequences were edited manually using SEQUENCER v.4.8 (GeneCodes Corp., Ann Arbor, MI, USA). All sequences used in the present study have been submitted to GenBank (Table 1).

Host plants, in particular for Israel and neighbouring countries, were identified using Shmida (2005) and we consulted A. Shmida directly in case of doubts.
Table 1. GenBank accessions of the examined specimens, along collection data for new NCBI submissions

| Name                                                   | Collection data                                      | Col1   | 16S     | 28S     | Voucher no. |
|--------------------------------------------------------|------------------------------------------------------|--------|---------|---------|-------------|
| Amphicoma carcelli (Laporte, 1832)                      | Italy: Mt Retafani, Mt Simbruini, vi.2005            | EF487726 | EF487848 | EU084143 | 678442      |
| Amphicoma cornithia (Fairmaire, 1891)                   | China: Fujian, Mt Liang shan-ding, Wuping County,    | MN482662 | MN482584 | MN482623 | DA366       |
| Amphicoma meles Favbricius, 1792                        | Marocco: Khemisset pref., Pays Zer Zaiane 20 km N    | –      | MN482575 | MN482614 | 839437      |
| Amphicoma abdominalis aemiliana Ghidini, 1956           | Italy: Sasso Marconi (Bo) 4.v.2009                   | MN482638 | MN482559 | MN482598 | 836910      |
| Eulasia (Eulasia) arctos arctos (Pallas, 1781)          | Armenia: Khosrov Reserve, NE of Vedi 28-29.v.2009    | MN482657 | MN482579 | MN482618 | 840343      |
| Eulasia (Eulasia) korbi (Petrovitz, 1972)               | Armenia: Khosrov Reserve, NE of Vedi 28-29.v.2009    | MN482659 | MN482581 | MN482620 | 840348      |
| Eulasia (Eulasia) nitskollis (Reiche, 1862)             | Jordan: Salt-Al Aride, 435–200 m 16.iii.2008          | JN969192 | JN969128 | JN969220 | 834232      |
| Eulasia (Rudeulasia) chalybaea (Faldermann, 1835)       | Jordan: Salt-Al Aride, 435-200 m 16.iii.2008          | MN482643 | MN482564 | MN482603 | 836921      |
| Eulasia (Rudeulasia) daccordii Uliana & Sabatinelli, 2013| Jordan: Salt-Al Aride, 435-200 m 16.iii.2008          | MN482646 | MN482567 | MN482606 | 836927      |
| Eulasia (Rudeulasia) diptertennis (Reitter, 1890)       | Israel: Rishon Le Tsion 30.iii.2009                   | MN482646 | MN482567 | MN482606 | 836927      |
| Eulasia (Rudeulasia) fastosa (Reitter, 1890)            | Syria: 15 km Latakia 5.v.2009                         | MN482644 | MN482565 | MN482604 | 836923      |
| Eulasia (Rudeulasia) genei (Truqui, 1848)               | Jordan: 10 km S Salt, 410 m 26.iii.2008               | MN482640 | MN482552 | MN482592 | 834239      |
| Eulasia (Rudeulasia) harmonia (Petrovitz, 1968)         | Syria: Tel Alnaqa 3.v.2009                           | MN482661 | MN482583 | MN482622 | 840443      |
| Eulasia (Rudeulasia) papaveris (Sturm, 1843)            | Jordan: Salt-Al Aride, 435-200 m 16.iii.2008          | MN482643 | MN482564 | MN482603 | 836921      |
| Eulasia (Rudeulasia) pietschmanni (Breit, 1920)         | Israel: Negev, Reim 12.ii.2009                       | MN482645 | MN482566 | MN482605 | 836924      |
| Eulasia (Rudeulasia) ritteri Uliana & Sabatinelli, 2010 | Turkey: Prov. Icel, Mut                              | EU084039 | EU487983 | EU084144 | 671327      |
| Eulasia (Rudeulasia) saccai (Faldermann, 1972)          | Jordan: Zabuia Nat Park, 943 m 10.iv.2008             | MN482628 | MN482558 | MN482597 | 836909      |
| Eulasia (Trichopleurus) bombylius (Fabricius, 1877)     | Jordan: 10 km S Salt, 410 m 26.iii.2008               | MN482628 | MN482549 | MN482589 | 836928      |
| Eulasia (Trichopleurus) viittata lineata (Fabickmann, 1920) | Jordan: 10 km S Salt, 410 m 26.iii.2008               | MN482629 | MN482550 | MN482599 | 836928      |
| Glaphyrus (Glaphyrus) aulicus (Chevalier, 1854)          | Jordan: Pella, 36 m                                  | MN482654 | MN482576 | MN482615 | 834938      |
| Glaphyrus (Glaphyrus) comosus Harold, 1869              | Syria: 23 km S Kassab, 455 m 30.ii.2008               | MN482629 | MN482549 | MN482588 | 834325      |
| Glaphyrus (Glaphyrus) opulentus Bedel, 1884             | Jordan: Pella arch remains, 36 m 18.iii.2008          | JN969195 | JN969131 | JN969223 | 834241      |
| Glaphyrus (Glaphyrus) orbachi Sabatinelli, Miessen & Rittner, 2012 | Jordan: Pella arch remains, 36 m 18.iii.2008          | MN482641 | MN482562 | MN482601 | 836916      |
| Glaphyrus (Glaphyrus) serratulae Fabricius, 1792        | Morocco: Cascades d’Ouzoud, Mc. 2008                 | MC2008 | MN482640 | MN482561 | 836913      |
| Glaphyrus (Hemiglaphyrus) caucasicus Kraatz, 1882       | Morocco: Cascades d’Ouzoud, Mc. 2008                 | MC2008 | MN482635 | MN482556 | 836908      |
| Glaphyrus (Hemiglaphyrus) orbachi Sabatinelli, Miessen & Rittner, 2012 | Armenia: Goravan sands, 3.3 km EES of Vedi           | MN482655 | MN482577 | MN482616 | 840429      |
| Glaphyrus (Hemiglaphyrus) serratulae Fabricius, 1792    | Lichnanthe rathvoni (Le Conte, 1863)                  | MN482624 | MN482586 | MN482602 | 836920      |
| Pygopleurus besucheti Baraud, 1989                      | West Bank; Maal Adumin 11.iii.2009                    | MN482642 | MN482563 | MN482602 | 836920      |
| Pygopleurus despectus (Petrovitz, 1958)                 | Syria: Misriasol                                     | MN482636 | MN482557 | MN482596 | 836908      |
| Pygopleurus diffusus (Petrovitz, 1959)                  | Greece: Neochori (S slope of Mt Ossa)                | MN482652 | MN482573 | MN482612 | 834909      |
| Pygopleurus israelitus (Muche, 1963)                    | Jordan: W Amman, Wadi Sir, 740–194 m 4.iii.2008      | MN482633 | MN482554 | MN482593 | 834240      |
| Pygopleurus kathem Sabatinelli, 2008                    | Jordan: Jordan Valley, Al Ardia, 207 m 29.ii.2008    | MN482631 | MN482552 | MN482591 | 834238      |
| Pygopleurus libanonensis (Petrovitz, 1958)              | Lebanon: Terbol 7.iii.2009                           | MN482647 | MN482568 | MN482607 | 836933      |
| Pygopleurus orientalis (Petrovitz, 1958)               | Jordan: 10 km S Salt, 410 m 26.iii.2008              | JN969196 | JN969132 | JN969224 | 834234      |
**Table 1. Continued**

| Name                                      | Collection data | 16S | 18S | 28S | Voucher no. |
|-------------------------------------------|-----------------|-----|-----|-----|-------------|
| *Pygopleurus ponticus* (Petrovitz, 1958) | Syria: Djebel Arab, Saleh 8.v.2009 | MN482599 | MN482587 | MN482619 | MN482560 | MN482599 | 836912 |
| *Pygopleurus syriacus* (Linnaeus, 1758) | Jordan: 10 kmS Salt, 410m 26.iii.2008 | MN482627 | MN482548 | MN482587 | 834233 |
| *Pygopleurus transcaucasicus* (Petrovitz, 1962) | Armenia: Khosrov Reserve, NE of Vedi 28-29.v.2009 | MN482658 | MN482571 | MN482619 | 840435 |
| *Pygopleurus scutellatus* (Brullé, 1832) | Greece: Loggastra 7.iii.2009 | MN482651 | MN482572 | MN482611 | 836939 |
| *Pygopleurus hirsutus* (Brullé, 1832) | Greece: Mystras 7.iii.2009 | MN482649 | MN482570 | MN482609 | 836936 |
| *Sericotrupes niger* (Linnaeus, 1758) | – JN969189 JN969125 JN969217 | 793042 |
| *Glaresis* | – DQ524560 DQ680923 JN969225 | 677911 |
| *Hybosorus* | – JN969198 JN969134 JN969228 | 793038 |
| *Ochodaeus montanus* (Fuente, 1912) | – JN969198 JN969134 JN969228 | 793038 |
| *Aegialia arenaria* (Fabricius, 1787) | – AY132412 EF487994 AY132443 | 670855 |
| *Cetonia aurata pisana* (Heer, 1841) | – EF487732 EF487937 JN969244 | 678437 |
| *Melolontha melolontha* (Linnaeus, 1758) | – EF487767 EF487850 EU084231 | 670877 |
| *Omaloplia ruricola* (Fabricius, 1775) | – EF487771 EF487790 EU084256 | 747065 |
| *Phyllopertha horticola* (Linnaeus, 1758) | – AY132398 EF487973 JN969259 | 703638 |

*Phylogenetic analysis and divergence time dating*

Multiple sequence alignment was conducted with the divide-and-conquer realignment technique as implemented in **SATÉ v.2.2.7** (Liu et al., 2012) using **MAFFT v.5.8** (Katoh et al., 2002, 2005) as aligner and **MUSCLE v.3.7** (Edgar, 2004a,b) as merger. **PARTITIONFINDER** was used to identify best-fitting substitution models and partition scheme under linked and unlinked branch lengths (Table S1; Lanfear et al., 2012, 2014) for the data which was initially split into **rrnL**, 28S, and three codon positions of **cox1**. The optimal setting was chosen based on the Bayesian information criterion.

Bayesian analysis of phylogenetic relationships and divergence times was conducted with **BEAST 2.3.0** (Bouckaert et al., 2014) using the partitions inferred by **PARTITIONFINDER** (Nylander et al., 2004; Brandley et al., 2005). A lognormal relaxed clock model (Drummond et al., 2006) was applied, setting the number of discrete rates to the number of branches. Two fossil priors were available for tree calibration (Fig. 2): *Creoglyphus* Nikolajev spp., being the oldest fossils of Glaphyridae (Krell, 2000, 2006; Nikolajev, 2005; Zhao et al., 2016) [node A, maximum age (range) for the origin of the Glaphyridae: 145.5–140.2 Ma] and *Lichnanthe defuncta* (Wickham) (Wickham, 1910; Yan et al., 2012) [node B, maximum age (range) for the split between Nearctic and western Palaearctic lineages: 37.2–33.9 Ma]. Age ranges of fossils were taken from fossil works database (http://fossilworks.org/bridge.pl; accessed 14 April 2014). Three other Lower Cretaceous fossil genera (*Creosteglyphus* Yan, Nikolajev & Ren, *Lithohypna* Nikolajev & Ren, and *Mesohypna* Nikolajev & Ren) are of same age as *Creoglyphus*. Also, they can be assigned based on available morphological evidence only to the stem lineage of Glaphyridae. Two fossil *Glaphyrus* species from Cenozoic and Lower Cretaceous (Zhao et al., 2016) cannot be associated with any of the extant genera due to their poor preservation and were thus not considered for calibration here. The North American fossil *Lichnanthe defuncta* whose genus is endemic to northern America, however, could be used as similar geographical distributions indicated the systematic position of the fossil.

Exponential priors were used to implement fossil calibration points for node A (offset 140.2 Ma) and node B (offset 33.9 Ma). The calibration distribution means were estimated by gamma distributed hyperpriors [node A: alpha = 10, beta = 0.5, i.e. 95% highest posterior density (HPD) for exponential prior mean varies about 20 Ma]. Age ranges of fossils were taken from fossil priors were available for tree calibration (Fig. 2): *Creoglyphus* Nikolajev spp., being the oldest fossils of Glaphyridae (Krell, 2000, 2006; Nikolajev, 2005; Zhao et al., 2016) [node A, maximum age (range) for the origin of the Glaphyridae: 145.5–140.2 Ma] and *Lichnanthe defuncta* (Wickham) (Wickham, 1910; Yan et al., 2012) [node B, maximum age (range) for the split between Nearctic and western Palaearctic lineages: 37.2–33.9 Ma]. Age ranges of fossils were taken from fossil works database (http://fossilworks.org/bridge.pl; accessed 14 April 2014). Three other Lower Cretaceous fossil genera (*Creosteglyphus* Yan, Nikolajev & Ren, *Lithohypna* Nikolajev & Ren, and *Mesohypna* Nikolajev & Ren) are of same age as *Creoglyphus*. Also, they can be assigned based on available morphological evidence only to the stem lineage of Glaphyridae. Two fossil *Glaphyrus* species from Cenozoic and Lower Cretaceous (Zhao et al., 2016) cannot be associated with any of the extant genera due to their poor preservation and were thus not considered for calibration here. The North American fossil *Lichnanthe defuncta* whose genus is endemic to northern America, however, could be used as similar geographical distributions indicated the systematic position of the fossil.

Exponential priors were used to implement fossil calibration points for node A (offset 140.2 Ma) and node B (offset 33.9 Ma). The calibration distribution means were estimated by gamma distributed hyperpriors [node A: alpha = 10, beta = 0.5, i.e. 95% highest posterior density (HPD) for exponential prior mean varies about 5 Ma; node B: alpha = 10, beta = 2, i.e. 95% HPD for exponential prior mean varies about 20 Ma]. We ran four independent analyses with 1×10⁸ generations; parameters and trees were logged every 10 000 samples. Convergence of parameters and stationarity of runs were assessed for each run separately in **TRACER v.1.6** (Rambaut & Drummond, 2013). Log files and species trees from independent runs were subsequently combined using **LOGCOMBINER 2.3.0**, after removing a burn-in of 10% and checked again in **TRACER**. Trees were summarized using **TREEANNOTATOR 2.3.0**. The .xml-file for a single Markov chain Monte Carlo run is provided as File S1.
Fig. 1. Feeding habits of Glaphyridae: (A) Pygopleurus israelitus (Muche, 1963), on Ranunculus sp.; (B) Eulasia (Eulasia) nitidicollis (Reiche, 1882), on Margerita sp.; (C) Glaphyrus (Glaphyrus) aulicus Chevrolat, 1854, on Sylilbus marianum; (D) Eulasia (Trichopleurus) vittata lineata Faldeman, 1835, on Sylilbus marianum; (E) Pygopleurus orientalis Petrovitz, 1958, on Daucus sp.; (F) Pygopleurus samai Keith, 2001, on Tulipa arvensis; (G) Amphicoma carceli (Laporte, 1832) on bark. [Colour figure can be viewed at wileyonlinelibrary.com].

Model-based phylogenetic analyses under maximum likelihood (ML) were performed in IQ-TREE (v.1.6.7; Nguyen et al., 2015; Chernomor et al., 2016). Partition schemes and substitution models were set according to the PARTITIONFINDER analysis. Individual evolutionary rates for each partition were allowed. The best tree out of 20 full tree searches was chosen by its likelihood. Node support was estimated by 1000 replicates of ultrafast bootstrapping (Hoang et al., 2018). The tree was rooted according to the BEAST analysis (see later).

Phylogenetic comparative analysis

For each glaphyrid species feeding type, flower taxon host, and host colour was coded. Colour preferences are also based
Fig. 2. Calibrated Bayesian phylogenetic tree of Glaphyridae. Scale bars indicate 95% confidence intervals; posterior support values > 0.5 are indicated below branches. Tree calibration points at nodes A and B are shown. Numbers in circles indicate current subgenus assignment of Eulasia species within the clade: 1, subgenus Eulasia; 2, Trichopleurus; 3, Rudeulasia. [Colour figure can be viewed at wileyonlinelibrary.com].

on results from plate trap experiments (Dafni et al., 1990). As no published data were available for many taxa, trait coding was principally based on our extensive field observation (G. Sabatinelli 2006–2019, unpublished data). Maximum parsimony reconstructions (Fitch algorithm) of ancestral feeding type and flower taxon and colour were done with PHANGORN v.2.4.0 (Schliep, 2011). Ambiguous character states were coded as such and inapplicable traits of non-feeders were treated as extra character state. As body size (as a proxy of body weight) might play a role in host choice in relation to the robustness of inflorescences and their stems, we analysed correlations of body size in relation to feeding behaviour, pollinated plant taxon, and the colour of the florescence with phylogenetic generalized least-squares regression (PGLS) in CAPER v.1.0.1 (Orme et al., 2018), taking the phylogenetic relationship of investigated species as inferred with BEAST into account. As no reliable mean values for body size were available, all analyses were done for minimum and maximum body sizes of all species. Because CAPER is not able to treat ambiguous character states, all ambiguities were treated as missing data. This seemed to be the cleanest way to handle such data with available phylogenetic comparative methods.

Results

Phylogenetic analysis and divergence time dating

In the tree from Bayesian inference (BI) Glaphyridae were recovered as monophyletic and sister to Ochodaeus (i.e. Ochodaeidae). All genera of Glaphyridae were monophyletic.

© 2020 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, Systematic Entomology, 45, 838–848
According to BI, the origin of Glaphyridae was c. 140 Ma, while crown group divergence was dated to have occurred c. 112 Ma, with Amphicoma positioned as basal lineage, being sister to all other Glaphyridae. It is the only genus that is distributed in two continents (Asian and Europe). The Nearctic Lichnanthe formed the sister to all other western Palaearctic Glaphyridae. Glaphyrus took a basal position within the latter and was thus sister to two other major Palaearctic clades enclosing Pygopleurus + Anthypna and Eulasia (Fig. 2). Divergence time between the Nearctic and Palaearctic clade was c. 97 Ma.

The Palaearctic groups (Pygopleurus, Eulasia and Glaphyrus) showed similar divergence times, despite the stem group of Glaphyrus originating slightly earlier (c. 64 Ma). Most extant species of Pygopleurus and Eulasia originated < 20 Ma. Among the three subgenera of Eulasia, only Trichopleurus Motschulsky and Eulasia (s.s.) were monophyletic. Rudeulasia Baraud was paraphyletic because the subgenera Eulasia and Trichopleurus nested within.

The IQ-TREE analysis had a very similar generic topology, with exception of the position of Anthypna meles (Fabricius), being nested within Pygopleurus (Fig. S1). Trichopleurus was positioned here as sister to the subgenus Eulasia + E. nitidicollis (Reiche), while in BI it was nested within two Rudeulasia species clades.

**Fig. 3.** Feeding habits of bumble beetles optimized on the tree topology, including: (A) type of feeding; (B) systematic group of flowers; (C) colour of flowers. [Colour figure can be viewed at wileyonlinelibrary.com].

**Phylogenetic comparative analysis**

Non-feeding, being present in Amphicoma and Lichnanthe (which also do not mate on flowers), was recovered as ancestral in the projection of the feeding mode (Fig. 3a) on the tree topology of BI. Superficial and deep feeding were inferred as derived characters with a unique origin. The first is encountered in the clade (Anthypna + Pygopleurus) + Eulasia, whereas the latter is restricted to the genus Glaphyrus. The type of feeding is also widely consistent with the morphology of mandibles of the species, showing an unarmed, blunt apex in non-feeding Amphicoma and Lichnanthe, but sharp teeth at the distal part of mandible in feeding Glaphyrus, Pygopleurus and Eulasia. The only exception is represented by the superficial feeder Anthypna, showing a moderately blunt tip similar to that of Lichnanthe (Fig. 3a).

By contrast, the systematic position of flower host and flower colour did not show a close link to the bumble beetle phylogeny (Fig. 3b). While several species of Eulasia and Glaphyrus feed on Cichoriodeae (Asteraceae), host use was unique for the latter genus only. Several species in Pygopleurus and Eulasia also showed multiple host groups with alternative colour properties, although the majority of included taxa are linked to red flowers of the poppy guild. Interestingly, we detected one well-supported monophyletic clade within Eulasia that mostly relates to red-coloured Papaveraceae. The reconstruction of
groups such as bees (c. 123 Ma; Cardinal & Danforth, 2013) or butterflies (119 Ma; Espeland et al., 2018). By contrast, in Pleurostict scarabs there were multiple (at least six) shifts from herbivory to pollen feeding (Ahrens et al., 2014). Similar to Glaphyridae, all occurred rather late in the Tertiary, from the Palaeocene to the Miocene.

The feeding type of first glaphyrid pollinators is still uncertain. Further evidence might come from a comprehensive comparative morphological analysis of all body traits between superficial and deep feeders. Deep feeding on flowers is likely to be linked to additional adaptations, such as the reinforcement of anterior legs for digging into the flowers, and the robustness of maxillary and labial palps (i.e. Glaphyrus), and thus distribution of these traits in ancestral and pollinating lineages might provide further insights. Such traits are also known in deep feeders of monkey beetles (Carabidae: Hoplini; Midgley, 1992; Ahrens et al., 2011). In the latter case, males compete for females on the flowers, which might also be the case in Glaphyrus. Interestingly, body size was not correlated with feeding behaviour, possibly due to the fact that there was considerable variation among the superficial feeders.

According to the reconstruction of ancestral feeding traits, Asteraceae (Cicharoidae) were the first hosts of Glaphyridae. Presumably, a further adaptive radiation was triggered by feeding on and pollination of red flowers (e.g. poppy guild), which arose at a later stage. It occurred for the first time between 30 and 40 Ma, whereby the clades that use red Ranunculaceae (Pygopleurus) are older than clades using exclusively red Papaveraceae (Eulasia spp.) (25–30 Ma). While the genus Ranunculus is known to have originated in the Oligocene (Emadzade et al., 2011), the red Ranunculus acting as host to the Pygopleurus species in the Near East has been dated to 3.9 Ma (Emadzade & Hörandl, 2011). This would mean that Pygopleurus species only subsequently used red Ranunculus species as flower hosts, and that a broad parallel host shift probably from red Papaver spp. to red R. asiaticus had occurred. Unfortunately, we had no access to dated phylogenies of Papaver (i.e. Papaveraceae) that might help in elucidating the origin of red flowers in this group in order to evaluate the incidence with Glaphyridae evolution. Nevertheless, as eastern Mediterranean Glaphyridae are among the most important pollinators of poppy guild flowers and also because considerable physiological and anatomical adaptation for vision of red colours has been found (Martinez-Harms et al., 2012), it has to be assumed that there always was a close evolutionary relationship between these flowers and Glaphyridae (i.e. species of Pygopleurus and Eulasia).

To explore the question of what determines the choice of flower host, we investigated the correlation between body size (as a proxy of body weight) and flower taxonomy, feeding behaviour and flower colour. Body weight (i.e. size) could play a role in host choice in relation to the robustness of flowers and their stem, in particular when considering that flowers are not only a food resource of Glaphyrids but also a mating site (Keasar et al., 2013). Sometimes many specimens are found on a single florescence. The correlation of body size with flower taxonomy and colour confirmed this expectation, as flowers of the same

| Trait correlation analysis between minimum/maximum body size and feeding traits/host characteristics from phylogenetic generalized least-squares regression analyses treating polymorphic characters as missing data showing coefficient of determination ($r^2$), F-statistics ($F_{stat}$), and P-value |
|---------------------------------------------------------------|
| r² | Fstat | P |
|-----------------------------------------------|
| Minimum body size                                    |
| Feeding behaviour                                   | 0.141 | 2.21 | 0.129 |
| Plant taxon                                        | 0.595 | 6.24 | 0.00281 |
| Florescence colour                                  | 0.426 | 4.94 | 0.00998 |
| Maximum body size                                   |
| Feeding behaviour                                   | 0.0116 | 0.158 | 0.854 |
| Plant taxon                                        | 0.443 | 5.57 | 0.00568 |
| Florescence colour                                  | 0.745 | 21.4 | 0.000001 |

Significant values are in bold.

Discussion

In this study, we present the first comprehensive phylogenetic analysis of Glaphyridae based on DNA sequences and extensive sampling of the entire group. While the sister-group relationship is somewhat uncertain, due to contrasting signals and evidence (e.g. Hunt et al., 2007; Lawrence et al., 2011; Gunter et al., 2016), Ochadidae were recovered here as sister to Glaphyridae, similar to other previous studies (e.g. Hunt et al., 2007; Ahrens et al., 2014; Bocak et al., 2014). Although the taxonomic sampling was not designed to address this question, it seems apparent from most studies and also from most comprehensive and recent evidence (McKenna et al., 2019) that Glaphyridae belong to the early-diverging lineages of Scarabaeidae, together with Hybosoridae and Ochadidae.

According to our results, pollen feeding originated in Glaphyridae only once, between 97 and 67 Ma, and this is largely consistent with results from Ahrens et al. (2014). An exact timing cannot be given, as it is not clear whether the early members of the stem lineage of Glaphyrus + Pygopleurus/Eulasia had already fed on pollen. According to current knowledge, this was much later than in other important pollinators.
taxon and colour tend to have similar morphology, being either more or less robust. As no reliable mean values for body size were available, all analyses were done with known minimum and maximum body sizes of the species. Both had similar patterns of correlation, although the correlation for maximum size was clearer, probably indicating the determinant measure for this relationship. Species on red flowers, i.e. poppy guild flowers, are generally smaller. Keasar et al. (2013) argue that adaptation on poppy guild flowers would be a mode of aggregation behaviour, which is in turn promoted by plant-mediated chemical protection of beetles from predation (Keasar et al., 2013). Most poppy guild taxa include phenologically early inflorescences, and thus pollinators might not be too abundant. Therefore, it is likely that plants adapted to new pollinator resources that are available in early spring, and that bumble beetles switched to new food resources.

Although the evolution of pollination and the coevolution with red poppy guild flowers make up only one chapter of the evolution of Glaphyridae, it is the one with the most important impact on ecosystems by the group. The evolutionary plasticity evident in adopting new food resources in relatively young geological timescales is impressive and, at the same time, surprising for such a comparatively ancient lineage, and merits further investigation at a finer scale in order to better explore the mechanisms of this interesting coevolutionary relationship.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. IQ tree of Glaphyridae. Branch support values from ultrafast bootstrapping are indicated near the respective nodes. A., Amphicoma; E., Eulasia; P., Pygopleurus; G., Glaphyris.

Table S1. Partition scheme and substitution models that were inferred by PARTITIONFINDER. The results of the analysis with linked branch lengths is shown, which was preferred based on the BIC.

File S1. BEAST extensible markup language (xml) file for one out of four MCMC-run repeats of the dating analysis.

Acknowledgements

The study was in part supported by the German Science Foundation (DFG; AH175/3 and AH175/6). We are grateful to the following persons and institutions for their help with collecting additional specimens: Prof. H. Özütkmen (Gazi University, Ankara), Prof. Ahmad Katbeh-Bader (University of Jordan), Dr A.L.L. Friedman (The Steinhardt Museum of Natural History, Tel Aviv University) and Dr Khouzama Knio (American University of Beirut, Natural History Museum, Beirut, Lebanon). We thank Prof. Avi Shmida (Dept of Ecology, Evolution & Behavior, the Institute of Life Sciences, and the Center for Rationality and Interactive Decisions, The Hebrew University of Jerusalem, Israel) and Prof. Tamar Keasar (Department of Biology and Environment, University of Haifa, Israel) for their advice on botanical aspects of the work. We thank Frank-Thorsten Krell as well as two anonymous referees for their helpful comments which helped to improve the manuscript. The authors declare there are no conflicts of interest.

References

Ahrens, D., Scott, M. & Vogler, A.P. (2011) A phylogeny of monkey beetles based on mitochondrial and ribosomal DNA (Coleoptera: Scarabaeidae: Hoplini). Molecular Phylogenetics and Evolution, 60, 408–415.

Ahrens, D., Schwarzer, J. & Vogler, A.P. (2014) The evolution of scarab beetles tracks the unfolding rise of angiosperms and mammals. Proceedings of the Royal Society B, 281, 20141470.

Averill, A.L. & Sylvia, M.M. (1998) Cranberry Insects of the Northeast, p. 112. University of Massachusetts at Amherst, Amherst.

Barth, F.G. (1985) Insects and Flowers: The Biology of a Partnership. Princeton University Press, Princeton, New Jersey.

Bocak, L., Barton, C., Crampton-Platt, A., Chesters, D., Ahrens, D. & Vogler, A.P. (2014) Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. Systematic Entomology, 39, 97–110.

Bouckaert, R., Heled, J., Kühnert, D. et al. (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology, 10, e1003537. https://doi.org/10.1371/journal.pcbi.1003537.

Brandley, M.C., Schmitz, A. & Reeder, T.W. (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology, 54, 373–390.

Browne, D.J. & Scholtz, C.H. (1995) Phylogeny of the Families of the Scarabaeoidea (Coleoptera) based on characters of the hindwing articulation, hindwing base and wing venation. Systematic Entomology, 21, 145–173.

Cardinal, S. & Danforth, B.N. (2013) Bees diversified in the age of eudicots. Proceedings of the Royal Society B, 280, 20122686. https://doi.org/10.1098/rspb.2012.2686.

Carlson, D.C. (1977) Taxonomic revision of Lichnanthe Burmeister with studies on the biology of L. rathvoni (LeConte) (Coleoptera: Scarabaeidae). PhD Thesis, Oregon State University, Corvallis, Oregon.

Carlson, D.C. (1980) Taxonomic revision of Lichnane Burmeister. The Coleopterists Bulletin, 34, 177–208.

Chernomor, O., von Haeseler, A. & Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology, 65, 997–1008. https://doi.org/10.1093/sysbio/syw037.

Dafni, A. & Potts, S.G. (2004) The role of flower inclination, depth, and height in the preferences of a pollinating beetle (Coleoptera: Glaphyridae). Journal of Insect Behavior, 17, 823–834.

Dafni, A., Bernhardt, P., Shmida, A., Ivri, Y., Greenbaum, S., O’Toole, C. & Losito, L. (1990) Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. Israel Journal of Botany, 39, 81–92.

d’Hotman, D. & Scholtz, C.H. (1990) Comparative morphology of the male genitalia of derived groups of Scarabaeoidea (Coleoptera). Elytron, 4, 3–39.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. 
\textit{PLoS Biology}, 4, e88. https://doi.org/10.1371/journal.pbio.0040088.

Edgar, R.C. (2004a) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. 
\textit{BMC Bioinformatics}, 5, 113.

Edgar, R.C. (2004b) MUSCLE: multiple sequence alignment with high accuracy and high throughput. 
\textit{Nucleic Acids Research}, 32, 1792–1797.

Emadzade, K. & Hörandl, E. (2011) Northern Hemisphere origin,
\textit{Northem Hemisphere origin,} 
Emadzade, K., Gehrke, B., Linder, H.P. & Hörandl, E. (2011) The Systematic 
\textit{Systematic Entomology} 
\textit{Systematic Entomology} 
Hunt, T., Bergsten, J., Levkanicova, Z. 
\textit{Systematic Entomology} 
\textit{Systematic Entomology}

Edgar, R.C. (2004a) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. 
\textit{BMC Bioinformatics}, 5, 113.

Edgar, R.C. (2004b) MUSCLE: multiple sequence alignment with high accuracy and high throughput. 
\textit{Nucleic Acids Research}, 32, 1792–1797.

Emadzade, K., Gehrke, B., Linder, H.P. & Hörandl, E. (2011) The Systematic 
\textit{Systematic Entomology} 
\textit{Systematic Entomology} 
Hunt, T., Bergsten, J., Levkanicova, Z. 
\textit{Systematic Entomology} 
\textit{Systematic Entomology}

Hawkins, S.J. (2006) Revision of the Chilean tribe Lichniini Burmeister, 1844 (Coleoptera: Scarabaeidae: Melolonthinae). 
\textit{Zootaxa}, 1266, 1–63.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. 
\textit{Molecular Biology and Evolution}, 35, 518–522. https://doi.org/10.1093/molbev/msx281.

Hunt, T., Bergsten, J., Levkanićova, Z. et al. (2007) A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. 
\textit{Science}, 318, 1913–1916.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. 
\textit{Nucleic Acids Research}, 30, 3059–3066.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. 
\textit{Nucleic Acids Research}, 33, 518–518.

Keasar, T., Harari, A.R., Sabatinelli, G. et al. (2010) Red anemone 
guild flowers as focal places for mating and feeding of Mediterranean glaphyrid beetles. 
\textit{Biological Journal of the Linnean Society}, 99, 808–817.

Keasar, T., Kishinevsky, M., Shmida, A., Gerchman, Y., Chinkov, N., Koplovich, A. & Katzir, G. (2013) Plant-derived visual signals may protect beetle herbivores from bird predators. 
\textit{Behavioral Ecology and Sociobiology}, 67, 1613–1622.

Krell, F.-T. (2000) The fossil record of Mesozoic and Tertiary Scarabaeoidea (Coleoptera: Polyphaga). 
\textit{Invertebrate Taxonomy}, 14, 871–905.

Krell, F.-T. (2006) Fossil record and evolution of Scarabaeoidea (Coleoptera: Polyphaga). 
\textit{Coleopterists Society Monograph}, 5, 120–143.

Krell, F.-T. (2007) Catalogue of Fossil Scarabaeoidea (Coleoptera: Polyphaga) of the Mesozoic and Tertiary - Version 2007. 
Technical Report 2007–2008. Denver Museum of Nature and Science, pp. 1–79.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. 
\textit{Molecular Biology and Evolution}, 29, 1695–1701.

Lanfear, R., Calcott, B., Kainer, D., Mayer, C. & Stamatakis, A. (2014) Selecting optimal partitioning schemes for phylogenomic datasets. 
\textit{Evolutionary Biology}, 14, 1–14.

Lawrence, J.F., Stipltinski, A., Seago, A.E., Thayer, M.K., Newton, A.F. & Marvaldi, A.E. (2011) Phylogeny of the Coleoptera based on morphological characters of adults and larvae. 
\textit{Annales Zoologici}, 61, 1–217.

Liu, K., Warnow, T.J., Holder, M.T., Nelesen, S.M., Yu, J., Stamatakis, A.P. & Linder, C.R. (2012) SAT-e-II: very fast and accurate simultaneous estimation of multiple sequence alignments and phylogenetic trees. 
\textit{Systematic Biology}, 61, 90–106.

Machatschke, J.W. (1959) Untersuchungen über die verwandtschaftlichen Beziehungen der Gattungen der bisherigen Glaphyrinae (Coleoptera: Lamellicornia). 
\textit{Beiträge zur Entomologie}, 9, 528–545.

Martinez-Harms, J., Vorobyev, M., Schorn, J. et al. (2012) Evidence of red sensitive photoreceptors in Pygopleurus israelitis (Glaphyrinae: Coleoptera) and its implications for beetle pollination in the southeast Mediterranean. 
\textit{Journal of Comparative Physiology A}, 198, 451–463.

McKenna, D.D., Shina, S., Ahrens, D. et al. (2019) The evolution and genomic basis of beetle diversity. 
\textit{Proceedings of the National Academy of Sciences of the United States of America}, 116, 24729–24737.

Medvedev, S.I. (1951) Plastinchatousye (Scarabaeidae), posdem. Melolonthinae, ch. 1 (chrushchi). Fauna SSSR, Zhestkokrylye. Tom 10, vyp. 1. Izd. Akad. Nauk SSSR, Leningrad, p. 512.

Medvedev, S.I. (1952) Plastinchatousye (Scarabaeidae), posdem. Melolonthinae, ch. 2 (chrushchi). Fauna SSSR, Zhestkokrylye. Tom 10, vyp. 2. Izd. Akad. Nauk SSSR, Leningrad, p. 274.

Midgley, J. (1992) Why do some hopliinid beetles have large hind legs? 
\textit{Journal of the Entomological Society of Southern Africa}, 55, 157–159.

Monaghan, M.T., Inward, D.G., Hunt, T. & Vogler, A.P. (2007) A molecular phylogenetic analysis of the Scarabaeinae (dung beetles). 
\textit{Molecular Phylogenetics and Evolution}, 45, 674–692. https://doi.org/10.1016/j.ympev.2007.06.009.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. 
\textit{Molecular Biology and Evolution}, 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Nikodym, M. & Bezdek, A. (2016) Scarabaeoidea, family Glaphyrinae. 
\textit{Catalogues of Palaeartic Coleoptera, Glaphyrinae – Scirotoidae – Dascilloidea – Buprestoidea – Byrrohoidae, Vol. 3 (ed. by I. Löbl and D. Löbl), pp. 87–97.} Brill, Leiden-Boston.

Nikolajev, G.V. & Ren, D. (2012) New species of the genus Lithophaga (Coleoptera, Scarabaeidae) from the Lower Cretaceous Yixian Formation, China. 
\textit{Phylogeny of Glaphyrinae}, 2811, 212–212.

Nikolajev, G.V. & Ren, D. (2013) Phylogeny of the Coleoptera based on morphological characters of adults and larvae. 
\textit{Annales Zoologici}, 61, 1–217.

Nikolajev, G.V., Wang, B. & Zhang, H.C. (2011) A new fossil genus of the family Glaphyrinae (Coleoptera: Scarabaeidae) from the Lower Cretaceous Xiyian Formation. 
\textit{Zootaxa}, 2811, 47–52.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004) Bayesian phylogenetic analysis of combined data. Systematic Biology, 53, 47–67.

Orme, D., Freckelton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N &Pearse, W. (2018) Caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 1.0.1. URL https://CRAN.R-project.org/package=caper [accessed on 15 June 2015].

Rambaut, A. & Drummond, A.J. (2013) TRACER v. 1.6. URL http://beast.bio.ed.ac.uk/Tracer

Ritcher, P.O. (1966) White Grubs and their Allies: A Study of North American Scarabaeoid Larvae, p. 219. Oregon State University Press, Corvallis, Oregon.

Robbins, P.S., Zhang, A., Averill, A.L., Linn, C.E. Jr, Roelofs, W.L., Sylvia, M.M. & Villani, M.G. (2006) Sex pheromone of the cranberry root grub Lichnanthe vulpina. Journal of Chemical Ecology, 32, 1663–1672.

Schliep, K.P. (2011) phangorn: phylogenetic analysis in R. Bioinformatics, 27, 592–593.

Scholtz, C.H. (1990) Phylogenetic trends in the Scarabaeoidea (Coleoptera). Journal of Natural History, 24, 1027–1066.

Scholtz, C.H. & Grebenikov, V.V. (2005) Scarabaeiformia Crowson, 1960. Handbook of Zoology. A Natural History of the Phyla of the Animal Kingdom. Vol. 4 Arthropoda: Insecta, Part 38. Coleoptera, Beetles Vol. 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim) (ed. by R.G. Beutel and R.A.B. Leschen), pp. 345–425. Walter de Gruyter, Berlin and New York, New York.

Shmida, A. (2005) MAPA’s Dictionary of Plants and Flowers in Israel, p. 493. MAPA Publisher, Tel Aviv.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America, 87, 651–701.

Smith, A.B.T., Hawks, D.C. & Heraty, J.M. (2006) An overview of the classification and the evolution of the major scarab beetle clades (Coleoptera: Scarabaeoidea) based on preliminary molecular analyses, Coleopterists Society Monographs, 5, 35–46.

Sommer, N. (2010) Wenn Käfer rot sehen: Untersuchungen zur Rot-sichtigkeit und Bestäubungsbiole bei mediterranen Vertretern der Familie Glaphyridae anhand molekularbiologischer sowie ethologischer Methoden. Diploma Thesis, University Vienna, Vienna, p. 80.

Thornhill, R. & Alcock, J. (1983) The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Massachusetts and London. URL http://dx.doi.org/10.4159/harvard.9780674433960.

Warren, D.L., Geneva, A.J. & Lanfear, R. (2017) RWTY (R We There Yet): an R package for examining convergence of Bayesian phylogenetic analyses. Molecular Biology and Evolution, 34, 1016–1020.

Westcott, R.L. (1976) Observations on the Biology and Ethology of Lichnanthe rathvoni LeConte (Coleoptera: Scarabaeidae) with Emphasis on Mating., University of Idaho, Department of Entomology Anniversary Publication, No. 11: 85–90.

Wickham, H.F. (1910) New fossil Coleoptera from Florissant, with notes on some already described. American Journal of Science, 29, 47–51.

Yan, Z., Nikolaev, G.V. & Ren, D. (2012) A new, well-preserved genus and species of fossil Glaphyridae (Coleoptera, Scarabaeoidea) from the Mesozoic Yixian Formation of Inner Mongolia, China. ZooKeys, 241, 67–75.

Zhao, H., Bai, M., Shih, C. & Ren, D. (2016) Two new glaphyrids (Coleoptera, Scarabaeoidea) from the Jehol Biota, China. Cretaceous Research, 59, 1–9.

Zunino, M. (1988) I Glaphyridae e la Filogenesi degli Scarabaeoidea (Coleoptera). Atti XV Congresso nazionale Italiano di Entomologia, L’Aquila, pp. 229–233.

Accepted 11 February 2020
First published online 9 March 2020

© 2020 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, Systematic Entomology, 45, 838–848