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Patterns of [FeFe] Hydrogenase Diversity in the Gut Microbial Communities of Lignocellulose-Feeding Higher Termites

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Hydrogen is the central free intermediate in the degradation of wood by termite gut microbes and can reach concentrations exceeding those measured for any other biological system. Degenerate primers targeting the largest family of [FeFe] hydrogenases observed in a termite gut metagenome have been used to explore the evolution and representation of these enzymes in termites. Sequences were cloned from the guts of the higher termites *Amitermes* sp. strain Cost010, *Amitermes* sp. strain JT2, *Gnathamitermes* sp. strain JT5, *Microcerotermes* sp. strain Cost008, *Nasutitermes* sp. strain Cost003, and *Rhyncotermes* sp. strain Cost004. Each gut sample harbored a more rich and evenly distributed population of hydrogenase sequences than observed previously in the guts of lower termites and *Cryptocercus punctulatus.* This accentuates the physiological importance of hydrogen for higher termite gut ecosystems and may reflect an increased metabolic burden, or metabolic opportunity, created by a lack of gut protozoa. The sequences were phylogenetically distinct from previously sequenced [FeFe] hydrogenases. Phylogenetic and UniFrac comparisons revealed congruence between host phylogeny and hydrogenase sequence library clustering patterns. This may reflect the combined influences of the stable intimate relationship of gut microbes with their host and environmental alterations in the gut that have occurred over the course of termite evolution. These results accentuate the physiological importance of hydrogen to termite gut ecosystems.

Hydrogen plays a pivotal role in the digestion of wood by termites (7, 11, 39). Concentrations in the guts of some species exceed those measured for any other biological system (18, 41, 43). The turnover of the gas in the gut has been measured in some species at daily fluxes as high as 33 m^3/m^3 gut volume (39). The environment is also spatially complex, comprising a matrix of microenvironments characterized by different hydrogen concentrations (14, 18, 26, 39).

Termites can be classified as belonging to one of two phylogenetic groups, higher termites and lower termites (25). Higher termites characteristically lack protozoa, which are abundant in the guts of lower termites, in their guts and have more highly segmented gut structures than do lower termites (17, 34, 35). Of the over 2,600 known species of termites, over 70% are higher termites (25, 49). They represent the largest and most diverse group of termites (24, 49). Yet, most of what we know about termite gut microbes comes from work done with lower termites, and comparatively little work has been done with the communities of higher termites (8–10, 12). The primary reason for this is that it was believed until recently that the gut microbes of higher termites played only a minor role in wood digestion (42, 46, 47). This changed with the recent publication of the gut metagenome of a higher termite where it was found that the gut microbial community harbors genes for reductive acetogenesis, polysaccharide degradation, and an abundance of [FeFe] hydrogenases, all pointing in the direction of a more active role in wood degradation (47). This previously underacknowledged role for the gut microbes has also found support in the findings of Tokuda and Watanabe (46).

Wood-feeding insects have shared a stable and intimate mutualism with their respective gut microbial communities for at least 20 million years (48). It has been proposed that the gut microbes of lower termites may "coevolve" with their respective hosts (4, 19, 21, 50). Past analyses of *nifH* genes from the guts of higher and lower termites and *Cryptocercus* have shown that genes from the same genus or family of termite tend to be more similar to one another than to those from more distantly related termites (19, 37, 50). Hongoh et al. found that the gut community composition is consistent within a genus of termites (21). Analyses of spirochete diversity in higher and lower termites have demonstrated a tendency of spirochete 16S sequences from the same genus of termite to cluster with one another, supporting the hypothesis that "spirochaetes are specific symbionts that have coevolved with their respective species of termites" (4, 36). Moreover, the composition of termite gut communities has been shown to vary substantially with host feeding habits, which are closely linked with phylogeny (32, 38, 44). In the case of wood roaches, a very close correspondence of host and symbiont phylogenies has lent strong support for the cospeciation of a *Cryptocercus* endosymbiont with its hosts (16, 19, 28). Noda et al. have reported the cospeciation of intestinal microorganisms with their termite hosts, thereby demonstrating the high stability of the association between a termite and its gut microbiota (33).

Here we report a phylogenetic analysis of [FeFe] hydrogenase genes cloned from the guts of higher termites. We have focused on family 3 [FeFe] hydrogenases, first defined by Warnecke et al., because they comprise the most highly represented group of hydrogenases observed in a *Nasutitermes* hindgut metagenome sequence (47). They were also the only group of hydrogenases observed in the *Nasutitermes* hindgut metagenome whose *in situ* translation was verified by mass spectroscopy (47). The objective was to better understand the diversity, adaptation, and evolution

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of these genes in these hydrogen-metabolizing ecosystems. Moreover, the influence of host ecosystem variations on the hydrogenase sequence composition of their associated microbial communities was investigated through cross-comparisons with sequence libraries reported previously for lower termite and wood roach samples (2).

MATERIALS AND METHODS

Termites. *Nasutitermes* sp. strain Cost003 and *Rhyncotermtes* sp. strain Cost004 were collected in the INBIO forest preserve in Guápiles, Costa Rica. Cost003 was collected at a height of 1.2 m from a *Psidium guajaba* tree and was believed to be feeding on deadwood. Cost004 was collected from a nest located under a bromeliad. Feeding trails leading from this nest to a pile of decaying wood and plant material suggested litter feeding. *Microcerotermes* sp. strain Cost008 was collected from the base of a palm tree about 100 m from the beach at Cahuita National Park in Costa Rica and appeared to be feeding on the palm tree. *Amitermes* sp. strain Cost010 was collected from the roots of dead sugarcane plants at a plantation in Costa Rica. *Amitermes* sp. strain JT2 and *Gnathamitermes* sp. strain JT5 were collected from subterranean nests at Joshua Tree National Park in the United States (permit no. JOTR:2008-SCI-002). Termites were identified in a previous study (38) using insect mitochondrial cytochrome oxidase subunit II (COXII) gene sequences and morphology.

DNA extraction and cloning. For each termite sample, DNA was extracted from single whole dissected guts and quantitated as described elsewhere (2, 31). Degenerate primers reported in a previous study (2) for the specific amplification of family 3 [FeFe] hydrogenases were used for the cloning of gut sequences as described previously (2). The degenerate primer sequences, which were ordered from IDT DNA, were WSI CCI CAR CAR ATG ATG G and CCI CKR CAI GCC ATI ACY TC for the forward and reverse primers, respectively, where "I" represents inosine.

Restriction fragment length polymorphism (RFLP) analysis and sequencing. Clones were selected for sequencing, and sequences were edited and verified as encoding hydrogenase as described elsewhere (2).

Sequences that analyses aligned poorly with other cloned hydrogenase sequences in our database were resequenced and analyzed manually for frameshift mutations or internal stop codons. Frameshift mutations were identified and manually corrected at the DNA level for three clones (see the footnotes to Table S1 in the supplemental material).

Phylogenetic analysis. Phylogenetic analyses were completed as described elsewhere (2). Cloned sequences and their operational taxonomic units (OTUs) used in these analyses are listed in Table S1 in the supplemental material. Trees were constructed using 173 unambiguously aligned amino acid positions with distance matrix (Fitch), maximum parsimony (Phylip PROTPARS), and maximum likelihood (PhylipProML) treeing methods. Phylograms presented in this paper were drawn using Phylip drawgram (20). The following sequences, all derived from gut symbionts of termites, comprised the outgroup used to construct Fig. 1 and 2 (3, 22): *Pseudotrichonympha grassii* (AB331668), uncultured parabasilid (AB331670), *Holomastigotoides mirabile* (AB331669), *Pseudotrichonympha grassii* (AB331667), *Treponema primitia* ZAS-1 (HndA1, HQ020732), *Treponema primitia* ZAS-2 (HndA2, HQ020741), *T. primitia* ZAS-2 (HndA3, HQ020740), and *T. primitia* ZAS-1 (HydA1, HQ020748). The following family 3 [FeFe] hydrogenase sequences reported elsewhere (3) were also used to construct Fig. 1 and 2: *Treponema primitia* strain ZAS-2 (HndA1, HQ020737) and *Treponema azotonutricium* strain ZAS-9 (HndA, HQ020755).

Diversity and sequence richness calculations. Chao1 sequence richness and Shannon diversity indices for each clone set were calculated using EstimateS version 8.0.0 for Macintosh computers, written and made freely available by Robert K. Colwell (http://viceroy.eeb.uconn.edu/EstimateS). The Shannon evenness index (30) was calculated from the Shannon diversity index. OTUs and their respective sequence abundances were used as inputs to the program.

Community comparisons. UniFrac (29) was used for quantitative comparisons of the higher termite [FeFe] hydrogenase sequence libraries with each other or with those prepared from lower termites and *Cryptocercus punctulatus* reported elsewhere (2). Maximum likelihood trees were constructed according to the methods described above and subsequently used as the input for UniFrac. One hundred seventy-three unambiguously aligned amino acids were used in treeing calculations. Each sequence library was designated a unique environment. The number of cloned sequences represented by each OTU was input to UniFrac to be used for calculating abundance weights. The environments were compared using the UniFrac jackknife and principal component analyses (PCAs). Normalized abundance weights were used in all calculations. The jackknife calculation was completed with 1,000 samplings and using 75% of the OTUs contained in the smallest environment sample as the minimum number of sequences to keep.

Nucleotide sequence accession numbers. Sequences have been deposited in the GenBank, DDBT, and EMBL databases under accession numbers HQ020957 to -1201.

RESULTS

Sequences cloned. Hydrogenase sequences representing as many as 44 sequence OTUs were cloned from each of the higher termites (Table 1). Table S1 in the supplemental material lists all cloned sequences, and their corresponding OTUs, analyzed in this study. The collector's curves for each sequence library are provided as Fig. S1 in the supplemental material. *Microcerotermes* was the only sample having 75% of all cloned sequences distributed among fewer than 7 OTUs. The Shannon diversity index and the Chao1 species richness index for each sequence library are listed in Table 1.

Phylogenetic analysis. In phylogenetic analyses comparing the cloned sequences to publically available [FeFe] hydrogenase sequences, all sequences in our database, with one exception (see footnote to Table S1 in the supplemental material), clustered within a single large clade to the exclusion of all nontermite bacterial sequences (data not shown). Comprising the sequences falling within the clade were family 3 [FeFe] hydrogenase sequences from a *Nasutitermes* gut metagenome (47) and from the genome sequences of two treponemes isolated from *Zootermopsis angusticolis*, *T. primitia* ZAS-2, and *T. azotonutricium* ZAS-9 (3). A maximum likelihood tree for all of the cloned [FeFe] hydrogenase sequences is provided as Fig. 1.

Upon inspection of phylogenetic groupings, the hydrogenase sequences appeared to cluster in a manner congruent with the phylogeny of their hosts. Moreover, hydrogenase sequences from a given termite sample tended to cluster with one another.

Sequence library cross-comparisons. A maximum likelihood tree comparing all of the family 3 hydrogenases cloned from the higher termite samples to those cloned previously from *C. punctulatus* and lower termite gut samples (2) is provided as Fig. 2. An apparent congruence between the phylogenetic clustering of the cloned hydrogenases and that of their respective hosts was observed in the UniFrac jackknife clustering of the samples (Fig. 3A). In this analysis, the clustering of the hydrogenase sequences was congruent with the phylogeny of their respective hosts reported by Legendre et al. and Inward et al. (23, 24, 27). Specifically, three unique coclustering groups could be distinguished corresponding to the three unique phylogenetic groupings sampled in this study, including *Cryptocercus*, lower termites, and higher termites. For instance, lower termites and *C. punctulatus* library sequences grouped with one another within clusters that appeared in all

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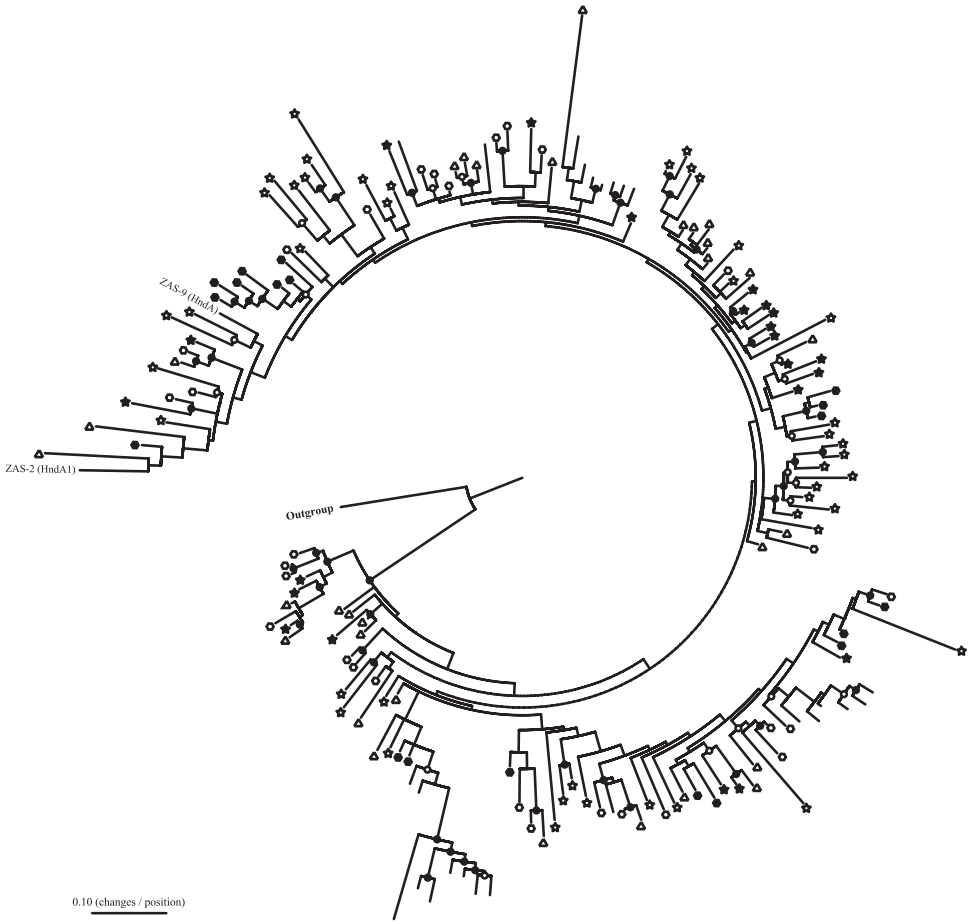

FIG 1 Phylogram for family 3 [FeFe] hydrogenases cloned from the guts of higher termites. The tree was calculated using a maximum likelihood (Phylip ProML) method with 173 unambiguously aligned amino acid positions. Open circles designate groupings also appearing in either parsimony (Phylip PROTPARS, 1,000 bootstraps) or distance matrix (Fitch) trees. Closed circles designate groupings appearing in trees constructed by all three methods. Each leaf represents an OTU. The termite host corresponding to each OTU is indicated by a shape or lack thereof: *Amitermes* sp. Cost010, triangle; *Amitermes* sp. JT2, solid black star; *Gnathamitermes* sp. JT5, polygon with white center; *Microcerotermes* sp. Cost008, solid black polygon; *Nasutitermes* sp. Cost003, no shape; *Rhyncotermes* sp. Cost004, star with white center. Hydrogenase sequences taken from *T. primitia* ZAS-2 and *T. primitia* ZAS-9 are labeled as ZAS-2 (HndA1) and ZAS-9 (HndA), respectively.

three treeing methods. Nodes appearing in all three treeing methods tended to have bootstrap values above 50% in parsimony analyses, whereas clusters appearing in only two methods or one method tended to have less bootstrap support. In only 3 instances was there a clustering appearing in all treeing methods that formed between higher termite library sequences and sequences originating from *C. punctulatus*, and this was never the case for any of the lower termite sequences. Moreover, the *C. punctulatus* and lower termite library sequences each had a tendency to fall within clusters appearing in multiple treeing methods that contain only *C. punctulatus* or lower termite sequences, respectively. This clustering was further supported by the UniFrac PCA of the sequences (Fig. 3B). There was a distinguishable separation between sequences from each of the three groups representing higher termites, lower termites, and *C. punctulatus*. Principal component 1, which accounted for the separation of higher termites from lower termites and *C. punctulatus*, explained 34.87% of the variation.

A UniFrac principal component analysis of the [FeFe] hydrogenase sequences cloned from higher termites is provided as Fig. 3C. Sequences from *Amitermes* sp. Cost010, *Amitermes* sp. JT2, and *Gnathamitermes* sp. JT5 clustered together. These samples could be distinguished from the others according to principal component 1, which explained 30.68% of the variation.

DISCUSSION

High [FeFe] hydrogenase sequence diversity in higher termites. The abundance of [FeFe] hydrogenases cloned from the guts of higher termites, representing as many as 44 OTUs in the case of *Rhyncotermes* sp. Cost004, emphasizes the physiological importance of these enzymes to these complex ecosystems. Moreover, these cloned sequences were found to belong to the largest family of [FeFe] hydrogenase sequences observed in a higher termite gut metagenome. There is good reason to believe that this is only a sampling of a much larger diversity, because only one of a total of 9 families reported in the *Nasutitermes* gut metagenome sequence was targeted in this analysis. The grouping of the sequences with one another to the exclusion of all other non-termite-associated bacterial [FeFe] hydrogenase sequences in our database may imply unique evolutionary responses to the termite gut ecosystem. Similar community-wide evolutionary adaptations of [FeFe] hy-

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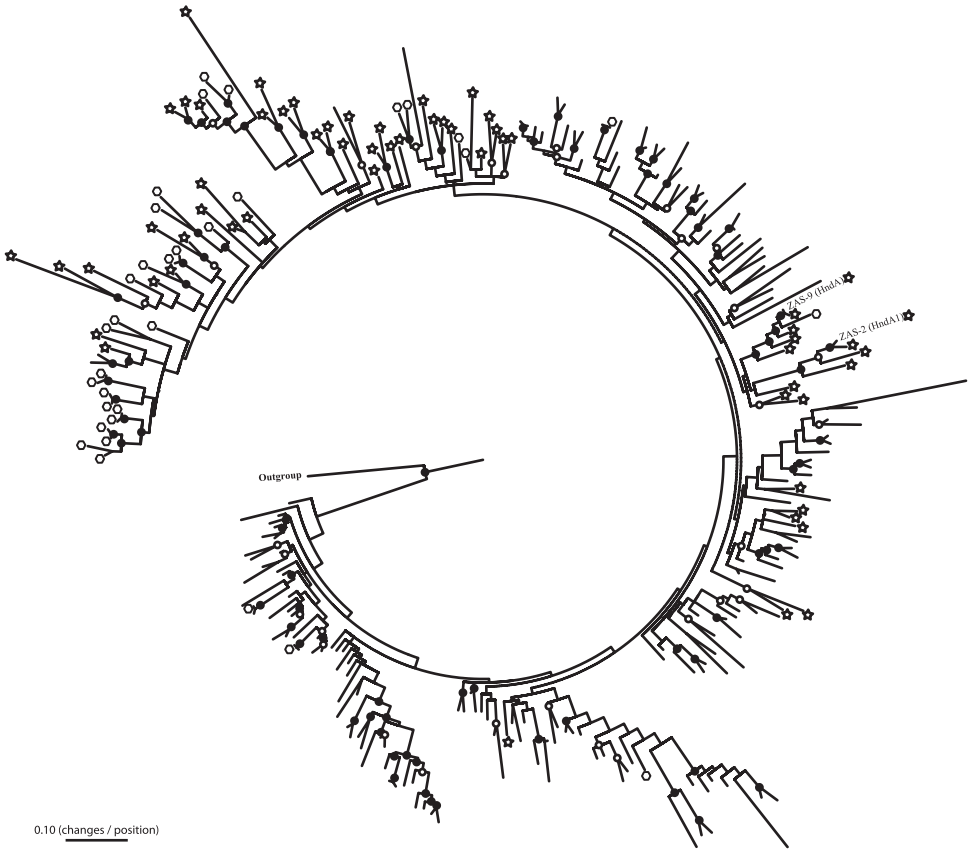

FIG 2 Phylogram comparing family 3 [FeFe] hydrogenases cloned from higher termites to sequences cloned previously from *C. punctulatus* and lower termites. The Fig. 1 caption describes the open and closed black circles and tree construction methods. Each leaf represents an OTU. Leaves and branches representing OTUs cloned from lower termites have a star at their end, those from *C. punctulatus* have a polygon at their end, and those from higher termites have nothing at their end. Hydrogenase sequences taken from *T. primitia* ZAS-2 and *T. primitia* ZAS-9 are labeled as ZAS-2 (HndA1) and ZAS-9 (HndA), respectively, with a star following the names.

TABLE 1 Analysis of diversity of [Fe] hydrogenase sequences cloned in this study

Host species	No. of RFLPs[a]	No. of OTUs[b]	Chao1 index[c]			Shannon index	
				95% CI			
			Mean	Lower bound	Upper bound	Mean[d]	Evenness[e]
Amitermes sp. Cost010	60	31	45	35.02	79.77	2.97	0.865
Amitermes sp. JT2	33	22	32.67	24.18	74.18	2.65	0.857
Gnathamitermes sp. JT5	44	30[f]	40.29	32.75	68.49	3.05	0.906
Microcerotermes sp. Cost008	36	21	29.1	22.84	56.57	2.33	0.765
Nasutitermes sp. Cost003	38	25	43	29.54	96.38	2.69	0.836
Rhyncotermes sp. Cost004	54	44	68.05	52.73	110.24	3.53	0.933

[a] Number of unique RFLP patterns observed. Ninety-six clones were selected from each clone library in each RFLP analysis.
[b] The number of OTUs was calculated using the furthest-neighbor method and a 97% amino acid sequence similarity cutoff.
[c] Chao1 species richness index calculated using the classic method in EstimateS. OTUs representing family 3 [FeFe] hydrogenases with their respective abundances were used as program inputs. 95% CI, 95% confidence interval.
[d] Shannon diversity index calculated using EstimateS. OTUs representing family 3 [FeFe] hydrogenases with their respective abundances were used as program inputs.
[e] The Shannon evenness index has been calculated by dividing the Shannon mean by the natural log of the number of family 3 OTUs.
[f] One of these OTUs represented family 7 [FeFe] hydrogenase sequences (see Table S1 in the supplemental material) and was not used in the calculation of the diversity indices.

drogenase sequences from unique ecosystems, as evidenced by sequence similarity and uniqueness, have been reported by Ballor and Leadbetter and by Boyd et al. (2, 5, 6).

The hydrogenases cloned from the higher termites tended to have a more even distribution and broader sequence diversity than sequences cloned from *C. punctulatus* or lower termites by Ballor and Leadbetter (2). For example, the higher termites analyzed in this study had an average of 29 OTUs per library and an average Shannon mean of 2.87 compared to the same parameters measured for the *C. punctulatus* and lower termite libraries reported previously as together having an average of 18 OTUs per library and an average Shannon mean of 2. A *t* test indicated that the Chao1 and Shannon diversity indices and OTU counts for the higher termite libraries were all greater than those for the lower termite and *C. punctulatus* libraries with a confidence greater than 95%. The Shannon evenness index too is found by a *t* test to be significantly higher at a confidence of over 95% in the higher termite libraries. This means that in the guts of higher termites not only there is a greater diversity of hydrogenase sequences than in lower termites or *C. punctulatus* but there is also a more evenly distributed representation of each individual OTU.

The *Microcerotermes* gut hydrogenase sequence library had the lowest diversity among the higher termite samples analyzed, and the *Rhyncotermes* sequence library had the highest. Both of these observations are in agreement with a study of formyltetrahydro-

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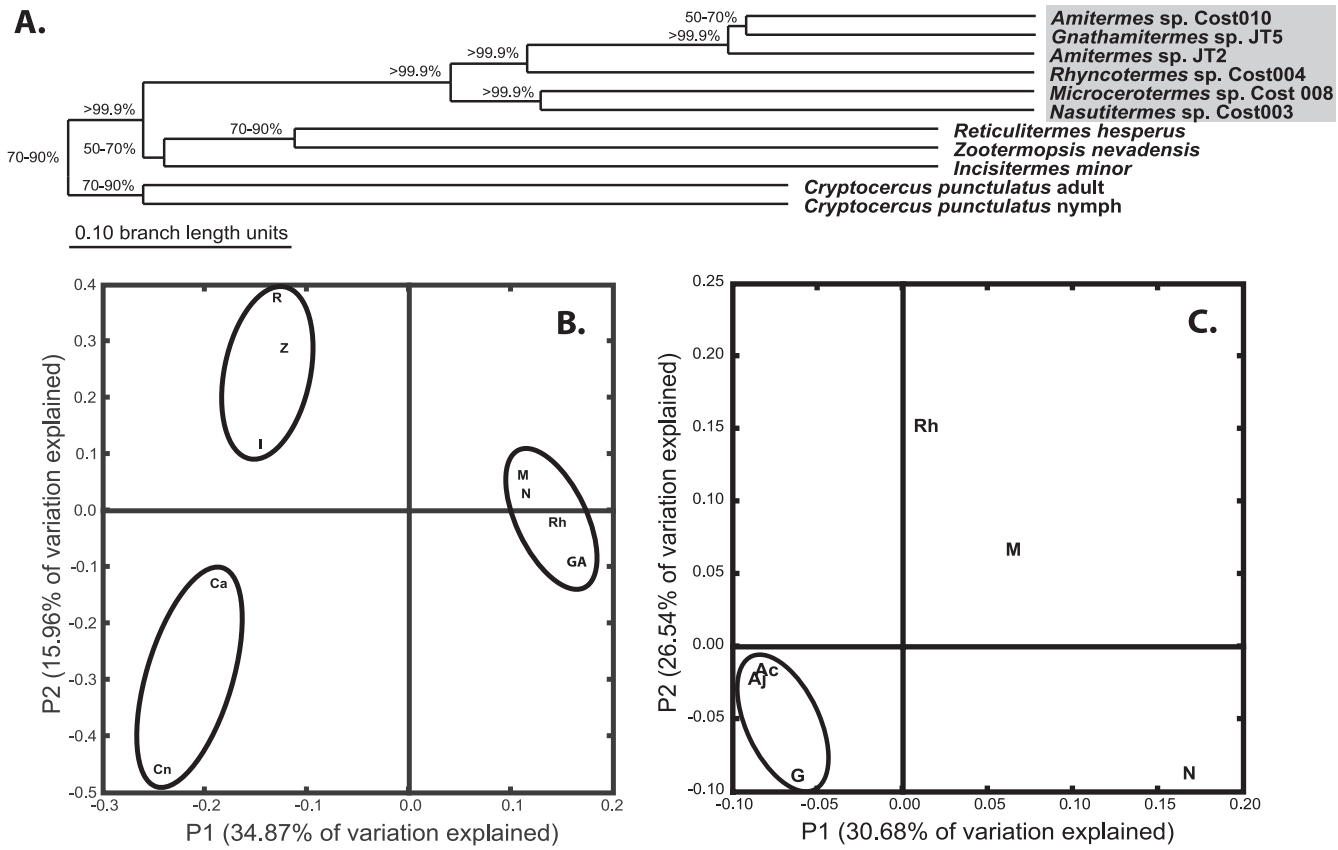

FIG 3 UniFrac analyses of family 3 [FeFe] hydrogenase sequences. (A) Jackknife clustering analysis. The maximum-likelihood tree shown in Fig. 2 and the OTUs with their respective abundance weights as listed in Table S1 in the supplemental material to this work and in Table S1 in the work of Ballor and Leadbetter (2) were used as inputs to UniFrac. The analysis was completed using normalized abundance weights and 1,000 samplings and keeping a number of sequences equal to 75% of the number of OTUs represented by the smallest sample analyzed. Each insect sample was designated a unique environment. The gray box highlights all higher termite environments. The numbers designate the percentage of samplings supporting a particular cluster. (B) UniFrac principal component analysis. The program inputs were as described for panel A. Principal components were calculated using normalized abundance weights. Each termite or *C. punctulatus* sample was designated a unique environment. Higher termite environments, lower termite environments, and *C. punctulatus* environments are each individually circled. P1, principal component 1; P2, principal component 2; Ca, *C. punctulatus* adult; Cn, *C. punctulatus* nymph; GA, a cluster of samples comprising *Amitermes* sp. Cost010, *Amitermes* sp. Cost003, and *Gnathamitermes* sp. JT5; I, *Incisitermes minor* isolate collection Pas1; M, *Microcerotermes* sp. Cost008; N, *Nasutitermes* sp. Cost003; R, *Reticulitermes hesperus* collection ChiA2; Rh, *Rhyncotermes* sp. Cost004; Z, *Zootermopsis nevadensis* collection ChiA1. (C) UniFrac principal component analysis. The maximum-likelihood tree shown in Fig. 1 and the OTUs with their respective abundance weights given in Table S1 in the supplemental material were used as inputs to UniFrac. Principal components were calculated using normalized abundance weights. Each termite sample was designated a unique environment. The circled environment corresponds to the most closely related higher termites analyzed in this study. P1, principal component 1; P2, principal component 2; Ac, *Amitermes* sp. Cost010; Aj, *Amitermes* sp. JT2; G, *Gnathamitermes* sp. JT5; M, *Microcerotermes* sp. Cost008; N, *Nasutitermes* sp. Cost003; Rh, *Rhyncotermes* sp. Cost004.

folate synthetase gene diversity reported by Ottesen and Leadbetter on the very same two gut samples analyzed in this study (38). The *Rhyncotermes* termites were gathered from a colony that appeared to be feeding on a compost pile containing a mixture of woody and leaf detritus, which may have the consequence of a broader diversity of bacteria within its gut environment. The remaining termites were gathered from subterranean nests and may have had a less varied diet.

If we can assume that functional variation is directly correlated with genetic variation, this observation of differences in evenness and diversity may imply that the absence of protozoa in higher termite guts has introduced important selective forces resulting in a broadening of bacterial hydrogenase functionality and, thereby, sequence diversity. This diversity may also stem from the more complex anatomy and segmentation of the higher termite gut than of lower termites or wood roaches, providing more ecological niches and, thereby, a broader diversity of hydrogenase sequences associated with microbes that have adapted to myriad microenvironments. Functional variation is known to be linked to increased ecosystem function (45). In their study of hydrogenase sequence diversity and phylogeny, Boyd et al. propose that an increase in phylogenetic diversity that they observed in slightly acidic geothermal springs may result in a more resilient community able to "better respond to change in both physical and chemical conditions in these environments due to seasonal hydrological and chemical changes" (5).

Congruence of [FeFe] hydrogenase and host phylogeny. [FeFe] hydrogenases cloned from closely related termites had a tendency to cluster with one another in phylogenetic analyses (Fig. 1). For example, sequences from both *Amitermes* gut samples tended to group together despite their being collected from locations separated by a great distance—California and Costa Rica.

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Sequence OTUs from a particular termite tended to group with one another rather than with sequences from other termites. In a phylogenetic analysis of the COII sequences used for molecular characterization of the termite samples, *Gnathamitermes* sp. JT5 and *Amitermes* sp. JT2 were found to be the most closely related of any of the higher termites used in this study (38). Correspondingly, there was a tendency for sequences from *Gnathamitermes* sp. JT5 to group with those from the *Amitermes* sp. samples. As one would expect, sequences taken from the genomes *T. primitia* ZAS-2 and *T. azotonutricium* ZAS-9, each isolated from the gut of a lower termite, did not group strongly with any of the sequences cloned from the higher termites (Fig. 1).

This congruence was further supported by phylogenetic comparisons of the higher termite sequences to lower termite and *Cryptocercus* sequences cloned previously (2). The observed lack of coclustering of the higher termite hydrogenase sequences with those from *Cryptocercus* or lower termites and the lack of clear segregation of the lower termite sequences from those of *C. punctulatus* is in agreement with the close evolutionary relatedness of these insects (23, 24, 27). A UniFrac principal component analysis using the maximum likelihood tree shown in Fig. 2 further supported these observations (Fig. 3B). Also, the jackknife clustering of the [FeFe] hydrogenase communities closely approximated previously proposed termite phylogenies (23, 24, 27).

UniFrac principal component and jackknife clustering analyses of a maximum likelihood tree of all higher termite sequences (Fig. 3C) revealed a close clustering of the *Amitermes* sp. and *Gnathamitermes* sp. JT5 samples. As mentioned above, these were the most closely phylogenetically related termites analyzed in this study. Their hydrogenase sequence libraries grouped with one another in over 99.9% of the samplings used to construct the jackknife-clustering tree shown in Fig. 3. This clustering was also apparent when the first and second principal components, collectively explaining 57.22% of variation, were plotted against each other.

The observed congruence between [FeFe] hydrogenase phylogeny and that of the host may imply that hydrogenases, and by extension their respective gut communities, have coevolved in an intimate relationship with their host termites. This provides further experimental support for previous proposals that termite or *Cryptocercus* gut microbes have coevolved with their host (1, 4, 19, 21, 36, 37, 40, 50). Perhaps this observation may be explained as a consequence of the influence of environmental changes in the gut, such as the presence or lack of protozoa or various anatomical alterations (34, 35), that have developed over the course of termite evolution. In particular, the gut compartments of higher termites facilitate dramatic changes in chemical composition along the length of the gut; for example, the P1 and P3 segments found in almost all higher termites and no lower termites are highly alkaline (pH $>$ 10) and hydrogen concentrations reach maxima in the ms and P3 segments (13–15). Schmitt-Wagner et al. have demonstrated that the composition of the microbial community varies from compartment to compartment in the guts of higher termites (40). Interestingly, Boyd et al. in their study of hydrogenase sequence distribution and diversity in geothermal springs found that geographic distance was the best predictor of phylogenetic relatedness of sequence communities, resulting most probably as a consequence of dispersal limitation (5). Care must be taken when making this comparison, however, because the cases reported by Boyd et al. are instances of covicariance explained entirely by geological constraints, whereas in the case of a termite gut there is an interaction of two biological entities where one influences the evolution of the other, which is an instance of coevolution (51). In this case, changes in the host are intimately linked to changes in the associated microbial community such that the evolution of one shapes the evolution of the other, hence, "coevolution." In light of the geographically constrained geothermal springs studied by Boyd et al. (5) and keeping in mind the above caution, the termite gut may be thought of as a "host-constrained" environment. In summary, surveying the representation of family 3 [FeFe] hydrogenase genes has begun to shed further light onto the evolutionary physiology of H_2 metabolism by the gut bacterial communities of termites.

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