

Molecular phylogeny of the Saprininae (Coleoptera: Histeridae): the evolution of psammophily or life in sand

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Received 13 December 2018; revised 17 January 2019; accepted for publication 17 January 2019

The subfamily Saprininae is a group of moderately large histerid beetles that exhibits remarkable morphological and biological diversity. Although all species are predaceous, feeding on larvae of flies and other arthropods, numerous ecologically diverse lineages appeared during the evolution of the group, including taxa adapted for life in sand. These psammophiles conquered the Earth's arid and sandy desert regions. They are endowed with profound morphological adaptations, and the beginning of their radiation largely coincides with the Palaeocene–Eocene Thermal Maximum, which constituted the peak of the long-term early Cenozoic global warming. Earth's temperature during that time was believed to be globally ~8 °C warmer than it is today, and the aridification played an important role in the desertification of the planet. Here, we present the first comprehensive phylogenetic analysis of Saprininae to date, based on 115 taxa of 37 (sub)genera, which represent all major Saprininae genera. Our analyses resulted in a well-resolved phylogeny of the group, partly congruent with earlier phylogenetic hypotheses. We find support for the monophyly of the Saprininae and four major clades within it. Ultra-psammophily has evolved at least three times independently, with convergent morphological features arising in different desert regions, as a response to similar selective pressures.

ADDITIONAL KEYWORDS: Coleoptera – evolution – Histeridae – phylogeny – psammophily – Saprininae.

INTRODUCTION

Sand is a naturally occurring granular material composed of finely divided rock and mineral particles. It is an important substrate from an ecological perspective owing to its structure, the shifting nature of the surface, the relative lack of shelter on the surface and its relative ease of penetration (Seely, 1991). Although the exact definition of sand varies, it is a soil type, more precisely a soil containing > 85% of sand-sized particles by mass (Minister of Supply and Services, Canada 1978). The size of sand particles should be between 0.074 and 4.75 mm according to the scientific Unified Soil Classification System. Large sandy deposits occur in dune systems, which are commonly present in desert environments and, for example, along the coasts of larger water bodies. They have a worldwide distribution covering almost

every latitude, from tropical to polar. Several large and threatened arthropod species are tied to inland sand areas, e.g. the ground beetle *Broscus cephalotes* (Linnaeus, 1758) and the zopherid beetle *Orthrocerus clavicornis* (Linnaeus, 1758) (Lönnberg & Jonsell, 2012).

Sand dunes provide habitat for many highly specialized, endemic species. A high ecological diversity and presently fragile and threatened environments characterize this habitat. Thus, many dune species are endangered, and numerous countries around the world implement large-scale dune protection programmes, with several dune systems holding high conservation status (e.g. in The Netherlands, USA, New Zealand and Turkmenistan). Coastal dunes have been referred to as 'nature's coastal defence', effectively protecting the coast from inundations and flooding (Queensland Government, Department of Environment and Heritage Protection, Australia), with plants playing a vital role in the process of their formation, trapping the deposited sand particles and acting as a windbreak.

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Perhaps owing to their inert appearance, sand dunes and dune structures are often overlooked regarding the diversity of life they support. Without being obvious, the constantly moving sand forms a very rich and productive habitat. Dune structures form by the continuous accumulation of windblown sand in hyper-arid regions or along the seashore, and for any animals they pose severe problems. Extreme and hostile microclimatic conditions often prevail on the surface (high temperature, low humidity and sand abrasion), but below there is a gradient of variable temperatures and humidity that organisms can choose (Henschel & Lubin, 1997).

As is the case the world over, the physical adaptations of the flora and fauna are often representative of the environment they inhabit. Species inhabiting dunes are morphologically adapted for life in such harsh conditions and are referred to as psammophiles or arenophiles. Psammophily is a derived lifestyle, and sand-dwelling organisms have evolved manifold adaptations. The colonization of sandy environments and speciation processes in organisms have been explained by various hypotheses (see, e.g. Ward & Seely, 1996). Among these adaptations can be body pilosity, pale or cryptic body coloration, cryptonephridism, high heat tolerance or low metabolic rate. To cite several arthropod examples, completely fringed anterior legs with setae in Namib Desert scorpions (Lawrence, 1978); complex setae, claws or keels that facilitate walking in or on sand in Namib Desert spiders (Henschel, 1997); strongly vaulted body, loss of protarsi and enlargement of protibiae in Histeridae: Sapriniinae beetles (Olexa, 1990; Lackner, 2010) are all among the morphological adaptations to psammophily. In Collembola, the body-size reduction, loss of furca, eyes and pigmentation, and presence of short legs and antennae were considered by Thibaud & Christian (1997) as morphological regressions correlated with psammophily. D'Haese (2000), however, did not fully subscribe to this opinion, because the same regressions also occur in Collembola species living in loamy soils. D'Haese (2000) suggested that these features could have served as adaptations to the sandy habitat that made the radiation possible. The diurnal, large-sized darkling beetles (Coleoptera: Tenebrionidae) of the Namib Desert are among the most intensively studied of all desert beetles (Lamb & Bond, 2013). They exhibit a large degree of specialization, with adaptive characters associated with substrate thermal and water relationships that are unknown from other deserts (Lamb & Bond, 2013). In fact, the tenebrionid genus *Onymacris* Allard, 1885 has become a model organism for coleopteran adaptations to desert life (Cloudsley-Thompson, 2001). According to Prendini (2001), who studied the scorpion genus *Brachisternus*, the morphological characters identified as psammophilous adaptations evolved

in a similar manner among many distantly related scorpion taxa.

How such adaptive modifications evolve, and what ancestral traits predispose taxa to switch to a psammophile existence, remains poorly understood. Given the overt phenotypic specialization associated with psammophily, a further key question arises: is this lifestyle an evolutionary 'dead end', effectively limiting subsequent potential evolutionary shifts to other ecological niches? A phylogenetic analysis by D'Haese (2000) argues against this hypothesis; in his study, the psammophily of the collembolan genus *Willemia* Börner, 1901 is presumed to be the ancestral living condition, with two reversions to the loam-confined life. In fact, there have been multiple studies investigating specialization as a possible 'dead end', where a lineage evolves to utilize a significantly narrower range of resources than related lineages (Haldane, 1921; Kelley & Farrell, 1998; Vámosi *et al.*, 2014). An integrated study combining molecular and morphological characters by Ojangurren-Affilastro *et al.* (2016) on the South American bothriurid scorpion genus *Brachistosternus* Pocock, 1893 concluded that psammophily evolved on at least four independent occasions. In a recent study on a small group of Histeridae: Sapriniinae beetles, it was concluded that psammophily evolved only once, but has been lost several times subsequently and even reversed to the presumed ancestral inquilinous lifestyle (Lackner, 2014a).

Here, we continue to investigate the Sapriniinae (Fig. 1). This is a moderately large beetle subfamily with a worldwide distribution (73 genera and subgenera, > 620 species; Lackner & Tarasov, 2019, in press). The zoogeographical distribution, taxonomy, morphology and biology of these predatory beetles have been the subject of intensifying research in the past 10 years (see, e.g. Lackner, 2010, 2014a, b; Lackner & Leschen, 2017 and the references therein). Although the monophyly of the subfamily is well supported by morphological characters (Lackner, 2014a), the efforts of previous authors (e.g. Reichardt, 1932) to disentangle their inter-relationships and elucidate their evolutionary history and ecologies were unsuccessful, and the inter-relationships remain poorly understood. The traditional classification of the Sapriniinae (e.g. Mazur, 2011) is largely based on an intuitive assessment of easily observable characters, without any phylogenetic background (cf. Lackner, 2014a). The first attempt to reclassify the subfamily was the morphology-based phylogenetic study by Lackner (2014a), which concluded that the Sapriniinae are monophyletic, but based on the low resolution of the cladogram, did not propose a new classification for the taxa within. Lackner's (2014a) study stated that: (1) the taxa closest to the root of the cladogram

are mostly inquilines; (2) inquilinity, the inferred plesiomorphic lifestyle of the subfamily, has undergone several transformations to other lifestyles during the



Figure 1. A representative of the Saprinae subfamily: *Philothis (Farabius) reichardti* Kryzhanovskij, 1966, holotypus, habitus, dorsal view.

evolutionary history of the subfamily; (3) psammophily, with a large number of species, occurred only once but was lost several times; (4) invasion of ant nests, in contrast, occurred three times independently (in different biogeographical regions; Lackner, 2014a; see also Lackner & Leschen, 2017); and (5) and invasion of termite nests also happened three times independently (twice in Africa and once in Australia). These results implied that Saprinae are a potential model for understanding how extreme ecological specialization channels subsequent life-history evolution.

Here, we present the first comprehensively sampled phylogeny of the Saprinae with the following goals: (1) to investigate the evolution of psammophily; and (2) to confirm or to rule out the role of morphological convergence within the group.

MATERIAL AND METHODS

TAXON SAMPLING

For this study, we sampled 115 Saprinae species and subspecies, representing 37 genera and subgenera (Fig. 2; Supporting Information, Table S1). Outgroups included four taxa of Histeridae: two species of Dendrophilinae: *Dendrophilus (Dendrophilus) punctatus punctatus* (Herbst, 1792) and *Dendrophilus (Dendrophilus) pygmaeus* (Linnaeus, 1758)] and two species of Abraeinae [*Abraeus (Abraeus) perpusillus* (Marsham, 1802) and *Chaetabraeus (Chaetabraeus) spec.* (Botswana)]; members of these subfamilies have been previously recovered as sister group or taxa related to Saprinae (Caterino & Vogler, 2002). As more distant outgroup representatives, we included representatives of Sphaeritidae [*Sphaerites glabratus* (Fabricius,

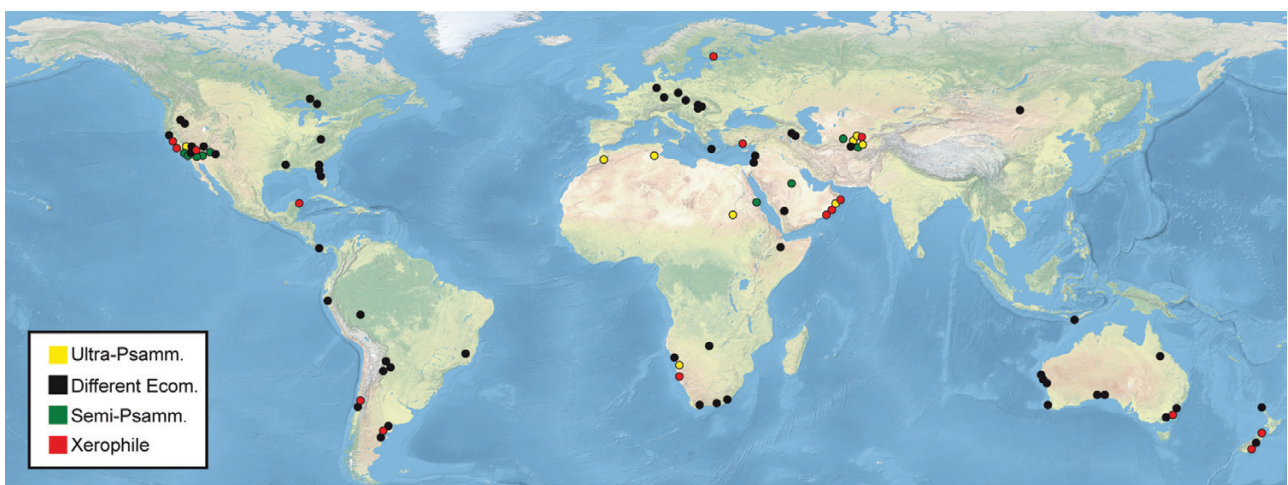


Figure 2. Map of the sampled taxa used in the present study.

1792)], Hydrophilidae [*Hydrobius fuscipes* (Linnaeus, 1758) and *Hydrophilus atterimus* (Eschscholtz, 1822)], Helophoridae (*Helophorus guttulus* Motschulsky, 1860) and Hydrochidae (*Hydrochus carinatus* Germar, 1824); taxa that either belong to the superfamily Histeroidea (*Sphaerites*) or to the sister superfamily (Hydrophiloidea).

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from 95% ethanol-preserved specimens using the DNeasy Blood & Tissue Kit (Quiagen, Hilden, Germany). The specimens were taken out from ethanol vials, pierced with a pin in their metathoracic or abdominal area and left to dry out for 12–24 h. Polymerase chain reaction was carried out in a total volume of 25 µL containing 5 µL Mango Buffer (5× reaction buffer, coloured; Bioline, Luckenwalde, Germany), 2 µL dNTPs (10 mM dNTP Mix; Bioline), 1.25 µL MgCl₂ (50 mM; Bioline), 1 µL of each primer (10 mM; Metabion International AG, Planegg, Germany) and 0.5 µL MangoTAQ (5 u/µL; Bioline). For primer sequences and thermocycling conditions see the Supporting Information (Tables S3 and S4). For some challenging samples, only a shorter fragment of the 5′ *COI* was amplified and sequenced.

MOLECULAR MARKERS

Three gene fragments were analysed in this study: the 5′ part and the 3′ part of the mitochondrial cytochrome oxidase subunit 1 (*COI*) and the partial nuclear 18S rRNA gene. For phylogenetic analyses, the sequences of the three fragments were concatenated and merged with previously published data for ten other Sapriniinae species. *Helophorus guttulus*, *Hydrochus carinatus*, *Hydrobius fuscipes*, *Hydrophilus atterimus* and *Sphaerites glabratus* were also downloaded from GenBank and added as outgroups (see Supporting Information, Table S1). In the alignment of concatenated sequences (2080 bp total length), 805 bp corresponded to the 3′ end of the *COI* gene, 658 bp to the 5′ end of the *COI* gene and 617 bp to the partial 18S gene.

PHYLOGENETIC ANALYSES

To identify ambiguous or random similarity in alignment, we applied Aliscore v.2.076 (Misof & Misof, 2009) with -e option and default settings, and subsequently masking problematic sites using Alicut v.2.3 (Kück *et al.*, 2010; https://github.com/mptrsen/scripts/blob/master/ALICUT_V2.3.pl). A maximum

likelihood analysis was conducted using IQ-TREE (Nguyen *et al.*, 2015; Trifinopoulos *et al.*, 2016). The substitution model was not defined a priori; instead, the implemented ModelFinder (Kalyanamoothy *et al.*, 2017) was applied to find the best models during analysis (see Supporting Information, Table S2) under the Bayesian information criterion. For estimates of support, 1000 replicates of ultrafast bootstrapping (Minh *et al.*, 2013) were performed. Owing to lack of known Sapriniinae fossils, we calibrated the analysis using the most recent work on evolutionary history of Coleoptera (Zhang *et al.*, 2018); as calibration points, we used the split between Histeroidea and Hydrophiloidea (214 Mya, SD = 12 Mya) and the most common ancestor of the family Histeridae (112 Mya, SD = 12 Mya). Divergence times at nodes were estimated with BEAST v.1.8.1 (Suchard *et al.*, 2018) using the fixed topology from maximum likelihood analysis, with the HKY+I+G model. The dataset was partitioned according to markers *COI* 3′, *COI* 5′ and 18S, applying an uncorrelated lognormal clock (for fragments of *COI* gene, we used molecular dating rates of 0.0115 mutations per lineage per million years (Brower, 1994) and a Yule speciation prior (Gernhard, 2008). The analysis was run for 100 million generations, with 10 000 sampling frequency. TRACER v.1.7 (Rambaut *et al.*, 2018) was used for checking a plateau. The maximum credibility tree was estimated using TREEANNOTATOR (Suchard *et al.*, 2018) after discarding the initial 30% of trees as burn-in.

RECONSTRUCTION OF THE ECOLOGICAL TRAIT

Reconstruction of the ancestral trait was performed using the ‘Discrete traits’ function in BEAST v.1.8.1. All analyses were run under settings described at <https://beast-classic.googlecode.com/files/ARv2.0.1.pdf> for 100 million generations, with sampling every 10 000 generations. The maximum credibility tree was estimated using TREEANNOTATOR after the effective sample size values were > 500, and 30% of trees were discarded as burn-in.

DEFINITIONS OF ECOLOGICAL UNITS

Sapriniinae are generally more common and diverse in arid than mesic areas but have likewise evolved other adaptations. When trying to delimit the biology of the groups living in dry conditions with particular regard to the morphological adaptations, we decided to divide them into three morphologically defined groupings: xerophiles, semi-psammophiles and ultra-psammophiles. We termed the fourth ecological unit ‘different ecomorph’.

1. *Xerophile*: according to Lincoln *et al.* (2003), a 'xerophile is an organism thriving in dry habitats'. In our study, we group into this category taxa that show traits of incipient adaptations to the environment they occupy. Specifically, this mostly means short to moderately long underside vestiture. These species are found on sandy soils, so-called psammobiomes, but can also be found away from them. They usually occur on carcasses, less often on dung.
2. *Semi-psammophile*: in this group, we include all taxa whose adaptations to psammophily are more pronounced, but not yet extreme. These taxa possess slightly to moderately dilated tibiae, a setose underside, and thin, long and straight

meso- and metatarsal claws. A peculiar group are the so-called psammolittoral taxa, which are found in damp sand, mostly on the beach under wrack or algae. These animals tend to have enlarged coxa and femora, incorporating a larger amount of muscle to offset the force used for digging in damp beach sand. When the mesocoxa are enlarged, the mesoventrite is reduced in size; in the case of enlarged procoxa, the prosternum is strongly compressed, forming a knife-like keel in extreme cases (Fig. 3D). Semi-psammophiles are rarely found away from sandy soils, but are not found buried deep inside the sand dunes.

3. *Ultra-psammophile*: in our study, we define the ultra-psammophiles as organisms that are found

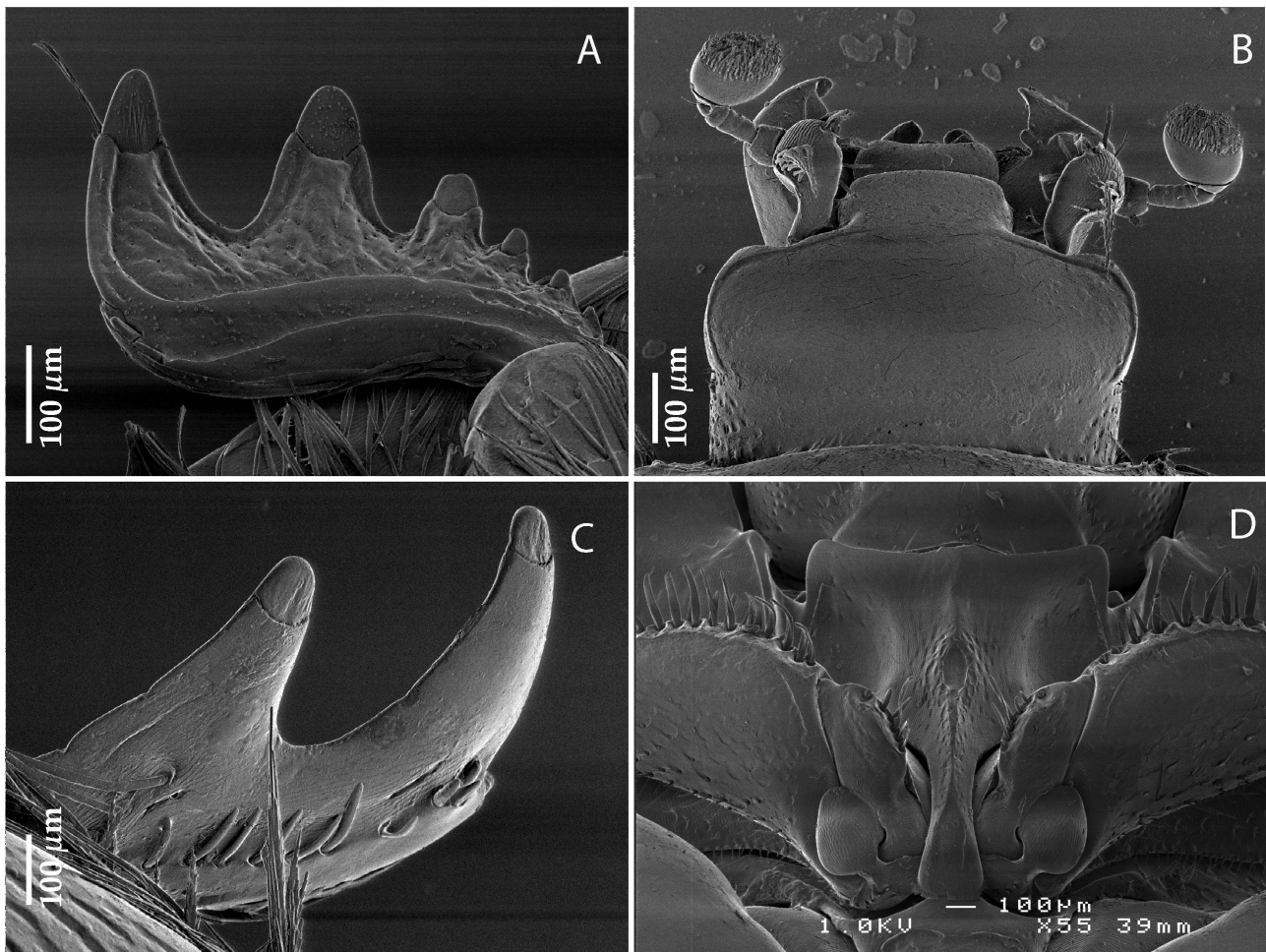


Figure 3. Examples of morphological adaptations to psammophily in the Sapriniinae beetles. A, enlarged protibia of a psammophilous saprinine beetle, *Terametopon (Psammoprinus) deserticola* Gomy & Vienna, 1997. B, flattened eyes of a psammophilous saprinine beetle, *Philothis (Philothis) arcanus* Reichardt, 1930. C, absent protarsus of a psammophilous saprinine beetle, *Philothis (Atavinus) atavus* Reichardt, 1931. D, strongly compressed prosternum of a semi-psammophilous beetle, *Pachylopus rossi* Kovarik, Verity & Mitchell, 1999.

exclusively in sand dune systems, buried deep in the sand. They are usually found only by rather laborious methods, exclusively near the roots of plants entombed by moving sand (Olexa, 1990). These beetles are extremely well adapted to the environment (they are also known as ‘sand-swimmers’ for their ability to slide down the falling sand), in which they burrow. They possess enlarged tibiae (Fig. 3A); flattened eyes, almost invisible from above (Fig. 3B); an extremely vaulted body (Fig. 1); atrophied or outright absent protarsi (Fig. 3C); reduced teeth on the outer margin of the protibiae; a setose underside (including elytral epipleuron); often reduced head and acute anterior pronotal angles; or compressed and usually setose prosterna (Fig. 3D).

4. *Different ecomorph*: here, we classify taxa that are not linked to the sandy soils or even to arid conditions. Among these can be (e.g. *Hypocaccus* Thomson, 1867) species found on shoals and gravel riverbanks, taxa that are inquilines of various mammals, birds, reptiles or social insects, in addition to free-living volant predators found on the steppe or other grassy or mesic areas. These taxa do not show any morphological adaptation to life in sand and are likely to represent the outgroup condition to all the above.

RESULTS AND DISCUSSION

The Saprininae are monophyletic, with high support (Fig. 4; for the complete tree including outgroups, see Supporting Information, Fig. S1); this confirms the results by Lackner (2014a). Four major clades are recognized, each with high support. When comparing our results with those of Lackner (2014a), who analysed morphological data, the composition of the amphipolar clade containing *Gnathoncus* Jacquelin-Duval, 1858 (mainly Holarctic Region) and *Tomogenius* Marseul, 1862 (Australian Region) is confirmed. These two taxa are represented in our analysis by two and three species each, respectively, and form a well-supported clade also containing the monotypic *Myrmetes* Marseul, 1862 (West Palaearctic), *Aphelosternus* Wenzel, 1962 (Nearctic: California) and *Saprinodes* Lewis, 1891 (Australia: NSW and Queensland). Lackner (2014a) included *Myrmetes* in the basal ‘grade’ along with *Gnathoncus* and *Tomogenius*, whereas the relationships of both *Aphelosternus* and *Saprinodes* were more remote. The Californian endemic inquiline *Aphelosternus* is recovered in the present analysis as sister to the Australopacific *Tomogenius*. *Saprinodes*, an enigmatic Australian endemic containing two species of unknown biology, was recovered by Lackner (2014a) in a clade with the monotypic Australian myrmecophile *Iridoprinus* Lackner & Leschen, 2017

[coded as ‘Saprininae gen. nov.’ by Lackner (2014a) and described formally by Lackner & Leschen (2017)]. Here, *Saprinodes* is recovered as sister to *Gnathoncus* + *Myrmetes*. All taxa of this clade (with the exception of *Saprinodes*, which is of unknown biology) are inquilines of birds, small rodents or ants.

The next large clade contains entirely New World taxa and is split into two smaller clades: one containing mostly members of the Nearctic inquiline genus *Geomysaprinus* Ross, 1940 and the other one containing mostly Neotropical *Euspilotus* Lewis, 1907. *Geomysaprinus*, represented in our analysis by two subgenera and ten species, was recovered as monophyletic, with very high support. The Argentinian taxon *Euspilotus* (*Euspilotus*) *lacordairei* (Marseul, 1855) was recovered as a sister group. The relationship between *E. (E.) lacordairei* and *Geomysaprinus* received low support and might be considered spurious. Lackner’s (2014a) morphological analysis recovered two included members of *Geomysaprinus* in two different lineages; our results, in contrast, strongly argue for the monophyly of the genus. Taxa included in the second clade containing mainly members of *Euspilotus* mostly received low support for their inter-relationships. Only several Argentinian and Chilean members of *Euspilotus* were recovered as monophyletic, whereas the position of Argentinian *Xerosaprinus* (*Xerosaprinus*) *dolatus* (Marseul, 1862), far removed from the rest of *Xerosaprinus* Wenzel, 1962, indicated a larger problem of *Euspilotus* being probably paraphyletic (Gerardo Arriagada, in litt.). Another member of the genus *Euspilotus*, *Euspilotus* (*Hesperosaprinus*) *alvarengai* Arriagada, 2012 from Guyana and Brazil, has been recovered as sister to the monophyletic attaphilic *Phoxonotus* Marseul, 1862, with very high support. *Chelyoxenus xerobatis* Hubbard, 1894, a southeastern USA gopher tortoise [*Gopherus polyphemus* (Daudin)] inquiline, has been recovered as sister to the *E. (H.) alvarengai* + (*Phoxonotus*) clade, albeit with very low support. The distribution of *Euspilotus* on the tree suggests non-monophyly of the genus; a result inferred already by Lackner (2014a) and confirming the opinion of DeMarzo & Vienna (1982).

The third large clade contains all the semi-psammophiles and psammophiles, while also containing several inquilines of small mammals and free-living taxa. This clade is almost identical to the one recovered by Lackner (2014a); its members share a synapomorphy of single vesicle inside the antennal club (for figures, see Lackner, 2010: figs 29–34). Their inter-relationships were almost unresolved by Lackner (2014a), with only several clades (e.g. *Philothis* Reichardt, 1930) supported by ‘stronger’ morphological synapomorphies. The present analysis depicts a basal position of the Middle-Asian semi-psammophile *Axelinus ghilarovi* Kryzhanovskij,

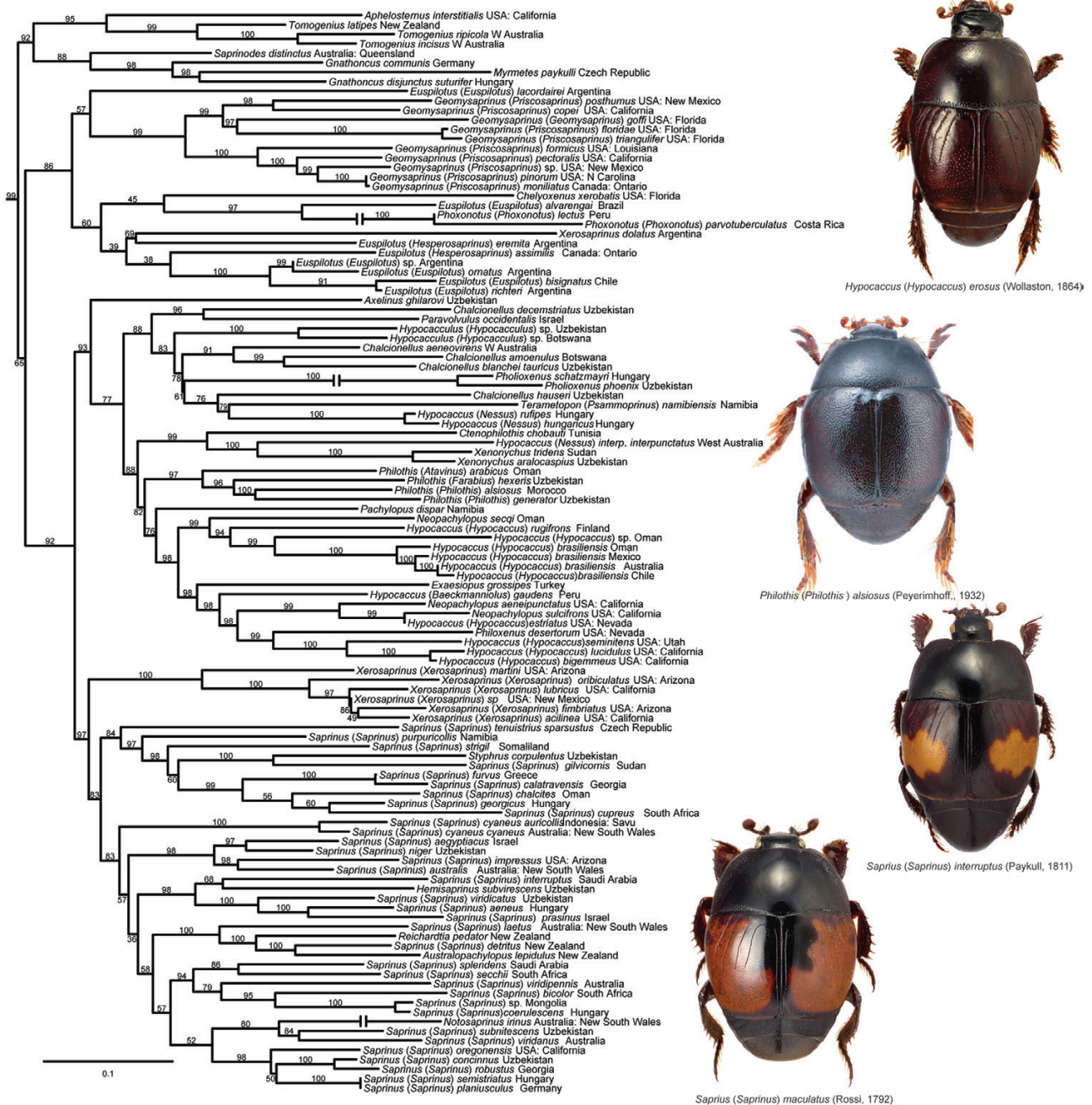


Figure 4. The phylogenetic hypothesis of the Sapriniinae inferred from the MAFFT-aligned dataset. Outgroups have been removed for better readability. Branch labels represent bootstrap support values.

1972 as sister to the rest, with high support. The rest of the clade is further split into two sub-clades; both equally highly supported. The distribution of the members of the genera *Chalcionellus* Reichardt, 1932 or *Hypocaccus* on the tree argues against their monophyly. On the contrary, monophylies of the fully ultra-psammophilic genera *Philothis* and *Xenonychus*

Wollaston, 1864 and the inquilineous *Pholioxenus* Reichardt, 1932 are confirmed. In order to resolve the inter- and intrarelations of species-rich genera, such as *Hypocaccus*, *Hypocacculus* Bickhardt, 1914, *Chalcionellus*, *Pholioxenus* (especially with regard to the African taxa) or *Paravolvulus* Reichardt, 1932, a much denser sampling would be required. Several taxa

that we would consider as important for the analysis (e.g. *Xenophilothis choumovitchi* Théron, 1962, *Pachylopus rossi* Kovarik, Verity & Mitchell, 1999, or members of *Dahlgrenius* Penati & Vienna, 1996) were likewise absent from the analysis (not sampled).

The fourth large clade contains mostly members of the genus *Saprinus* Erichson, 1834, with monophyletic Nearctic xerophile *Xerosaprinus* sister to the rest. The genus *Saprinus* is the most species-rich and widespread taxon of the subfamily (Mazur, 2011). Although presumed to be non-monophyletic (see, e.g. Lackner, 2014a), our analyses show its monophyly, with moderately high support. With 34 sampled species, *Saprinus* was the most densely sampled taxon in our analysis. Its members are mostly free-living predators, with several members [Palaeartic *Saprinus interruptus* (Paykull, 1811) and *Saprinus gilvicornis* Erichson, 1834] found mostly in sandy soils, showing adaptations to xerophily; whereas two New Zealand monotypic genera (*Reichardtia* and *Australopachylopus*) adapted for the psammo-littoral way of life. We refrain from further comment on the relationships within *Saprinus* at this stage, given the insufficient sampling; further comments and taxonomic changes will be addressed in a separate study (L.T., M.M., K.C. & B.M., unpublished).

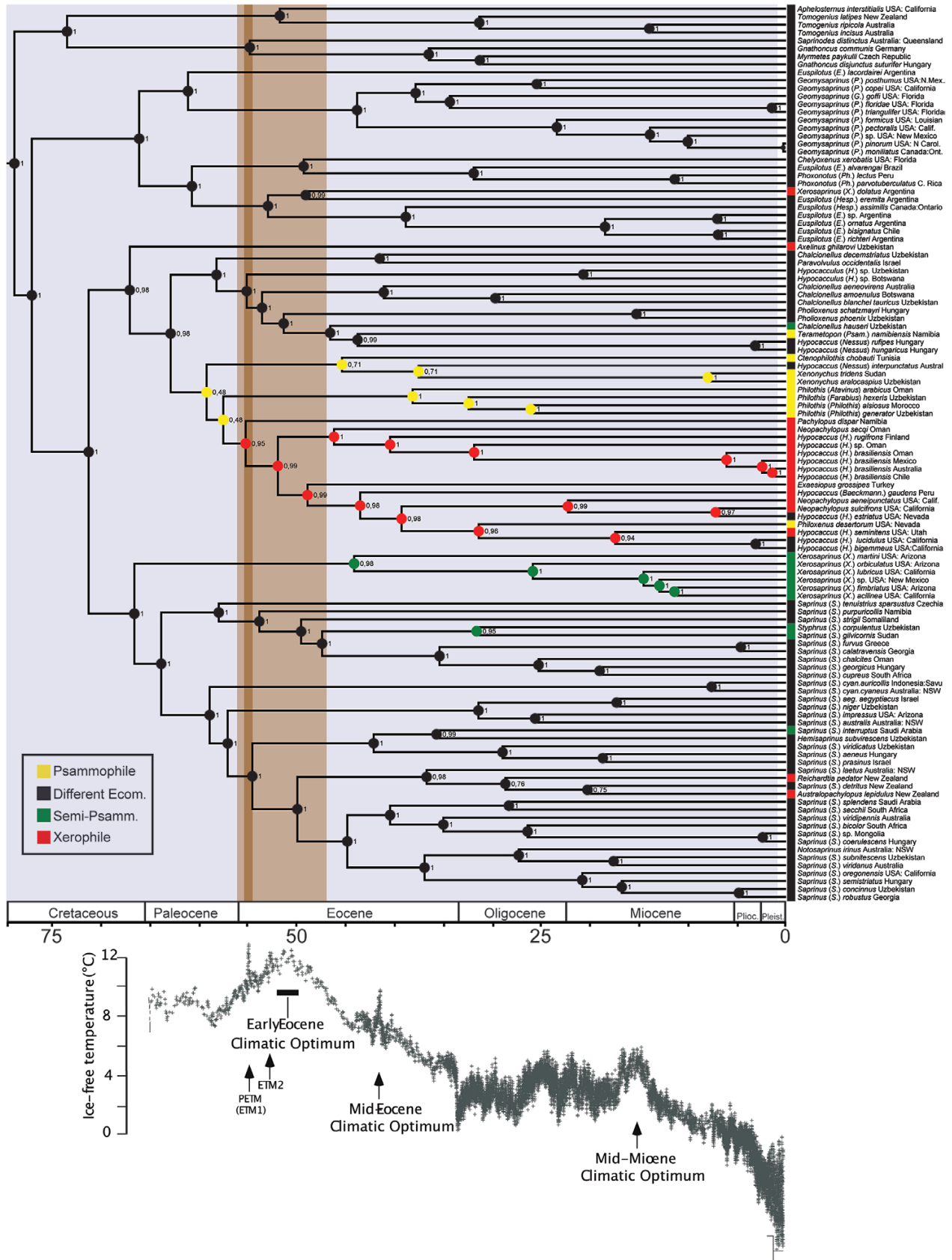
ECOLOGY

Evolution of the psammophily in the Sapriniinae

To interpret the evolutionary history of organismal characters, phylogenies are regarded as the best tool (Chen *et al.*, 2015). We reconstructed the phylogeny of the Sapriniinae to address the evolution of psammophily in the group. The phylogenetic analysis was used with psammophily evolution traced on the preferred cladogram (Fig. 5; for the complete tree including 95% height density posterior, see Supporting Information, Fig. S2). Optimization of the psammophily characters revealed that the ancestral ecomorphotype was either free living or xerophilic. Crown Sapriniinae first appeared ~80 Mya, in the early Cretaceous (Supporting Information, Fig. S2). Psammophily first evolved before the Palaeocene–Eocene boundary, with rapid radiation corresponding to the Palaeocene–Eocene Thermal Maximum (~55.5 Myr) and continuing in the Early Eocene Climatic Optimum (EECO). After the EECO, the radiation and occurrence of psammophilous clades continued, with appearance of new taxa around the Eocene–Oligocene boundary. Ultra-psammophily of free-living Sapriniinae has occurred three times independently during the evolution of the group: (1) Namib Desert, after the EECO (*Terametopon*, Namibia); (2) Sahara Desert, Arabian Desert, Kyzyl-Kum and

Kara-Kum, before the Palaeocene–Eocene Thermal Maximum, during and continuing after the EECO (*Philothis*, *Xenonychus* and *Ctenophilothis*); and (3) Sonora Desert, around the Eocene–Oligocene boundary (*Philoxenus desertorum*, USA + Mexico).

1. *Namib Desert ultra-psammophiles: Terametopon* Vienna, 1987. Nested deeply within Old World *Hypocacculus*, *Chalcionellus* etc., and sister to Central and East European *Hypocaccus* (*Nessus*) *rufipes* (Kugelann, 1792) and *Hypocaccus* (*Nessus*) *hungaricus* Lackner & Seres, 2018, respectively, is the sole representative of Namibian taxon *Terametopon*, *Terametopon* (*Psammoprinus*) *namibiensis* Mazur, 1993. The genus, revised recently (Lackner, 2009), contains two subgenera and seven species and is characterized by a strongly vaulted body, strongly narrowed pronotum, setose underside (including elytral epipleuron), long protarsal claws and enlarged protibiae, all of which are common morphological adaptations to psammophily. Members of the nominotypical subgenus possess a curious frontoclypeal projection (for figures, see Lackner, 2009: figs 4, 20, 28, 44), unique in the subfamily. The beetles are extremely rare in collections, mostly known from several specimens only. They are found in sand dunes, and occasionally, trapped by pitfall traps (T.L., personal observation). As mentioned by Lackner (2009), the free-living ancestor of the taxon has probably invaded the dunes of the Namib Desert and speciated there in isolation. The Namib Desert contains one more ultra-psammophilous taxon, the monotypic *Paraphilothis* Vienna, 1994, which was unfortunately not sampled.
2. *Deserts of the Palaeartic Region (Sahara, Arabian Desert, Kyzyl-Kum, Kara-Kum etc.): taxa Philothis, Xenonychus and Ctenophilothis* Kryzhanovskij, 1987. A large clade containing mostly psammolittoral and semi- and ultra psammophilous taxa from the Old World contains the most specialized psammophiles known hitherto. The monophyletic genus *Philothis*, with three subgenera (*Philothis* s.s., *Farabius* Reichardt, 1930 and *Atavinus* Olexa, 1990) has been revised by Olexa (1990), with two subsequent species described (Gomy, 1992; Mazur, 1994). *Philothis* is spread across the Sahara, Arabian Desert, sand dunes of Azerbaijan, Kara-Kum and Kyzyl-Kum Deserts, with a single species described from the dunes of eastern Iran. These beetles contain arguably the strongest morphological adaptations to life in sand: reduced or outright absent protarsi, strongly compressed prosterna, shovel-like protibiae, strongly dilated meso- and metatibiae, flattened eyes, etc. (for figures, see Olexa, 1990: figs 14–22, 24–27). It seems likely that their ancestor came from the psammolittoral stock,



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Figure 5. Dated tree of the Sapriniinae, with reconstructed ancestral states of ecological preferences.

because their sister taxa are mostly found on the beach, in the sand. Other ultra-psammophile taxa of this grade are the monophyletic *Xenonychus* (two species, widely distributed from the Canary Islands across the Sahara, beaches of the Mediterranean Sea east to Kyzyl-Kum and Kara-Kum Deserts in ex-Soviet Middle Asia) and *Ctenophilothis* (two species, Sahara). Both taxa were recently revised (Lackner, 2012, 2013). Several more psammophilous Sapriniinae occur in the described area; these were, unfortunately not sampled (e.g. *Reichardtiolus* Kryzhanovskij, 1956, *Ammostyphrus* Reichardt, 1924 or *Xenophilothis* Kryzhanovskij, 1987).

3. *Sonora Desert (USA: California, Arizona, Nevada and Utah; Mexico): Philoxenus* Mazur, 1991. The sole Nearctic ultra-psammophile, monotypic *Philoxenus* is apparently derived from within psammolittoral North American *Hypocaccus* stock. Among the morphological adaptations are a strongly vaulted body, setose underside, enlarged tibiae and compressed prosternum (for figure, see Mazur, 1991: fig. 4a–c). In deserts of North America, there is at least one more dune-adapted taxon, *Monachister* Mazur, 1991, which was unfortunately not sampled.

The tree and ancestral state reconstruction indicate that extreme psammophily came from generalist ancestors; the sand adaptation arose in conditions where dunes were present. The tree topology in the Sahara + Arabian Desert + Kyzyl-Kum and Kara-Kum clade indicates that extreme psammophily gave rise to beach-dwelling taxa, but the low support for this clade could mean that it was, in fact, the other way around. This would probably be the preferred scenario and the more logical explanation: an ancestor, which had already conquered the beach environment, moving to inland dunes equipped with pre-adaptations for more extreme sandy environments. The major constraint to life in hot, dry sand is desiccation, and psammophilous Sapriniinae probably overcome it by behavioural traits of flying after the sunset. In fact, no psammophilous taxon of the subfamily has yet been observed during the hot hours of day outside. Mating behaviour is completely unknown, as are the immature stages. In contrast, there are several records of psammophilous Sapriniinae flying to light (e.g. Kanaar, 2008; Lackner, 2014b), suggesting nocturnal habits of these beetles.

Morphological characters, including underside pilosity, enlargement of tibiae, reduction or loss of protarsi, flattened eyes, compressed prosterna, strongly vaulted body and the ability to move rapidly through the sand, could be considered derived adaptations caused by the selection pressures of the harsh environment. These attributes have enabled convergent Sapriniinae evolution of extreme psammophily, exploiting major world deserts, with the exception of the Atacama Desert

in Chile and dune systems of Australia, where these beetles have not (yet) been discovered. The greatest radiation of psammophilous Sapriniinae has been documented in the Palaearctic Region, where they spawned several lineages with numerous species across the Sahara Desert, Arabian Peninsula and the deserts of former Soviet Middle Asia (Mazur, 2011). These taxa are likewise yet to be discovered in Mongolia (Gobi Desert) or India and Pakistan (Thar Desert).

This study is the first of its kind for the family Histeridae (Coleoptera), in which specialized habits abound. We do not propose a new classification of the subfamily or nomenclatural changes, because these will be the subjects of a following paper (L.T., M.M., K.C. & B.M., unpublished).

CONCLUSIONS

1. The present study confirms the monophyly of the Sapriniinae with very high support, relative to a small diversity of outgroups.
2. Inside the subfamily, four well-supported major clades have been recovered.
3. Ultra-psammophily is thought to have evolved at least three times independently: in Palaearctic, Afrotropical and Nearctic Regions. This result is contradictory to the findings of Lackner (2014a), where psammophily was observed to have evolved only once and subsequently lost several times.
4. Crown Sapriniinae appeared in the early Cretaceous, ~80 Mya. The ancestral ecomorphotype of psammophilous lifestyle was either xerophilic or free living. Psammophily first evolved before the Palaeocene–Eocene boundary, with rapid radiation corresponding to the Palaeocene–Eocene Thermal Maximum (~55.5 Mya), and continued during the Early Eocene Climatic Optimum.

ACKNOWLEDGEMENTS

The following persons are duly acknowledged for their help with procuring the specimens used in this study: John Nunn (Dunedin, New Zealand), Gábor Seres and Tamás Németh (both Budapest, Hungary), Alexey Tishechkin (Sacramento, CA, USA), Martin Fikáček, David Král, Jan Horák and Matthias Seidel (all Prague, Czech Republic), Bill Warner (Phoenix, AZ, USA), Jan Pražák (Hradec Králové, Czech Republic), Peter Koniar and Ivan Smatana (both Košice, Slovakia), Martin Švarc (Liberec, Czech Republic), Matt Gimmel (Santa Barbara, CA, USA), Gerardo Arriagada (Santiago de Chile, Chile), Richard Leschen (Auckland, New Zealand), Pete Kovarik (Columbus, OH, USA) and Paul Skelley (Gainesville, FL, USA). We thank the photographers J. Romsauer (Párkány = Štúrovo, Slovakia) and F. Slamka (Bratislava,

Slovakia), and G. Seres (Budapest, Hungary) for the help with the habitus photographs. This study received support from the Systematics Research Fund funded by the Linnean Society of London and the Systematics Association (London, UK), and from the Alexander von Humboldt Foundation, itself part of the Federal Ministry for Education and Research (Berlin, Germany). Two anonymous reviewers are thanked for their helpful comments and suggestions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Complete dataset used in the present study.

Table S2. Evolutionary models of the genes calculated using IQ-TREE.

Table S3. Primers.

Table S4. Thermocycling conditions.

Figure S1. The phylogenetic hypothesis of the Sapriniinae inferred from the MAFFT-aligned dataset, including outgroups and full branch length.

Figure S2. Estimated time tree of the Sapriniinae, including outgroups; node bars represent 95% height density posterior.