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# Multistate Characters and Diet Shifts: Evolution of Erotylidae (Coleoptera)

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*Abstract.*—The dominance of angiosperms has played a direct role in the diversification of insects, especially Coleoptera. The shift to angiosperm feeding from other diets is likely to have increased the rate of speciation in Phytophaga. However, Phytophaga is only one of many hyperdiverse lineages of beetles and studies of host-shift proliferation have been somewhat limited to groups that primitively feed on plants. We have studied the diet-diverse beetle family Erotylidae (Cucujoidea) to determine if diet is correlated with high diversification rates and morphological evolution by first reconstructing ancestral diets and then testing for associations between diet and species number and diet and ovipositor type. A Bayesian phylogenetic analysis of morphological data that was previously published in Leschen (2003, Pages 1–108 *in* Fauna of New Zealand, **47**; 53 terminal taxa and 1 outgroup, 120 adult characters and 1 diet character) yielded results that are similar to the parsimony analyses of Leschen (2003). Ancestral state reconstructions based on Bayesian and parsimony inference were largely congruent and both reconstructed microfungal feeding (the diet of the outgroup Biphyllidae) at the root of the Erotylidae tree. Shifts among microfungal, saprophagous, and phytophagous diets were most frequent. The largest numbers of species are contained in lineages that are macrofungal feeders (subfamily Erotylinae) and phytophagous (derived Languriinae), although the Bayesian posterior predictive tests of character state correlation were unable to detect any significant associations. Ovipositor morphology correlated with diet (i.e., acute forms were associated with phytophagy and unspecialized forms were associated with a mixture of diets). Although there is a general trend to increased species number associated with the shift from microfungal feeding to phytophagy (based on character mapping and mainly restricted to shifts in Languriinae), there is a large radiation of taxa feeding on macrofungi. Cycad feeding is scattered in more deeply diverged taxa and may have preceded the evolution of angiosperm feeding in some groups. Preliminary analysis of diet mapped onto higher beetle phylogenies suggests that about half of the major Coleoptera lineages may have had fungus-feeding ancestors. We discuss the roles of stochastic models and prior distributions of the reconstruction of ancestral character states in the context of the current data. [Bayesian estimation; character correlation; Coleoptera; insect diet; mycophagy; parsimony; phytophagy.]

The long history of Insecta that began in the Palaeozoic was influenced in large part by their association with plants, and the rise of the angiosperms or flowering plants, is thought to have promoted the enormous diversity of species (Farrel, 1998). Host plant shifting and diet specialization (see Brues, 1936), combined with millions of years of evolution, appears to have created a supergroup unmatched amongst terrestrial Eukaryotes. Indeed, insects suck, chew, parasitize, bore, store, and even cultivate their foods to a highly sophisticated degree of specialization, and much of the evolution of the group appears to be related to the way in which insects interact with their environment by feeding (Brues, 1936).

One of the oft-cited cases of insect radiations is that of Coleoptera, a group that first appeared in the Permian (Ponomarenko, 1969, 2002) and has since dominated the terrestrial insect biota by the shear number of described species. Although the first beetles appearing in the fossil record are thought by some to have been wood-inhabiting (Ponomorenko, 1969; Crowson, 1975), some of the most primitive groups of extant beetles have mouthparts that are associated with feeding on materials such as microfungi and algae such as Myxophaga and primitive Staphylinoidea (e.g., Crowson, 1981; Lawrence, 1989), and this type of microphagous feeding mechanism may have been the ancestral feeding type (Leschen, 1993; Betz et al., 2003). However, ancestral state reconstruction at the base of Coleoptera is elusive because of uncertain subordinal relationships (see reviews in Beutel, 2005; Leschen, 2006), and the uncertainty with reconstructing ancestral states at phylogenetically deep nodes (Cunningham, 1999). For these reasons it may be preferable to reconstruct primitive diet behaviors for groups with better resolved relationships and at lower taxonomic levels with relatively more complete taxonomic sampling to better understand how evolution occurred in diet-diverse groups.

Based on phylogenies of Phytophaga (Curculionoidea and Chysomeloidea), Farrell (1998) predicted that about half of the modern beetle species are associated with the radiation of angiosperms dating to the Cretaceous. Each repeated shift to angiosperm feeding was correlated with a high rate of species diversification, a pattern that agrees with the study by Mitter et al. (1988). Whereas the case for an angiosperm-induced diversification of beetles seems to be clear for Phytophaga, does this pattern exist in other groups of beetles? There are many groups of beetles that do not have a direct association with plants that also have an impressive number of species, such as the primarily predatory family Staphylinidae, estimated to be the second largest beetle family after the Curculionidae. Diet-diverse groups like members of the Staphylinoidea (Newton, 1984; Leschen, 1993; Hansen, 1997) and Cucujoidea (e.g., Nitidulidae: Lawrence, 1991; Leschen, 1999a; Erotylidae: Leschen, 2003) have an enormous range of feeding strategies, in addition to phytophagy, and examining some of these groups may help to understand the evolution of broad-scale diet or host shifts.

An independent test for exploring whether phytophagy has promoted a higher diversification rate within the Coleoptera is to examine groups outside of Phytophaga. In particular, we propose that examination of groups that contain lineages with mixed diets will provide a fresh insight into the origin and diversification of

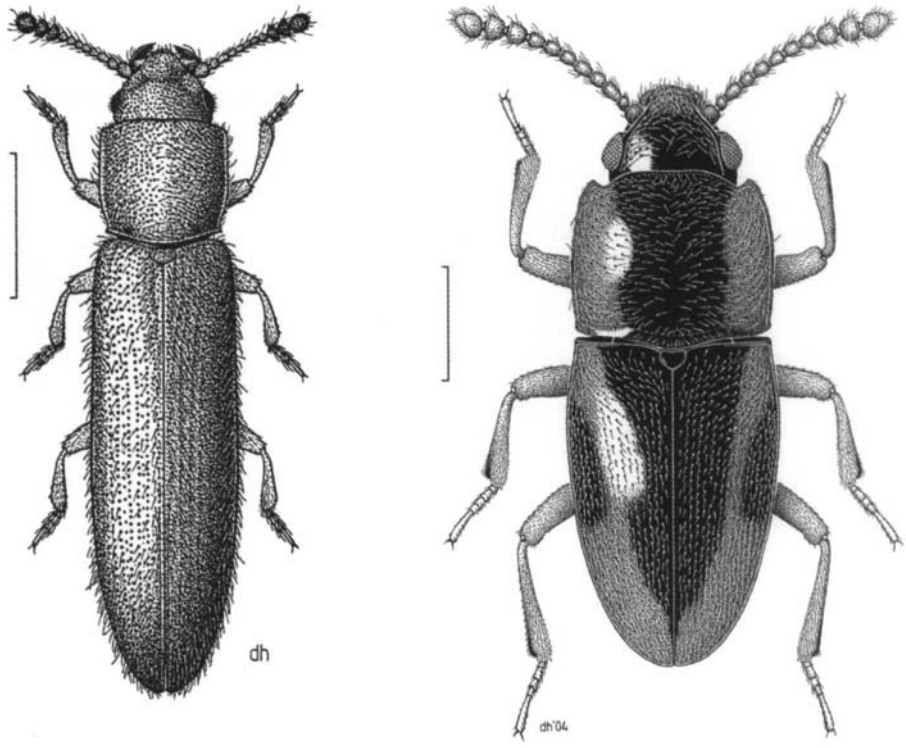


FIGURE 1. Habitus illustrations of *Hapalips prolixus* (Sharp) (Languriinae), left, and *Cryptodacne synthetica* Sharp, right, from New Zealand.

phytophagous groups. In this study we determine the origins and shifts of diet in the diverse cucujoid beetle family Erotylidae (Fig. 1).

Erotylidae, commonly known as pleasing fungus or lizard beetles, are distributed worldwide and includes approximately 3200 species in 280 genera placed among six subfamilies (Wegrynzowicz, 2002; Leschen, 2003). The classification of the group was most recently revised by Leschen (2003), who included the family Languriidae in Erotylidae based on a parsimony analysis of the genera (see also Leschen and Wegrzynowicz, 1998; Wegrynzowicz, 2002; and Robertson et al., 2004). Major problems still exist with the erotylid classification including the paraphyly of Xenoscelinae and Loberinae (full details are discussed in Leschen, 2003). The family contains phytophagous, mycophagous, and saprophagous species, and a few other taxa that feed on pollen and in dead wood. Most species are free living, but *Chasmatodera* and *Bancous* (Erotylinae from Africa) are found in the nests of social insects (Skelley, 1999), and some species of *Loberopsyllus* (Cryptophilinae from the Neotropics) are phoretic on cricetine rodents (Leschen and Ashe, 1999). *Lepidotoramus* (Cryptophilini from South America) may be endoparasitic on Lepidoptera pupae (Leschen, 1997). The highest numbers of species are contained in the basidiomycete fungus–feeding Erotylinae and the mainly phytophagous-feeding Languriinae, whereas other subfamilies tend to have fewer species with mixed diets. This variation of diet and species number makes erotylids an ideal group for assessing character correlations and changes in species diversity.

We refer to shifts in diets that are correlated with a high number of species as "megashifts." This term is used to discriminate between shifts among diets that result in higher diversification rates from those where there is little change or a decreased rate of diversification. Mitter et al. (1988) included Erotylidae in their study, but as two separate families reflecting the taxonomy of the group at that time, and Languriinae provided one sister-group comparison in favor of an increased rate of speciation in a group that shifted to phytophagy. We examine speciation rate and host shifting in more detail for Erotylidae with the data provided in a recent study that includes the most comprehensive taxon and morphological character sampling of the family (Leschen, 2003).

Morphological characters associated with feeding and oviposition may correlate with shifts in diet and food texture. In the superfamily Staphylinoidea, a diet of hard or compact tissues requires a mandibular morphology not present in taxa that feed on soft textured diets (Leschen, 1993; Hansen, 1997; Betz et al., 2003). Similarly, the elongate rostrum in many weevils (Curculionoidea), the cephalic analogue of the ovipositor, may have facilitated the exploitation of plants, mainly angiosperms (Anderson, 1995). The ovipositors of Erotylidae are variable, especially in the form of the gonocoxites (Fig. 2), and we determine whether this structure is correlated with feeding type.

Previously, character evolution was often examined by using parsimony approaches (Farris, 1983; Wenzel and Carpenter, 1994; Leschen, 1999b), whereby ancestral states are reconstructed by optimizing observed

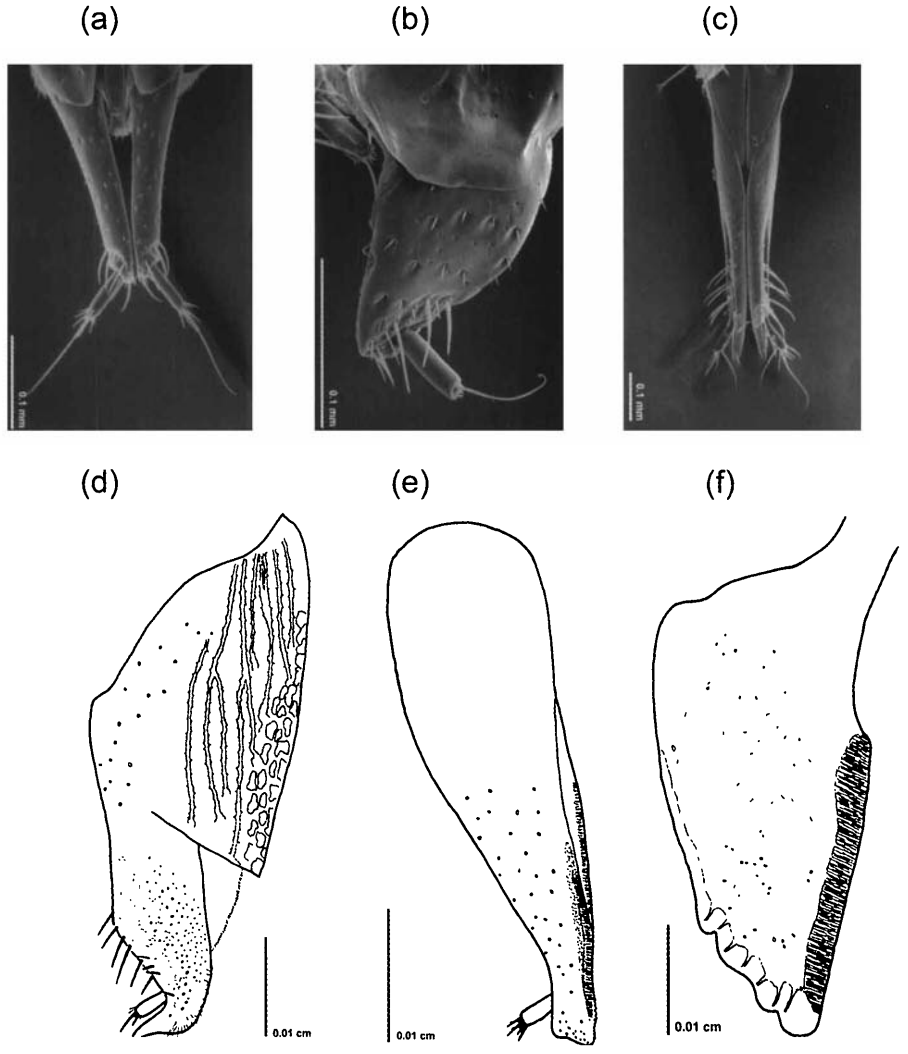


FIGURE 2. Illustrations and electron micrographs of erotylid gonocoxae. a, *Hapalips prolixus* (Sharp) (Languriinae, New Zealand); b, *Loberonotha olivascens* (Broun) (Xenoscelinae, New Zealand); c, *Nomotus* sp. (Languriinae, Costa Rica); d, *Macrophagus robustus* Motsculsky (Xenoscelinae, Palaearctic); e, *Thallisella crotchi* Gorham (Languriinae, Central America); f, *Platoberus* sp. (Languriinae, Costa Rica).

character state changes onto a topology. However, it is becoming increasingly accepted that estimates of ancestral character states are associated with a large amount of uncertainty that may or may not be accounted for by a particular method (e.g., Cunningham, 1999; Huelsenbeck et al., 2000). This uncertainty arises from three sources: (1) uncertainty in the underlying tree and branch lengths (Huelsenbeck et al., 2000); (2) misspecification of the selected model of character evolution (Lewis, 2001); and (3) multiple internal reconstructions that are plausible under the assumed model (Mooers and Schluter, 1999). A convenient method of accommodating this uncertainty is to adopt a model-based framework (Cunningham, 1999). This shift in emphasis has led to a growth in stochastic modeling of nonmolecular characters (e.g., Pagel et al., 1994, 2004; Schluter et al., 1997; Huelsenbeck et al., 2003; Ronquist, 2004), especially in a framework of Bayesian inference.

Several fundamental questions remain unanswered regarding ancestral character state reconstruction in general and model-based approaches specifically. Morphological studies usually employ discrete binary characters for tree estimation (Wiens, 2001) and this approach to character coding is usually carried over into character state reconstruction. By coding characters as binary there are more observations per transition type than with multistate characters. Recent studies have shown that different methods of character coding can have a dramatic effect on ancestral state reconstructions (Hibbett, 2004), and therefore careful determination of character states for complex behavioral characters is imperative. A further concern is that most ancestral character state reconstructions of nonmolecular characters use branch lengths estimated from molecular characters. The desirability of this approach is currently unknown. Studies that use morphological character states to jointly estimate topology, branch lengths, and ancestral character states are rare. Here we use both parsimony and model-based approaches to reconstruct the evolution of diet shifts and morphology in Erotylidae beetles

using only one behavioral and 120 morphological characters. We contrast the two approaches to character state reconstruction and discuss the role of model assumptions and prior distributions in the context of the current data.

## Materials and Methods

### *Phylogenetic Reconstruction*

The data matrix consists of 120 adult morphological characters and 1 behavioral character (diet type) and 53 terminal taxa rooted with the outgroup Biphyllidae based on the outgroup information of Wegrzynowicz (2002), Leschen (2003), and Leschen et al. (2005). This revised data matrix is essentially that of Leschen (2003) but includes the additional behavioral character and a new genus from equatorial Africa, to be described elsewhere. The data matrix is located at http://www.landcareresearch.co.nz/research/ and http://systematicbiology.org/. All of the genera (Appendix 1) are included as terminal taxa except for Erotylinae (a group of over 2500 species; P. Skelley, personal communication), which is represented by four tribes (Dacnini, Erotylini, Megalodacnini, Tritomini) and higher Languriinae (Languriini), which is represented by *Anadastus*, *Dasydactylus*, *Languria*, and *Nomotus*, a group of over 750 species (Leschen and Wegrzynowicz, 1998) that now includes all members of the previously recognized Cladoxenini (*Cladoxena*, *Crotchia*, *Microlanguria*, *Paracladoxena*, and *Penolanguria*).

Phylogenetic relationships that were previously reconstructed by Leschen (2003) using parsimony are analyzed using Bayesian methods (e.g., Larget and Simon, 1999; Huelsenbeck et al., 2002; Pagel and Meade, 2004) with the program BayesPhylogenies (Pagel and Meade, 2004). We used the $k$-states model, which is a continuous-time Markov model generalized for $k$-state data (Pagel, 1994; Lewis, 2001; Pagel et al., 2004). We restricted the rate matrix such that all transition types had the same rate, because preliminary analyses under more complex models yielded posterior distributions that deviated little from the prior distributions and convergence was poor. These findings are also consistent with the recommendation of Pagel et al. (2004) that at least 10 taxa per parameter are required for reliable parameter estimation. We assumed that the rate of evolution for each character followed a four-category discrete approximation to the gamma distribution (Yang, 1994). Uniform priors were used for the topology, gamma shape parameter (1 to 100), and transition rates from the $q$-matrix (1 to 100). A flat dirichlet prior was used for the character state frequencies. For the branch lengths we used an exponential distribution truncated at 10 character state changes per character, where the mean was set to the sum of branch lengths on the tree divided by the number of nodes. We ran a single chain for 20 million cycles with a thinning interval of 10,000 and a burn-in of 5 million cycles. This analysis was performed multiple times and we compared clade posterior probabilities between pairs of runs and the marginal likelihood across all runs for convergence. A sparse thining interval was used to reduce the autocorrelation on topology and because of the long chain lengths. Bayes factors were calculated using the harmonic means of the marginal likelihood, and the strength of support for one model over another was measured using the scale from Kass and Raftery (1995).

We performed parsimony-bootstrap analyses using PAUP* version 4.0b10 (Swofford, 1998) with heuristic tree searches including random addition sequence (1000 replicates) and character states that were treated as unordered. Bootstrap analyses were performed with 100 pseudoreplicates, start trees obtained by stepwise addition with 1000 random addition replicates. Because of time constraints, the 100th pseudoreplicate had to be abandoned, and the reported values are based on 99 pseudoreplicates.

### *Determination of Diets*

Diet is a complex character composed of several character states that needs to be defined accurately to reflect ecological association (Wenzel, 1992; Miller and Wenzel, 1995) and the reconciliation of the homology among these character states may be difficult. Exact determination of diet for Erotylidae is problematic because of the lack of natural history observations reported in the literature, poor knowledge of larvae (which indicate larval diet and female oviposition preferences), lack of obvious correlations between adult mouth part structures and diet, and the high number of species in the family. In some cases it may be satisfactory to assume diet from an exemplar taxon, but this may not be appropriate in diverse taxa. For example, in the molecular study of Erotylidae based on exemplars, Robertson et al. (2004) assumed that *Toramus* fed on Zygomycetes based on a single observation, even though members of this genus feed on Ascomycetes as well as other fungi (e.g., Leschen and Wegrzynowicz, 1998; Leschen, 2003).

Categorizing diets as cladistic character states for Erotylidae and other beetles is not straightforward. Lawrence (1989) divided fungal feeding taxa into *microphagous* (feeding on loosely organized material) and *macrophagous* (feeding on compact material) feeding mechanisms based on mouth part morphology (see also Betz et al., 2003). These classes were modified by Leschen (1993). Based on criteria discussed by Lawrence (1989) and Leschen (1993), we recognized the following classes of diet for coding in the data matrix. *Mycophagy* or fungus feeding was determined by the presence of fungal hyphae, conidia, and/or spores in the gut. We divided mycophagy into two states reflecting the use of two very different forms of fungal fructifications. *Microfungi* do not form large fruiting bodies and tend to form loosely connected hyphae and conidia and spores not born on large fruiting bodies. *Macrofungi* form large fruiting bodies and tend to be formed by fungal taxa belonging to the Agaricales, Polyporales, and Ascomycetes (Xylariales). *Phytophagy*, live plant feeding, was determined by the presence of any form of plant tissue. We decided to class *dead wood* and *pollen feeding* separately from phytophagy,

because these represent different phytophagous specializations. Specific host information is lacking for many phytophagous groups, although we discuss cycad associations exhibited by some or all members of *Xenocryptus*, *Pharaxonotha*, *Hapalips*, and *Nomotus*. *Saprophagy* is a mixed diet of plant and fungal material. The gut contents of saprophagous taxa may have a mix of food types or may consist of material that is unrecognizable.

Diet was determined by direct observation of live material and analysis of gut contents following the methods of Newton (1984) and Leschen (1993). In most cases we relied primarily on gut analysis of adult specimens and scored the character states based on the presence of particular types of tissue present. A total of 152 dissections on microslides and glycerin mounts were made and are listed in Leschen (2003). Additional biological information reviewed by Lawrence (1991), Skelley et al. (1991), Leschen and Wegryznowicz (1998), Leschen (1997, 2003), and Robertson et al. (2004) also contributed to the scoring of diet character states, which are provided in Appendix 1. Diets are unknown for several taxa (*Atomarops*, *Stengita*, *Pseudhapalips*, *Penolanguria*, *Cladoxena*, *Bolerus*, *Stenodina*, *Othniocryptus*, *Protoloberus*, and *Xenoscelis*), whereas five terminals have polymorphic diet characters: *Toramus* (microfungi and saprophagy), *Crowsonguptus* (pollen and saprophagy), *Paracladoxena* (microphagy and pollen), *Leucohimatium* (microfungi and saprophagy), and *Macrophagus* (pollen and saprophagy). All taxa with polymorphic character states were coded as missing for the posterior predictive tests due to software limitations (see below).

### *Gonocoxite Morphology*

Ovipositors function mainly to place the egg by the female into or on the larval substrate. The ovipositor is a complex organ, composed of several segments mainly or exclusively derived from segment XI (Beutel and Lawrence, 2005). We have examined the proximal gonocoxites (= valvifers) from the ovipositor, which usually bear a stylus. The gonocoxae in Erotylidae are very diverse and the overall shape of the gonocoxite (Fig. 2) may correlate with diet, assuming that ovipositional sites and larval and adult diets are the same. Leschen (2003) recognized six morphological types (see character 92): (0) narrow (Fig. 2a), (1) dilated (Fig. 2b), (2) acute (Fig. 2c), (3) sinuate (Fig. 2d), (4) *Platoberus* type (Fig. 2f), (5) *Thallisella* type (Fig. 2e). A narrow gonocoxite refers to the condition of a typical shaft-like coxite that bears a terminal gonostyle and setae along its flanks. The dilated condition refers to a gonocoxite that is wider than long and often dorsoventrally flattened. The remaining gonocoxal forms appear to be used for depositing eggs directly onto larval feeding substrates. A gonocoxite that is acute with an elongate shaft is present in most Languriinae, whereas a sinuate form occurs in *Acryptophagus*, *Leucohimatium*, *Macrophagus*, and *Othniocryptus*. Two other forms are coded here that are quite different from the remaining forms and are referred to as the *Platoberus* and *Thallisella* types. We suggest that the acute gonocoxite may be used for inserting eggs into plant host tissue.

### *Species Richness*

Coding species number per clade has been variously handled in phylogenetic studies depending on the question addressed. Including all species in a group as terminal taxa is necessary to assess tree balance and apply parametric models (e.g., Ree, 2005). Simple nonparametric counts of species per clade have been used in other studies, such as that of Mitter et al. (1988; see also Brooks and McLellan, 1991). Because sampling all species in a hyperdiverse clade such as Erotylidae is presently impossible, for this study we chose to tally all numbers of species per clade (erotyline tribes) or terminal taxon (genus) and classified groups into those that are hyperdiverse, with values falling above the geometric mean of species number and groups that are hypodiverse falling below the mean. These classes were then treated as two-state characters and coded in the data matrix.

### *Parsimony and Bayesian Character State Reconstructions*

We used the Bayesian consensus topology as a reference tree to obtain parsimonious character mappings under ACCTRAN and DELTRAN optimizations (Maddison et al., 1984). The reconstructions were optimized by using the MPR (most parsimonious reconstructions) option in MacClade 4.0 (Maddison and Maddison, 2004).

The posterior distribution of topologies and branch lengths were used to estimate rates of evolution and character states at selected internal nodes using the program BayesMultistate 1.0.1 (Pagel et al., 2004). We assumed a uniform prior probability distribution bounded between 1 and 100 for parameters from the rate matrix ($q_{ij}$). We ran a single chain of MCMC for $1 \times 10^8$ cycles with a burn-in of $1 \times 10^7$ cycles and a thinning interval of $1 \times 10^5$ using the 1500 post-burn-in trees generated by the program BayesPhylogenies. This analysis was repeated multiple times to ensure that convergence was reached. Posterior probabilities for each character state at each node were given conditional on that node existing. The joint posterior probability of a particular node existing and a given state at the node is simply the product of the two marginal probabilities (Pagel et al., 2004).The reconstructions of the feeding type and gonocoxite characters were performed on trees that were in turn estimated using those characters. Although this approach has been criticized (e.g., de Queiroz, 1996), it is appropriate to include those characters in tree reconstruction because we are estimating the joint posterior probability of the topology and character state assignments to internal nodes (Ronquist, 2004).

### *Posterior Predictive Test for Character Correlation*

Host associations may be tightly linked with morphology and we hypothesized that gonocoxite structure of the ovipositor will correlate with diet type because of female preferences for larval feeding. Moreover, change in

speciation rate could also be correlated with evolutionary novelty or a shift to a new diet, and here we also examined the correlation of diet and species richness.

We used posterior predictive tests (Gelman et al., 1995; Nielsen, 2002; Huelsenbeck et al., 2003), as implemented in SIMMAP 1.0$\beta$2.0.8 (Bollback, 2006), to examine the degree of correlation between feeding type, morphology of the gonocoxite, and species richness for each terminal taxon. This test involves sampling character histories under a stochastic model consistent with the states at the tips (Nielsen, 2002). Two test statistics were employed: the association statistic (Huelsenbeck et al., 2003) and the mutual information content (Chiu and Kolodziejczak, 1991; Gutell et al., 1992).

The time duration that a particular state exists on the phylogeny is known as the dwell time. The association test statistic, $D$, measures the difference in expected and observed frequencies that character states from two characters that occur together on a tree. The test statistic is obtained as follows. We calculate the expected frequency ($a_{ij}^{(e)}$) that character state $i$ from character 1 is associated with character state $j$ from character 2 by multiplying the frequency that state $i$ occurs with the frequency that state $j$ occurs. The observed frequency of association ($a_{ij}^{(o)}$) is obtained by calculating the dwell time on the tree that states $i$ and $j$ co-occur. The difference between $a_{ij}^{(o)}$ and $a_{ij}^{(e)}$ is $d_{ij}$, which measures the degree of association between the two states only. By summing over states we obtain the test statistic, $D$.

The mutual information content test statistic is calculated as, $m_{ij} = f_{ij} \log_2 \frac{f_{ij}}{f_i f_j}$, where $f_{ij}$ is the fraction of time the states $i$ and $j$ are associated on the phylogeny, and $f_i$ and $f_j$ are the frequencies of the two states. The overall test statistic, $M$, is obtained by averaging over all state associations.

The posterior predictive test generates a null distribution of $D$ and $M$ from the output of a Bayesian MCMC analysis and then compares the $D$ and $M$ values calculated from the original data to this null distribution. We took 100 post-burn-in trees generated by BayesPhylogenies and their branch lengths and generated character mappings for the feeding type, gonocoxite morphology, and species richness, using 10 realizations from the priors, and 1 realization from each tree and character. We used the branch lengths as estimated from the BayesPhylogenies analysis by turning off the "rescale tree length" option. We used a discrete gamma distributed prior (Schultz and Churchill, 1999; Huelsenbeck et al., 2003) on the rate parameter with 50 rate categories and $\alpha$ and $\beta$ both set to 1.603, such that the overall rate is 1.0. The value selected for $\alpha$ and $\beta$ was the mean of the alpha shape parameter for the gamma-distributed of among-character rate variation estimated by BayesPhylogenies. For the bias parameter we used an equal prior where $\pi(0)$ and $\pi(1)$ were both set to 0.5, which corresponds to the assumption that rates between states are equal.

We also reconstructed the transformation rates between the six feeding type character states using stochastic mapping (Nielsen, 2002; Huelsenbeck et al., 2003) as implemented in SIMMAP 1.0$\beta$2.0.8 (Bollback, 2006). This method operates by taking a random sample of states at internal nodes based on the probabilities of those states estimated using the method of Felsenstein (1981). A realization of the evolutionary process is then simulated along each branch consistent with those states. By repeating this process over different topologies, weighted by their posterior probabilities, we can obtain expected transformation rates between the different character states. This was achieved by taking 10 realizations from the prior and 1 realization for each tree for the feeding type character.

## RESULTS

### *Phylogenetic Analysis*

The 95% credibility intervals on the topology from the Bayesian analysis contained 1426 unique topologies from 1500 post-burn-in trees. The mean of the posterior distribution on the shape parameter from the gamma distribution was 1.603, indicating only weak among-character rate variation. The Bayes factor between the equal-rate model and the gamma-rate model was 8.6864, suggesting "strong" support from the data for the gamma model (Kass and Raftery, 1995). Split posterior probabilities were very similar between pairs of identical runs, an example of which is shown in Figure 3, consistent with convergence of the MCMC algorithm. The majority-rule consensus topology (including compatible nodes with less than 50% support) estimated from the Bayesian analysis (Fig. 4) is very similar to some of the trees estimated from the parsimony analyses (Leschen, 2003). Though a detailed examination of the Bayesian consensus tree shows that most higher taxa are paraphyletic compared to the reference tree in Leschen (2003), the Bayesian reference tree shows a monophyletic Pharaxonothinae + Xenoseclinae and a paraphyletic Thallisellini (Languriinae) and Loberinae. The posterior probabilities tended to be higher than the bootstrap proportions, as noted in other studies (e.g., Wilcox et al., 2002; Huelsenbeck and Rannala, 2004).

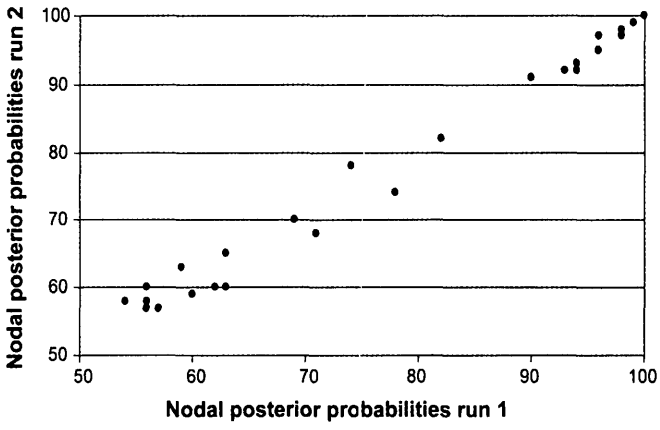


FIGURE 3. Graph showing an example of convergence of two independent MCMC analyses. Correspondence between nodal posterior probabilities that received greater than 50% support is shown.

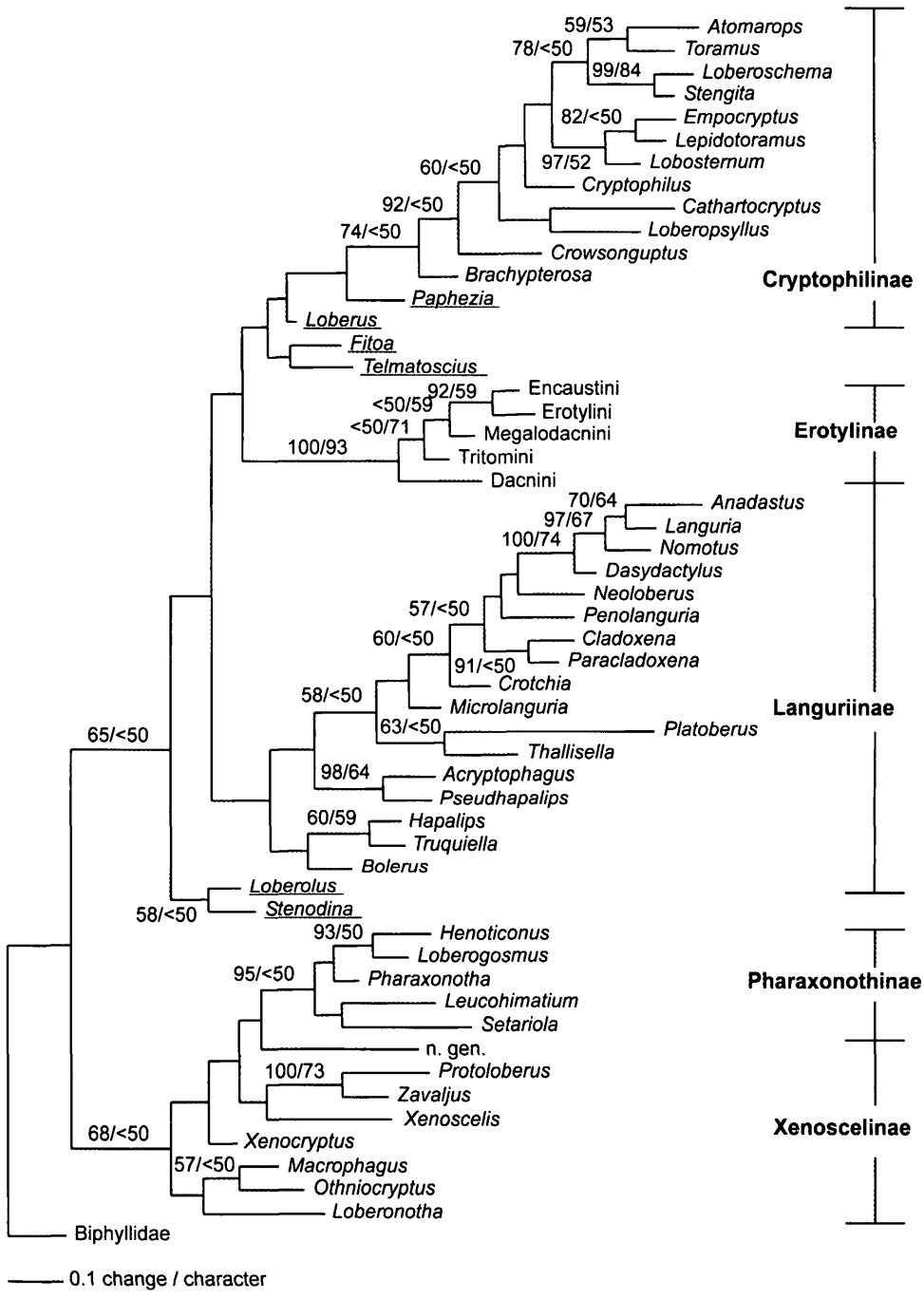


FIGURE 4. Bayesian consensus tree estimated under the *k*-states+Γ model. Numbers above nodes are posterior probabilities followed by parsimony bootstraps. Only nodes where one of the measures of support yielded a percentage greater than 50% are indicated. The branch length scale shows the expected number of character state changes per character. Higher taxa are indicated and Loberinae are underlined.

*Parsimony and Bayesian Character State Reconstructions*

The total number of reconstructions of the diet character using the MPR option in MacClade is 675 for the tree shown in Figure 5. The large number of reconstructions is due to the ambiguous changes present along the major branches of Languriinae and Pharaxonothinae + Xenoscelinae and unknown and polymorphic states for many terminals. Within Erotylidae, the basal diet is microfungal feeding. The character-state graph (Fig. 6) shows extensive diet shifts (19 shifts under DELTRAN including those in terminal taxa with polymorphic diets), most occurring among microfungal feeding, saprophagy, and phytophagy. Under ACCTRAN nodes 2, 17, 18, and 26 became ambiguous, 3 of which are resolved as microphagy and 1 as phytophagy under DELTRAN (Table 1). The optimal states for nodes 11 and 12 shift from dead wood feeding to phytophagy when the optimization method is changed from DELTRAN to ACCTRAN.

Optimal Bayesian character reconstructions are largely congruent (Fig. 5 and Table 1) with parsimony

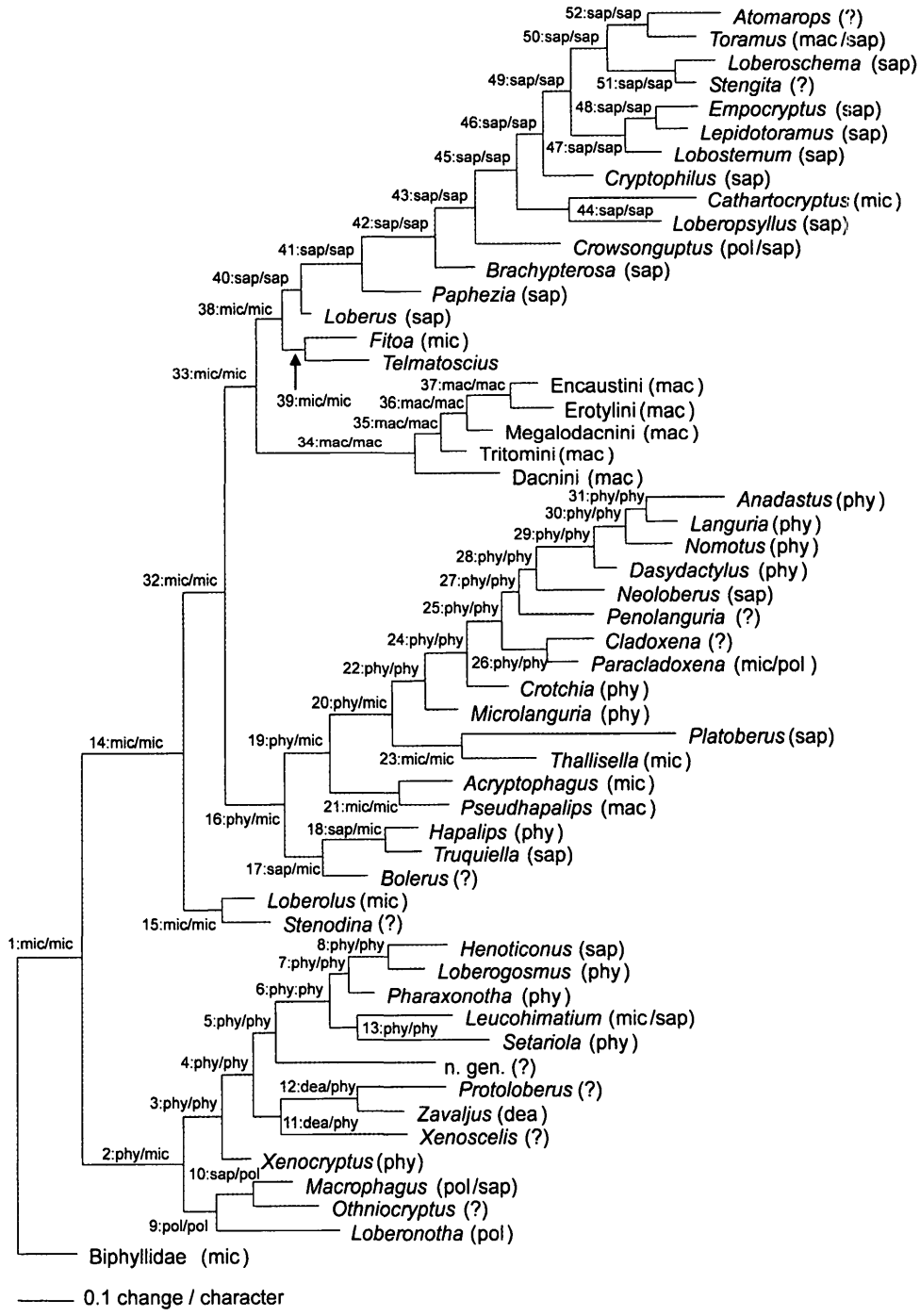


FIGURE 5. Bayesian consensus tree from Figure 4 with observed character states and optimal states for each node estimated with Bayesian inference and parsimony. The following abbreviations for the feeding character states are used: mic (microfungi), mac (macrofungi), sap (saprophagy), phy (phytophagy), pol (pollen), and dea (dead wood). The branch length scale shows the expected number of state changes per character.

reconstructions, with 42 of 52 nodes having the same state under both methods (note that nodes 11 and 12 are congruent with Bayesian reconstructions using ACCTRAN only). The Bayesian analyses reconstructed microfungi at the root of the tree with a posterior probability of 0.686 (Table 1, Fig. 5, node 1). The next best supported character state is phytophagy, although with posterior probability of only 0.114. The remaining states are listed in Table 1 and Figure 5. Figure 7 shows the marginal distribution of the character state transition parameter, showing strong deviation from the uniform prior distribution.

The number of reconstructed changes under parsimony and the relative transformation rates from the stochastic mapping (Figs. 6 and 8) are in general agreement. For example, the most frequent character

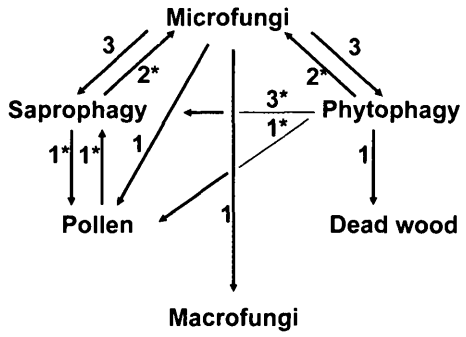


FIGURE 6. Character state graph for observed diet shifts of Erotylidae using DELTRAN optimization of characters. The asterisks indicate those terminal shifts that involve multistate taxa.

transformation type from the stochastic mapping is from phytophagy to saprophagy. This same transformation type also occurred three times under the parsimony reconstructions. In general, the observed character state changes under parsimony tend to be those with higher rates under the stochastic character mapping indicating that a general correlation exists (Fig. 8).

*Posterior Predictive Test for Character Correlation*

The posterior predictive test for association between feeding type and gonocoxite morphology using the character association test statistic yielded significant results for three pairwise comparisons: narrow gonocoxae with phytophagy, dilated gonocoxae with microfungal feeding, and sinuate gonocoxae with pollen feeding. All of the above correlations were significantly negative, indicating that the two respective states are associated with each other less often than expected under the null model. When the mutual information content test statistic was used, the following significant results were obtained: dilated gonocoxae with microfungal feeding, dilated gonocoxae with phytophagy, and sinuate gonocoxae with pollen feeding. As with the association test results, these posterior predictive $P$-values were all significantly negative. When a Bonferroni correction was applied, all of the posterior predictive $P$-values became nonsignificant, with the exception of dilated gonocoxae with microfungal feeding correlation using the mutual information content test. None of the comparisons between feeding type and species richness were significant under either test statistic.

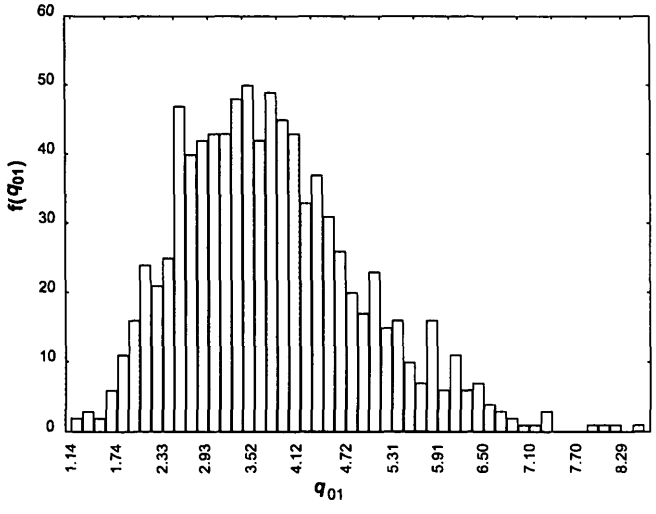


FIGURE 7. Marginal distribution of the rate coefficient ($q_{01}$). Note how the shape differs strongly from a uniform prior probability distribution bounded between 1 and 100.

## DISCUSSION

*Phylogenetic Relationships*

Results from the Bayesian phylogenetic analyses of the erotylids presented here are similar to those presented in Leschen (2003), where most of the higher groupings are poorly supported and the existing subfamilies are paraphyletic. The Bayesian analysis is not based on a new data matrix, but a slightly modified one that includes one behavioral character (diet) and an additional in-group taxon (n. gen. 1) and we do not recommend any change to the classification established by Leschen (2003) until a more thorough study of additional character data is undertaken and combined with the existing data matrix.

*Ancestral States and Diet Shift Frequency*

Microfungal diet, the diet coded for the outgroup Biphyllidae, is placed at the root of the erotylid tree, under both parsimony and Bayesian ancestral state reconstructions. Diet shifts are more frequent among microfungal feeding, saprophagy, and phytophagy, where there are back-shifts (reversals to feeding on microfungi). Although ancestral microfungal feeding precedes most shifts, the transition to dead wood association in *Zavaljus* is derived from phytophagy. Though pollen feeding is derived from microfungal feeding in *Loberonotha*, *Macrophagus* (polymorphic), and *Othniocryptus* (unknown), pollen feeding is also derived from saprophagous (*Crowsonguptus*) and phytophagous (*Paracladoxena*) ancestors in these polymorphic terminals.

Shifts among saprophagy, pollen, and microfungal diets do not necessarily require changes in mouthpart morphology (Leschen, 1993), but the shifts to feeding on live plants from microfungal feeding seem unlikely to occur because these diets are physically different in form and texture and may require completely different mouth part morphologies (see Leschen, 1993, and Betz et al., 2003). It is possible that plant tissues used by more primitive erotylids are softer than those tissues used by many Languriinae that feed on leaves and stems of herbaceous plants. The adult mouth parts of basal erotylids are not markedly different among the taxa, whereas those of higher Languriini tend to be acute with an almost straight outer edge (see Leschen, 2003). A closer study of diets among basal taxa could reveal that phytophagous diets may be specialized on softer plant tissues like those available in the male cones of cycads that, for example,

TABLE 1. Posterior probabilities of feeding type character states for node in the Bayesian consensus tree. For each node the optimal character state is in bold. The optimal parsimony states under DELTRAN are also given. The asterisks indicate nodes with ambiguous reconstructions under ACCTRAN.

| Node | Microfungi (0) | Phytophagy (1) | Pollen (2) | Saprophagy (3) | Macrofungi (4) | Dead wood (5) | Parsimony |
|---|---|---|---|---|---|---|---|
| 1 | **0.686** | 0.114 | 0.071 | 0.092 | 0.025 | 0.012 | Microfungi |
| 2 | 0.037 | **0.563** | 0.201 | 0.148 | 0.024 | 0.027 | Microfungi* |
| 3 | 0.012 | **0.933** | 0.005 | 0.021 | 0.008 | 0.021 | Phytophagy |
| 4 | 0.074 | **0.541** | 0.027 | 0.135 | 0.043 | 0.179 | Phytophagy |
| 5 | 0.063 | **0.738** | 0.024 | 0.122 | 0.037 | 0.016 | Phytophagy |
| 6 | 0.028 | **0.881** | 0.005 | 0.076 | 0.008 | 0.003 | Phytophagy |
| 7 | 0.006 | **0.947** | 0.002 | 0.039 | 0.004 | 0.002 | Phytophagy |
| 8 | 0.026 | **0.576** | 0.011 | 0.362 | 0.017 | 0.007 | Phytophagy |
| 9 | 0.029 | 0.036 | **0.682** | 0.225 | 0.019 | 0.008 | Pollen |
| 10 | 0.049 | 0.061 | 0.173 | **0.672** | 0.032 | 0.013 | Pollen |
| 11 | 0.106 | 0.132 | 0.044 | 0.173 | 0.070 | **0.475** | Phytophagy |
| 12 | 0.050 | 0.062 | 0.021 | 0.081 | 0.033 | **0.754** | Phytophagy |
| 13 | 0.212 | **0.355** | 0.027 | 0.346 | 0.042 | 0.018 | Phytophagy |
| 14 | **0.531** | 0.228 | 0.009 | 0.198 | 0.029 | 0.006 | Microfungi |
| 15 | **0.875** | 0.037 | 0.012 | 0.048 | 0.020 | 0.008 | Microfungi |
| 16 | 0.195 | **0.505** | 0.024 | 0.239 | 0.027 | 0.195 | Microfungi |
| 17 | 0.079 | 0.350 | 0.033 | **0.463** | 0.052 | 0.022 | Microfungi* |
| 18 | 0.020 | 0.415 | 0.008 | **0.538** | 0.013 | 0.006 | Microfungi* |
| 19 | 0.369 | **0.491** | 0.019 | 0.090 | 0.023 | 0.009 | Microfungi |
| 20 | 0.088 | **0.675** | 0.017 | 0.201 | 0.014 | 0.006 | Microfungi |
| 21 | **0.783** | 0.064 | 0.021 | 0.084 | 0.034 | 0.014 | Microfungi |
| 22 | 0.010 | **0.957** | 0.004 | 0.024 | 0.004 | 0.002 | Phytophagy |
| 23 | **0.673** | 0.062 | 0.021 | 0.196 | 0.033 | 0.014 | Microfungi |
| 24 | 0.050 | **0.848** | 0.021 | 0.068 | 0.009 | 0.004 | Phytophagy |
| 25 | 0.209 | **0.342** | 0.088 | 0.320 | 0.029 | 0.012 | Phytophagy |
| 26 | **0.614** | 0.042 | 0.258 | 0.055 | 0.022 | 0.009 | Phytophagy* |
| 27 | 0.047 | **0.457** | 0.020 | 0.431 | 0.031 | 0.013 | Phytophagy |
| 28 | 0.037 | **0.495** | 0.015 | 0.418 | 0.024 | 0.010 | Phytophagy |
| 29 | 0.002 | **0.993** | 0.001 | 0.003 | 0.001 | 0.000 | Phytophagy |
| 30 | 0.003 | **0.990** | 0.001 | 0.004 | 0.002 | 0.001 | Phytophagy |
| 31 | 0.005 | **0.980** | 0.002 | 0.008 | 0.003 | 0.001 | Phytophagy |
| 32 | **0.613** | 0.052 | 0.009 | 0.276 | 0.043 | 0.006 | Microfungi |
| 33 | **0.600** | 0.020 | 0.007 | 0.327 | 0.041 | 0.004 | Microfungi |
| 34 | 0.001 | 0.002 | 0.001 | 0.002 | **0.994** | 0.000 | Macrofungi |
| 35 | 0.001 | 0.001 | 0.000 | 0.002 | **0.996** | 0.000 | Macrofungi |
| 36 | 0.002 | 0.003 | 0.001 | 0.003 | **0.991** | 0.001 | Macrofungi |
| 37 | 0.002 | 0.002 | 0.001 | 0.003 | **0.991** | 0.001 | Macrofungi |
| 38 | **0.581** | 0.016 | 0.005 | 0.386 | 0.008 | 0.003 | Microfungi |
| 39 | **0.976** | 0.007 | 0.002 | 0.009 | 0.004 | 0.002 | Microfungi |
| 40 | 0.002 | 0.003 | 0.001 | **0.991** | 0.002 | 0.001 | Saprophagy |
| 41 | 0.010 | 0.013 | 0.004 | **0.963** | 0.007 | 0.003 | Saprophagy |
| 42 | 0.007 | 0.008 | 0.005 | **0.973** | 0.004 | 0.002 | Saprophagy |
| 43 | 0.017 | 0.015 | 0.013 | **0.944** | 0.008 | 0.003 | Saprophagy |
| 44 | 0.294 | 0.068 | 0.023 | **0.564** | 0.036 | 0.015 | Saprophagy |
| 45 | 0.020 | 0.011 | 0.004 | **0.956** | 0.006 | 0.003 | Saprophagy |
| 46 | 0.012 | 0.010 | 0.003 | **0.967** | 0.005 | 0.002 | Saprophagy |
| 47 | 0.003 | 0.004 | 0.001 | **0.989** | 0.002 | 0.001 | Saprophagy |
| 48 | 0.004 | 0.005 | 0.002 | **0.985** | 0.003 | 0.001 | Saprophagy |
| 49 | 0.025 | 0.017 | 0.006 | **0.941** | 0.009 | 0.004 | Saprophagy |
| 50 | 0.115 | 0.048 | 0.016 | **0.784** | 0.026 | 0.011 | Saprophagy |
| 51 | 0.041 | 0.051 | 0.017 | **0.852** | 0.027 | 0.011 | Saprophagy |
| 52 | 0.335 | 0.057 | 0.019 | **0.546** | 0.030 | 0.013 | Saprophagy |

are the diet of *Xenocryptus* and some *Pharaxonotha* (see below).

The shift to macrofungal feeding from microfungal feeding in Erotylinae is a specialized shift that accompanies many changes in morphology, including the structure of larval and adult mouth parts (Leschen, 2003). Although there are a few taxa outside Erotylinae that feed on rather large-bodied sporocarps of Xylariaceae (e.g., some *Toramus*), these feed only on spores as adults and larvae. So, in parallel with plant feeding shifts in Languriini, the Erotylinae also have features that are consistent with a diet shift from microfungal feeding ancestors that correlates with changes in mouthpart morphology.

There is an overall pattern of derived fixation of diet in crown groups of Cryptophilinae (saprophagy), Languriini (phytophagy), Erotylinae (macrofungi), and to a lesser extent in Pharaxonothinae + Xenoscelinae. Fixation of diet seems to indicate some level of constraint or conservatism in these lineages after the ancestral shifts have occurred. Further phylogenetic study of the taxa, especially to adequately test the monophyly of Pharaxonothinae + Xenoscelinae and the placement of

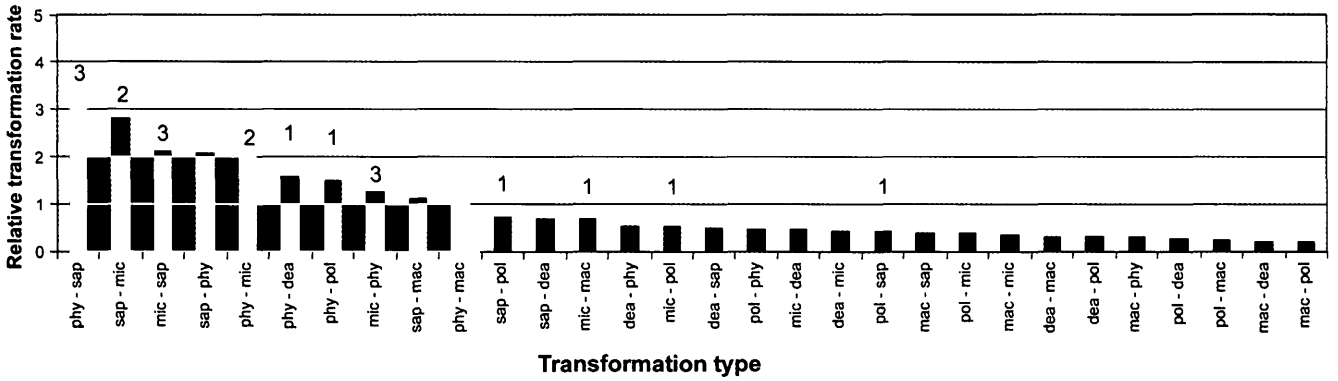


FIGURE 8. Graph of relative rate of character state transformation for the feeding type characters. The number of parsimony reconstructions is given above each bar for which at least one observed change was observed. Character state abbreviations are the same as in Figure 5.

Loberinae, as well as natural history data to code unknown terminal taxa, will improve our understanding of diet shifts in the group.

### *Correlation of Diet and Gonocoxite Structure*

Based on the Bayesian character correlation analysis, those taxa that presumably lay eggs directly on substrates or into existing spaces and having narrow or dilated gonocoxites, display a wide variety of diets (Table 2). On the other hand, species that presumably insert eggs into the substrate with acute gonocoxites tend to be phytophagous, although this association was not significant and an acute gonocoxite also occurs in the tribe Dacnini (Erotylinae), which is mycophagous. The dual occurrence of the acute ovipositor in Dacnini and languriines may be correlated with shifts to new diets, or, at least, novel methods of resource use of a diet that may be already present in the ancestors of each clade. Dacnini are the only taxon we know of that may drill into the fungal substrate using complex ridges along the shaft of the ovipositor (see Boyle, 1956).

### *Correlations among Diet and Species Number, Specialization, and Radiation in Erotylidae*

The Erotylinae feed on macrofungi and contains approximately 2500 species and the more derived phytophagous members of the tribe Langurini containing 750 species. Feeding on macrofungi is positively

TABLE 2. Test statistics and *P*-values (in parentheses) for associations between feeding type and gonocoxite morphology. A negative value indicates that the two states are associated less often than expected under the null model. A positive value indicates that the two states are associated more often than expected under the null model. Posterior predictive *P*-values in bold are significant at the 0.05 level.

| | Microfungi | Phytophagy | Pollen | Saprophagy | Macrofungi | Dead wood |
|---|---|---|---|---|---|---|
| Character association test | | | | | | |
| Narrow | 0.033 | −0.022 | −0.024 | 0.006 | 0.000 | 0.007 |
| | (0.92) | **(0.02)** | (0.11) | (0.78) | (0.72) | (0.88) |
| Dilated | −0.056 | −0.024 | 0.079 | 0.004 | −0.001 | −0.002 |
| | **(0.02)** | (0.08) | (0.95) | (0.73) | (0.49) | (0.38) |
| Acute | −0.010 | 0.005 | −0.001 | 0.007 | 0.000 | 0.000 |
| | (0.11) | (0.78) | (0.49) | (0.86) | (0.59) | (0.56) |
| Sinuate | −0.006 | 0.060 | −0.040 | −0.014 | 0.002 | −0.003 |
| | (0.34) | (0.95) | **(0.03)** | (0.18) | (0.66) | (0.34) |
| "Platoberus" | 0.029 | −0.015 | −0.010 | −0.002 | −0.001 | −0.001 |
| | (0.97) | (0.18) | (0.20) | (0.36) | (0.63) | (0.42) |
| "Thallisella" | 0.010 | −0.004 | −0.005 | 0.000 | 0.000 | −0.001 |
| | (0.84) | (0.29) | (0.31) | (0.76) | (0.65) | (0.48) |
| Mutual information content test | | | | | | |
| Narrow | 0.058 | −0.012 | −0.009 | 0.016 | 0.003 | 0.019 |
| | (0.95) | (0.07) | (0.08) | (0.73) | (0.68) | (0.78) |
| Dilated | −0.061 | −0.023 | 0.166 | 0.013 | 0.001 | 0.001 |
| | **(<0.001)** | **(0.03)** | (0.99) | (0.83) | (0.66) | (0.62) |
| Acute | −0.003 | 0.016 | 0.005 | 0.023 | 0.001 | 0.001 |
| | (0.10) | (0.87) | (0.72) | (0.80) | (0.52) | (0.59) |
| Sinuate | −0.006 | 0.123 | −0.024 | −0.002 | 0.004 | 0.001 |
| | (0.14) | (1.00) | **(0.02)** | (0.21) | (0.61) | (0.51) |
| "Platoberus" | 0.054 | −0.003 | −0.005 | 0.003 | 0.000 | 0.000 |
| | (0.85) | (0.09) | (0.13) | (0.63) | (0.63) | (0.48) |
| "Thallisella" | 0.018 | 0.001 | 0.002 | 0.006 | 0.001 | 0.001 |
| | (0.82) | (0.68) | (0.67) | (0.73) | (0.53) | (0.66) |

TABLE 3. Test statistics and *P*-values (in parentheses) for associations between feeding type and species richness. A negative value indicates that the two states are associated less often than expected under the null model. A positive value indicates that the two states are associated more often than expected under the null model. Posterior predictive *P*-values in bold are significant at the 0.05 level.

| | Microfungi | Phytophagy | Pollen | Saprophagy | Macrofungi | Dead wood |
|---|---|---|---|---|---|---|
| Character association test | | | | | | |
| Hypodiverse | 0.001 | 0.031 | 0.003 | 0.018 | −0.066 | 0.005 |
| | (0.75) | (0.83) | (0.65) | (0.84) | (0.07) | (0.71) |
| Hyperdiverse | −0.010 | −0.031 | −0.003 | −0.018 | 0.066 | −0.005 |
| | (0.25) | (0.17) | (0.35) | (0.16) | (0.93) | (0.29) |
| Mutual information content test | | | | | | |
| Hypodiverse | 0.015 | 0.047 | 0.004 | 0.027 | −0.034 | 0.008 |
| | (0.64) | (0.88) | (0.67) | (0.77) | (0.12) | (0.6) |
| Hyperdiverse | −0.007 | −0.021 | 0.001 | −0.014 | 0.209 | 0.000 |
| | (0.34) | (0.12) | (0.47) | (0.18) | (1.00) | (0.42) |

associated with hyperdiversity, whereas the remaining diets, including phytophagy, are positively associated with hypodiversity (Table 3), although none of these associations were significantly supported. This result reflects the high numbers of species/terminal taxa in the mycophagous Erotylinae and the relatively widespread distribution of phytophagy in both hyper- and hypodiverse groups.

The extraordinary high number of species in Erotylidae is probably not correlated with a single shift to one diet, as phytophagy is for other insects (Mitter et al., 1988; Farrell, 1998), but rather it could be due to two separate megashifts. The shift from microfungi to macrofungi diets was abrupt and "complete" as indicated by the fixation of macrofungal feeding in the whole of Erotylinae. This contrasts with the pattern of shifts from microfungal feeding to phytophagy, which has occurred as frequently as the shifts from microfungal feeding to saprophagy.

Among the Languriinae and the Pharaxonothinae + Xenosclinae clades there were relatively high rates of diet shifting, but each of these clades differs in their diet-shift histories. The fixation of phytophagy in derived Languriinae followed extensive shifting between saprophagy and phytophagy, whereas in Pharaxonothinae + Xenosclinae clade there was little diet fixation and appreciable speciation for most groups. Most Pharaxonothinae and Xenosclinae genera have only one to three species and the only genus among these taxa that has a relatively high number of species is *Pharaxonotha*, which has many species that feed on cycads worldwide. There could have been widespread extinction in Pharaxonothinae and Xenoscelinae, resulting in scant numbers of species per genus, leaving a hypodiverse present-day remnant of a vast radiation. Because so few fossil erotylid beetles are known (Leschen, 2003), it is difficult to determine the geological history of the group.

In contrast to most Erotylinae that are more or less restricted to macrofungal diets, saprophagy is the main diet in members of Cryptophilinae and Loberinae. In these groups, the world diversity has not been described, but the numbers are likely to greatly supercede the known numbers of the worldwide genera *Loberus* (Loberinae, 75 species) and *Toramus* (Cryptophilinae, 44 species). It is possible that the hyperdiversity in these groups may not be exclusively related to diet, but to habitat specialization as well. To attribute "success" of a clade to one diet shift over another, therefore, is not justifiable for these saprophagous groups and, in general, underscores problems in testing theories of adaptive zones and key innovations.

### *Evolution of Plant Associations*

Details about taxonomic host shifts in the entire Erotylidae cannot be rigorously discussed due to the lack of specific host data (Leschen, 2003), though this has been done to a limited extent for the better known Erotylinae (Skelley et al., 1991; Robertson et al., 2004). Though angiosperm and fungal records exist, erotylids have also been associated with cycads and conifers. It has been shown in studies of phytophagous groups (weevils, leaf beetles, and cerambycids) that cycad and other primitive plant associations preceded angiosperm associations (Anderson, 1995; Farrell, 1998), which is consistent with fossil plant history. A closer look at Erotylidae show some parallels.

The Holarctic genus *Zavaljus* is found in rotting logs in boreal forests and its basal placement in the erotylid tree is suggestive of an old association with conifers. On the other hand, cycad associations are scattered across the erotylid tree amongst at least five genera. Among the basal Pharaxonothinae and Xenoscelinae, there are three genera that occur on cycads (note that there are undescribed African taxa that are cycad specialists). The widely distributed genus *Pharaxonotha* is probably paraphyletic (Leschen, 2003; P. Skelley, personal observation) and composed of species that may be saprophagous (like the type species of the genus, *P. kirschii* Reitter) and others that are cycad specialists (e.g., Tang, 1987; Norstog et al., 1992). The genus *Xenocryptus* has at least three Australian species found on cycads and one South African species with unknown habits (Leschen, 2003). Among Languriinae, *Hapalips* is a widespread genus but there are a few records of this genus from cycads (Sen Gupta, 1968; Leschen, 2003), whereas at least two species of *Nomotus* have been recorded from cycads (Windsor et al., 1999). The distribution of cycad feeding at the base of the tree in Pharaxonothinae and Xenoscelinae, and in some

*Hapalips* at the base of Languriinae, seems to provide evidence that cycad associations may be primitive within the group, consistent with the fossil record and phylogenetic patterns seen in some other studies of Coleoptera. However, only species of *Xenocryptus*, *Pharaxonotha*, and undescribed taxa from South Africa have been consistently collected from cycads, and additional phylogenetic work on the entire family is needed to determine if these associations with cycads are secondary host shifts or more ancient.

### *Ancestral Diets in Major Coleoptera Lineages*

The erotylids are an ideal group to examine the evolution of diet because of the extraordinary number of habitats they inhabit and diversity of foods consumed. Because the family diverges early in the history of the large superfamily Cucujoidea (Leschen et al., 2005), there may be similar patterns of shifts among diets to be seen in other major groups of beetles. A shift from mycophagy or saprophagy to phytophagy is also suggested to occur independently in the series Staphyliniformia, Elateriformia, Scarabaeiformia, and Cucujiformia (which includes the hyperdiverse Phytophaga) by Mitter et al. (1988). However, these workers based their results on noncladistic interpretations of beetle phylogeny and reconstructing the ancestral diets of major lineages of Coleoptera in this manner is problematic (see the introductory section).

We have examined the character optimizations of the following diets on the cladograms listed in Table 4: algae-feeding, dead wood, mycophagy, phytophagy, predatory, saprophagy. Of the 14 sets of optimizations, 6 are ambiguous and the rest are unequivocal at the ancestral node. Three of the reconstructions have fungus feeding at their bases, and another four have saprophagous ancestors, which would have also included some form of facultative mycophagy in their diet (see above descriptions of diet). Therefore, about half of the major lineages of beetles may have had mycophagous ancestors.

The presence of phytophagy at the base of cucujiform lineages (the last three taxa listed in Table 4) suggests that much of the higher Coleoptera have shifted extensively in habitats filled with living and dead plant matter, promoting opportunistic shifts to phytophagy, a conclusion supported in other studies. Whether ancestral shifts have facilitated an increased number of species per clade requires more detailed study of each lineage, especially because most lineages arose in the Triassic and have had over 200 million years to shift among available diets.

### *Methodological Issues*

This study has raised a number of methodological issues with reconstructing ancestral states on phylogenies. In this study parsimony and Bayesian model–based approaches are largely congruent in their reconstructions of the feeding-type character state, but the Bayesian results may be giving correlations that are not consistent with the data for states that are less common and are associated with clades of a few taxa. For example, the specialized gonocoxites of the *Platoberus* type and *Thallisella* type only occur each in their respective taxa; however, both of these states are positively correlated with a wide variety of diets (Table 2), although none of these associations are significant. The stochastic mappings will generate some character histories whereby the *Platoberus* type and *Thallisella* type appear in other regions of the tree. This represents a form of model misspecification because we believe that because these states are specific to their respective taxa, they will not have evolved elsewhere in the erotylid phylogeny. In this case an asymmetric model may be more realistic. An alternative approach would be to use a highly constrained prior whereby the *Platoberus* type and *Thallisella* type can only be associated with their respective taxa. However, none of the positive associations between the *Platoberus* type and *Thallisella* type with various feeding types are significant, and so this effect has not seriously misled our analyses. On the other hand, the parsimony reconstructions will only reconstruct the *Platoberus* type and *Thallisella* type states at nodes that are direct descendants of *Platoberus* and *Thallisella*. Because the current software does not allow us

TABLE 4. Ancestral diets of major groups of Coleoptera determined by parsimony mapping. For all studies, the reference tree (parsimony) with all characters (treated as unordered) and taxa were used and diets were based on Lawrence et al. (1999). Geological periods are derived from Carpenter (1992) and Ponomorenko (2002).

| Taxon (first fossil record) | Diet | Reference |
|---|---|---|
| Adephaga (Triassic) | Predatory | Buetel, 1995 |
| Myxophaga (no record) | Algae-feeding | Beutel et al., 1998 |
| Basal polyphaga (Triassic) | Mycophagy/saprophagy | Beutel and Leschen, 2005 |
| Scarabaeoidea (Triassic) | Mycophagy/saprophagy | Scholtz and Chown, 1995 |
| Staphyliniformia (Triassic) | Saprophagy | Hansen, 1997 |
| Hydrophiloidea (Jurassic) | Predatory/saprophagy | Beutel and Leschen, 2005 |
| Staphylinoidea (Jurassic) | Predatory/saprophagy | Beutel and Leschen, 2005 |
| Staphylinidae (Jurassic) | | |
| Omaliine group | Saprophagy | Newton and Thayer, 1995 |
| Tachyporine group | Predatory | Ashe, 2005 |
| Elateriformia (Jurassic) | Mycophagy/phytophagy | Lawrence et al, 1995 |
| Byrrhoidea (Jurassic) | Dead wood/phytophagy | Costa et al, 1999 |
| Cucujoidea/Cleroidea (Jurassic) | Phytophagy/saprophagy | Leschen et al., 2005 |
| Curculionoidea (Triassic) | Phytophagy | Marvaldi et al, 2002 |
| Chrysomeloidea (Triassic) | Phytophagy | Farrell and Sequiera, 2004 |

to specify a model or prior distribution that takes this rare states effect into account, this problem can only currently be handled by combining parsimony approaches with model-based approaches.

The inferences that we have made here using the Bayesian approach are clearly conditional on the evolutionary model being a good approximation to the actual process that has generated the data. We have assumed the characters are evolving with a range of rates that are constant across the tree, which we have approximated with a discrete gamma distribution. However, this assumption may be incorrect for at least some morphological characters. Many morphological characters are in fact likely to evolve under a covarion-type process (Fitch and Markowitz, 1970) where characters are strongly constrained for much of their history but become briefly unconstrained or positively selected during a speciation event. It is not clear how well current stochastic evolutionary models capture this process and what effect this potential form of model misspecification has on the reconstruction of phylogenies and ancestral states from morphological characters. A further problem unique to ancestral character state reconstruction using Markov models is the necessity of obtaining branch lengths to optimize the likelihood function or approximate posterior probabilities. Under the stochastic models implemented here, character state changes are assumed to be more likely to occur on the longer branches. If we accept that many morphological changes will occur during speciation events or the origin of higher taxa, then it is possible that branch lengths optimized from morphological characters are more accurate than branch lengths optimized from molecular characters.

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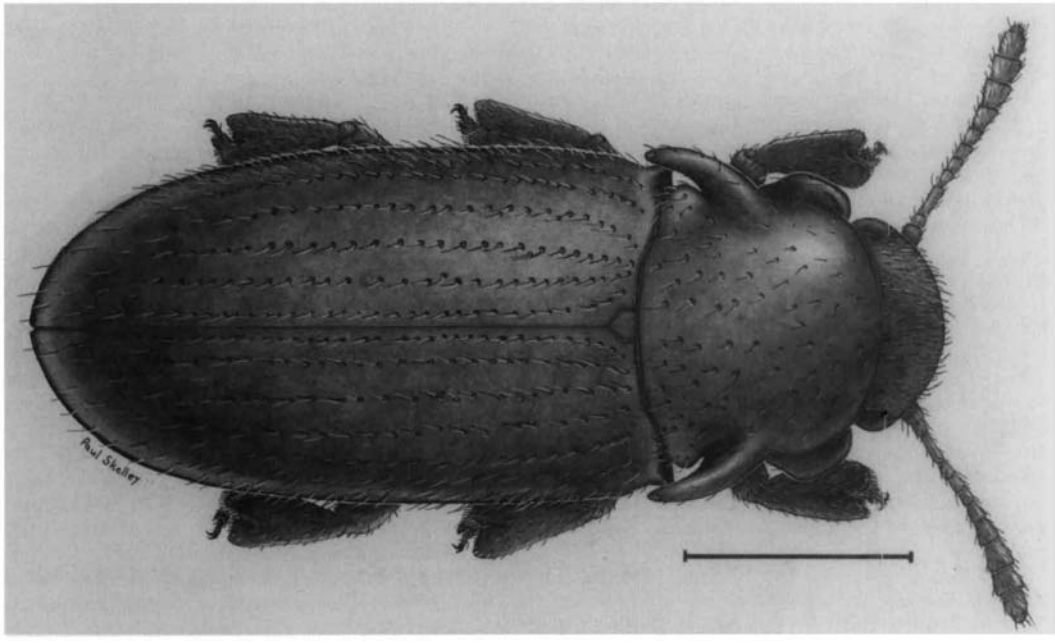
APPENDIX 1. Terminal taxa, their species number, diet, and gonocoxite structure. Details about the scoring of morphological characters for terminal taxa, and the classification of the family, are described in detail by Leschen (2003). For each terminal taxon below, the number of recorded species/diversity scoring in data matrix, diet, form of gonocoxite are listed.

Biphyllidae (200/1, microfungi, narrow), Dacnini (338/1, macrofungi, acute), Encaustini (152/1, macrofungi, narrow), Erotylini (751/1, macrofungi, narrow), Megalodacninae (100/1, macrofungi, narrow), Tritomini (618/1, macrofungi, narrow), *Acryptophagus* (1/0, microfungi, sinuate), *Anadastus* (268/1, phytophagy, acute), *Atomarops* (3/0, ?, sinuate), *Bolerus* (16/0, ?, narrow), *Brachypterosa* (1/0, saprophagy, narrow), *Cathartocryptus* (8/0, microfungi, dilated), *Cladoxena* (4/0, ?, acute), *Crotchia* (20/0, phytophagy, acute), *Crowsonguptus* (4/0, pollen/saprophagy, narrow), *Cryptophilus* (15/0, saprophagy, dilated), *Dasydactylus* (24/0, phytophagy, acute), *Empocryptus* (15/0, saprophagy, narrow), *Fitoa* (1/0, microfungi, narrow), *Hapalips* (57/1, phytophagy, narrow), *Henoticonus* (1/0, saprophagy, narrow), *Languria* (18/0, phytophagy, acute), *Lepidotoramus* (1/0, saprophagy, dilated), *Leucohimatium* (8/0, microfungi/saprophagy, sinuate), *Loberogosmus* (1/0, phytophagy, ?), *Loberolus* (1/0, microfungi, narrow), *Loberonotha* (1/0, pollen, dilated), *Loberopsyllus* (4/0, saprophagy, narrow), *Loberoschema* (7/0, saprophagy, dilated), *Loberus* (75/1, saprophagy, narrow), *Lobosternum* (1/0, saprophagy, ?), *Macrophagus* (1/0, pollen/saprophagy, sinuate), *Microlanguria* (14/0, phytophagy, acute), *Neoberolus* (3/0, saprophagy, acute), *Nomotus* (5/0, phytophagy, ?), *Othniocryptus* (1/0, ?, sinuate), *Paphezia* (1/0, saprophagy, narrow), *Paracladoxena* (20/0, microfungi/pollen, acute), *Penolanguria* (15/0, ?, acute), *Pharaxonotha* (11/0, phytophagy, narrow), *Platoberus* (10/0, saprophagy, *Platoberus*-type), *Protoloberus* (1/0, ?, narrow), *Pseudhapalips* (1/0, macrofungi, sinuate), *Setariola* (1/0, phytophagy, dilated), *Stengita* (1/0, ?, narrow/dilated), *Stenodina* (1/0, ?, narrow), *Telmatoscius* (1/0, microfungi, narrow), *Thallisella* (13/0, microfungi, *Thallisella*-type), *Toramus* (43/0, macrofungi/saprophagy, dilated), *Truquiella* (1/0, saprophagy, narrow), *Xenocryptus* (2/0, phytophagy, dilated), *Xenoscelis* (1/0, ?, narrow), *Zavaljus* (1/0, dead wood, narrow), n. gen. 1 (1/0, ?, dilated).




*Bancous perplexa* (Skelley). *Bancous* Pic (3 spp.) and *Chasmatodera* Arrow (2 spp.) are two very unusual erotyline genera from Africa and Asia. They have a highly modified prothorax with strange invaginations and processes while the legs are dilated, characters which do not occur in other members of the fungus-feeding Erotylini. One species of *Bancous* has been collected from the nest of the fungus-growing species *Protermes prorepens* (Sjöstedt) in the Belgian Congo (Skelley 1999). Illustration by Paul Skelley; Scale bar = 1.0 mm.