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Phylogenetic Relationships, Evolution, and Systematic Revision of the Septate Gregarines (Apicomplexa: Eugregarinorida: Septatorina)

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ABSTRACT: A phylogenetic hypothesis was constructed with the use of ssu rDNA sequence data from 27 eugregarine species parasitizing a variety of arthropod hosts and habitats. The data were used to address higher-level character transitions, identify clades, recognize supraspecific taxonomic groups, assess the existing gregarine classification, and assess the effects of host metabolic pattern and habitat transitions on the radiation of the septatorinid gregarines. Suprageneric character transitions for association form, association timing, syzygy, gametocyst dehiscence, and oocyst liberation are defined. New character search based on the proposed phylogeny produced a morphological character set that correlates strongly with the sequence data. These morphological character sets are mapped to the new septatorinid phylogeny. Existing superfamily groups within Septatorina were recovered and a new superfamily recognized. At the family level, the monophyly of Actinocephalidae and Stylocephalidae is confirmed and the polyphyly of Gregarinidae is partially resolved with the recognition of Blabericolidae. At the generic level, the monophyly of *Protomagalhaensia* and *Xiphocephalus* is confirmed, the polyphyly of *Leidyana* is partially resolved with the recognition of *Blabericola*, and the polyphyly of *Gregarina* is revealed but cannot be resolved without additional taxonomic data. High-level diversification of Septatorina resulted from adaptations of the gametocyst, allowing colonization of both terrestrial and sweet-water habitats. Major radiations within the group correlate with host metamorphic pattern, suggesting that evolutionarily, gregarine species track niche resources along lines of transmission; they do not necessarily track host species in evolutionary time. Gregarine assemblages within a single host species may be either vicariant assemblages, (i.e., products of coevolutionary, phylogenetic effects), or ecotypic assemblages, (i.e., products of ecological fitting and host switching). The following systematic or nomenclatural acts are committed. Stenophoroidea and Gregarinoidea are emended. Diagnoses of Stenophoroidea, Gregarinoidea, and Sphaerocystidae are revised. Stylocephaloidea n. supfam., Blabericolidae n. fam., and *Blabericola* n. gen. are recognized and erected. *Blabericola princisi* n. comb., *Blabericola cubensis* n. comb., *Blabericola haasi* n. comb., and *Blabericola migrator* n. comb. are recognized. *Schneideria*, *Neoschneideria*, and *Paraschneideria* are removed from Sphaerocystidae and placed in Actinocephalidae. *Protomagalhaensia* is removed from Hirmocystidae and placed in Blabericolidae. *Pyxinia* is removed from Stylocephaloidea: Actinocephalidae and placed in Stenophoroidea: Monoductidae.

KEY WORDS: Actinocephalidae, adaptive radiation, *Amoebogregarina nigra*, Apicomplexa, *Blabericola cubensis*, *Blabericola haasi*, *Blabericola migrator*, Blabericolidae, *Colepismatophila watsonae*, Eugregarinorida, *Geneiorhynchus manifestus*, *Gregarina basiconstrictonea*, *Gregarina blattarum*, *Gregarina coronata*, *Gregarina cuneata*, *Gregarina diabrotica*, *Gregarina kingi*, *Gregarina niphandrodes*, *Gregarina polymorpha*, *Gregarina tropica*, Gregarine, Gregarinidae, Gregarinoidea, Hirmocystidae, *Hoplorhynchus acanthatholius*, *Leidyana erratica*, Monoductidae, *Neoschneideria*, *Paraschneideria*, *Paraschneideria metamorphosa*, phylogeny, *Prismatospora evansi*, *Protomagalhaensia*, *Protomaghalensia granulosae*, *Protomaghalensia wolfi*, *Pyxinia crystalligera*, *Schneideria*, Septatorina, Sphaerocystidae, *Stenophora robusta*, Stenophoroidea, Stylocephalidae, Stylocephaloidea, *Stylocephalus giganteus*, *Xiphocephalus ellisi*, *Xiphocephalus triplogemmatus*.

The ideal systematic arrangement for any group is a natural classification that provides organization, ease of use, and predictivity. The current gregarine (Protista: Apicomplexa: Eugregarinida) classification provides some degree of organization, but the pattern of organization breaks down at the generic and specific levels. It is certainly not a natural classification below the family level and thus is predictive only at relatively high levels. The system is difficult to use because it lacks rank or clade-level emphasis of cardinal characters (i.e., unique diagnostic characters) and depends upon unique suites of nonunique and often extrinsic characters at almost all levels. It is a pragmatic solution to the problem of organizing taxa in a hyperdiverse group when the overwhelming majority of taxa remain to be described.

The current gregarine classification is a consensus arrangement developed by several major systematists over the course of almost 100 yr. Unfortunately, there has been little agreement on the relative weight of characters or the correlation between cardinal characters and rank levels. Watson (1916, 1922) used epimerite and oocyst structures as cardinal characters of genera and heavily weighted the timing of association in combination with epimerite and oocyst structures as cardinal characters of families. Grassé (1953) clearly distinguished and separated the Eugregarina from the Archigregarina and Neogregar-

ina based on fundamental patterns of asexual reproduction in the life cycle. His work was the first to delineate modes of association and gametocyst dehiscence among gregarines carefully, but his arrangement of Eugregarina placed heavy weight on host association and the site of initial development (intracellular vs. extracellular). Accordingly, septate and aseptate forms were often placed in the same family and at all levels supraspecific taxa were defined by unique combinations of nonunique and often extrinsic characters. Chakravarty (1959) separated septate and aseptate gregarines to erect the current concepts of the suborders Septatorina and Aseptatorina, respectively. His arrangement of Septatorina was based explicitly on 3 character sets. They were, in order of decreasing priority, gamont morphology, gametocyst structure and mode of dehiscence, and oocyst "structure and other characters." Levine (1979, 1985, 1988) accepted Chakravarty's (1959) use of a protomerite–deutomerite septum as the cardinal character of the Septatorina and attempted to reconcile the existing arrangements of Watson (1916, 1922); Grassé (1953); and Chakravarty (1959) largely by consensus, with additional weight given to host association. Clopton (2002) was largely an attempt to update Levine (1988): most revisions of the arrangement reflected a reduced weight of host association and clarification of characters distinguishing genera.

Problems in generic- and family-level placement are not uncommon in gregarine systematics and arise from confused definition of the life-cycle characters used to diagnose superfamily groups. Traditionally, septate gregarine systematics has been reductive in its approach, separating most gregarine families and genera into 1 of 2 superfamilies based on the timing of the association of gamonts. Stenophoricae is fundamentally diagnosed by late association, and thus "solitary" gamonts, in combination with gametocyst dehiscence and oocyst liberation by simple rupture of the gametocyst wall. In contrast, Gregarinicae is fundamentally diagnosed by "precocious association"; thus well-developed trophozoites and gamonts are rarely encountered outside a reproductive association. Among the Gregarinicae, gametocysts generally dehisce or liberate their oocysts through "spore tubes" formed from the gametocyst wall itself. Given the limited precision with which these characters have been defined, it is not surprising that intermediate forms exist.

Watson (1915) erected *Leidyana* to comprise gregarine species united by the following characters: "sporonts solitary, epimerite a simple globular knob, dehiscence by spores ducts, spores dolioform." Systematic revisions of the septate gregarines have retained Watson's (1915) nonrestrictive diagnosis of *Leidyana*, but with some ambivalence, associating the group either with the modern Stenophoricae (Chakravarty, 1959; Levine, 1985, 1988; Clopton, 2002) or Gregarinicae (Watson, 1915, 1916; Watson [Kamm], 1922; Grassé, 1953; Hoshide, 1958; Kudo, 1966). *Leidyana* sensu stricto is unplaceable in the current classification: Association places it within Stenophoricae but gametocyst dehiscence places it within Gregarinicae. This demonstrates the fundamental problem of the current systematic arrangement: Authors have no difficulty in ascribing new species to the genus, but experts cannot confidently place the genus in a superfamily. Such problems rise more from a lack of precise definition of character transitions than from artificiality of the classification, at least at higher levels.

Molecular phylogenetics has made considerable progress in resolving basal relationships among major groups of protists. Where such analyses exist they can be particularly useful in identifying monophyletic groups, resolving the utility and relationships of cardinal character transitions, and guiding new character search, especially at higher taxonomic levels. To date there are few molecular analyses that include the septate gregarines, although the high level phylogenetic relationships of the Eugregarinida are fairly well resolved.

Molecular evidence indicates the Alveolata are a monophyletic group comprising 3 large protistan groups: ciliates, dinoflagellates, and the apicomplexans (Barta et al., 1991; Wolters, 1991). Within the alveolates, the apicomplexans and dinoflagellates are sister groups to the exclusion of the ciliates (Fast et al., 2001, 2002; Leander and Keeling, 2004). Eugregarinida is a monophyletic group within the monophyletic Apicomplexa (Lang-Unnasch et al., 1998; Carreno et al., 1999; Barta, 2001; Barta et al., 2001; Fast et al., 2002). With *Cryptosporidium*, they form basal groups within the larger clade that includes both coccidian and malarial parasites of vertebrates (Carreno et al., 1999; Mathew et al., 2000; Barta, 2001; Tenter et al., 2002; Toso and Omoto, 2007). Alternative phylogenetic arrangements have been hypothesized for relationships among Archigregarina, Neogregarina, and Eugregarina (Septatorina and Aseptatorina). The most convincing is that of Barta (2001), which postulates the sisterhood of Aseptatorina and Septatorina within the sisterhood of

Eugregarinida and Neogregarinida. Other analyses postulate sisterhood of the neogregarines and septatorinids to the exclusion of Aseptatorina (Leander, Clopton, and Keeling, 2003); sisterhood of the Septatorina and Aseptatorina as descendants of neogregarines and, in turn, archigregarines (Leander, Harper, and Keeling, 2003); sisterhood of the aseptatorinids and neogregarines to the exclusion of the septatorinids and marine aseptate gregarines (Leander et al., 2006); and the isolation of the neogregarines and nonmarine aseptatorinids in a clade separate from 1 containing Septatorina, marine aseptate gregarines, and Archigregarina (Leander, 2007). Despite these difficulties, the sisterhood of gregarines and *Cryptosporidium* is clear and well supported. Eugregarinida arose in a marine environment and subsequently invaded terrestrial hosts; thus the relationship among Eugregarinida and their hosts probably dates to the Permian (Perkins et al., 2002). Evolutionary relationships among eugregarine macrotaxa are implied by the current systematic arrangement, but no phylogenetic hypothesis exists for the group.

In order to assess relationships and evolutionary patterns within Septatorina, a phylogenetic hypothesis was constructed with the use of genomic ssu rDNA sequence data from 27 septatorinid species parasitizing a variety of arthropod hosts and habitats. The resulting phylogeny is used to address higher-level cardinal character transitions, identify clades, recognize supraspecific taxonomic groups, assess the existing gregarine systematic arrangement, and assess the effects of host metabolic pattern and habitat transitions on the radiation of the septatorinid gregarines.

MATERIALS AND METHODS

Specimen collection

Gregarines were collected from arthropod hosts between 1999 and 2008 and preserved for molecular analysis either during the course of ongoing taxonomic survey or solely for use in this study. Voucher slides for each parasite taxon are deposited in the collections of the Harold W. Manter Laboratory (HWML) of the University of Nebraska State Museum, University of Nebraska, Lincoln, Nebraska, U.S.A. Collections are detailed by taxon below.

Species maintained in laboratory colony: Yellow mealworm beetles, *Tenebrio molitor* (Coleoptera: Tenebrionidae), have been maintained in our laboratory since 1995. Gamonts of *Gregarina cuneata* and *Gregarina polymorpha* were collected from the intestines of larval beetles. Gamonts of *Gregarina niphandrodes* were collected from intestines of adult beetles. discoid cockroaches, *Blaberus discoidalis* (Blattaria: Blaberidae: Blaberinae), lobster cockroaches, *Nauphoeta cinerea* (Blattaria: Blaberidae: Oxyhaloinae), and Madagascar hissing cockroaches, *Gromphadorhina portentosa*, (Blattaria: Blaberidae: Oxyhaloinae) have been maintained in our laboratory since 1997. Gamonts of *Gregarina cubensis* and *Protomagalhaensia granulosae* were collected from intestines of discoid cockroaches. Gamonts of *Leidyana hassi* and *Protomagalhaensia wolfi* were collected from intestines of lobster cockroaches. Gamonts of *Leidyana migrator* were collected from intestines of Madagascar hissing cockroaches. Colony stocks of German cockroaches, *Blattella germanica* (Blattaria: Blattellidae: Blattellinae), and red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae), obtained from Carolina Biological Supply (Burlington, North Carolina, U.S.A.) were used to establish research colonies in our laboratory in 2005. Gamonts of *Gregarina blattarum* and *Gregarina basicontrictonea* were collected from the intestines of German cockroaches and adult red flour beetles, respectively. Colony stocks of *Tribolium freemani* (Coleoptera: Tenebrionidae) were built from subcultures provided in 2008 through the kindness of Dr. John Janovy, Jr. at the University of Nebraska, Lincoln, Nebraska, U.S.A., who obtained the original colony stocks from the United States Department of Agriculture (USDA) Post-Harvest Grain Marketing Laboratory, Kansas State University, Manhattan, Kansas, U.S.A., in January 1999. The Janovy cultures of *Trib. freemani* are the same from which *Gregarina cloptoni* was originally described and our colony stocks were the source of gamonts and trophozoites of *Gre. cloptoni*. Gametocysts of *Paraschneideria metamorphosa* were collected from the intestines of late-stage pupae of *Sciara coprophila* (Diptera: Nematocera: Sciadophoridae) collected from the departmental greenhouse at Peru State College, Nebraska, U.S.A., in January 2008. Gamonts and trophozoites of *Pyxinia crystalligera* were collected from laboratory stocks of *Dermestes maculata* (Coleoptera: Dermestidae) originally obtained from colonies maintained in the Biology Department at Sam Houston State University, Huntsville, Texas, U.S.A. Trophozoites, gamonts, and gametocysts of *Colepismatophila watsonae* were collected from immature and adult common silverfish, *Lepisma saccharina* (Thysanura: Lepismatidae), maintained in a laboratory colony at Peru State College started from wild material collected from outbuildings on the residence of Dr. T. J. Cook, Walker Co., Texas, U.S.A. (30°45′06″N; 95°25′42″W) in June 2008.

Species obtained from field collections: Trophozoites, gamonts, and gametocysts of *Gregarina diabrotica* and *Gregarina coronata* were collected from individuals of the striped cucumber beetle, *Acalymma vitattum* (Coleoptera: Chryosomelidae), and the southern corn rootworm beetle, *Diabrotica undecimpunctata howardi* (Coleoptera: Chryosomelidae), respectively, collected on wild buffalo gourd near Duck Creek north of the city of Peru, Nemaha County, Nebraska, U.S.A. (40°30′04″N; 95°45′16″W) in September 2007 and 2008. Trophozoites, gamonts, and gametocysts of *Amoebogregarina nigra* were collected from adult differential grasshoppers, *Melanoplus differentialis* (Orthoptera: Acrididae) collected near Duck Creek north of the city of Peru, Nemaha County, Nebraska, U.S.A. (40°30′04″N; 95°45′16″W) in October 2006 and 2007. Trophozoites, gamonts, and gametocysts of *Gregarina kingi* and *Leidyana erratica* were collected from adults of the field cricket, *Gryllus pennsylvanicus* (Orthoptera: Gryllidae) collected

from leaf mulch in Auburn, Nemaha County, Nebraska, U.S.A. (40°23′77″N; 95°50′78″W) in August 2007. Trophozoites, gamonts, and gametocysts of *Geneiorhynchus manifestus* were collected as part of the original type series collection (Clopton et al., 2007) from naiads of the common green darner, *Anax junius* (Odonata: Aeshnidae) collected from Beaver Slide Pond, Big Sandy Creek Unit, Big Thicket National Preserve, Polk County, Texas, U.S.A. (30°38′49″N; 96°17′64″W) in February 2006. Trophozoites, gamonts, and gametocysts of *Prismatospora evansi* were collected from naiads of the common green darner, *An. junius* (Odonata: Aeshnidae) collected from Collins Pond, Big Sandy Creek Unit, Big Thicket National Preserve, Polk County, Texas, U.S.A. (30°47′78″N; 94°12′81″W) in February 2006. Trophozoites, gamonts, and gametocysts of *Stylocephalus giganteus* were collected from adults of *Eleodes obscura* (Coleoptera: Tenebrionidae) collected on the Daryl Fisher Ranch, Keith Co., Nebraska, U.S.A. (41°14′37″N; 101°32′84″W) in August 2003. Trophozoites, gamonts, and gametocysts of *Xiphocephalus ellisi* were collected from adults of *Eleodes opaca* (Coleoptera: Tenebrionidae) collected near Beckius Windmill, Keith County, Nebraska, U.S.A. (41°11′17″N; 101°31′77″W) in August 2003. Trophozoites, gamonts, and gametocysts of *Xiphocephalus triplogemmatus* were collected from adults of *Eleodes tricostata* (Coleoptera: Tenebrionidae) collected on the Nevens Ranch, Keith County, Nebraska, U.S.A. (41°12′40″N; 101°25′09″W) in August 2003. Trophozoites and gamonts of *Stenophora robusta* were collected from adults of the flat-backed greenhouse millipede *Oxidus gracilis* (Diplopoda: Polydesmida: Paradoxosomatidae) collected from garden beds surrounding the residence of Dr. Marv Snyder in Omaha, Douglas County, Nebraska, U.S.A. (41°14′16″N; 95°58′57″W) in August 2007. Trophozoites, gamonts, and gametocysts of *Gregarina tropica* were collected as part of the original type series collection (Clopton et al., 2008a) from nymphs and adults of the brown-winged earwig, *Vostox brunneipennis* (Dermaptera: Labiidae) collected near Turkey Creek in the Turkey Creek Unit, Big Thicket National Preserve, Tyler County, Texas, U.S.A. (30°33′06″N; 94°19′03″W) in March 2007. Trophozoites, gamonts, and gametocysts of *Hoplorhynchus acanthatholius* were collected from adults of the common civil bluet damselfly, *Enallagma civile* (Odonata: Coenagrionidae) collected from Optimist Lake, Auburn, Nemaha County, Nebraska, U.S.A. (40°23′94″N; 95°50′24″W) during July–August 2007.

Collection and preservation of specimens: Individual intestines were dissected to *Tenebrio molitor* muscle saline (TMS; Belton and Grundfest, 1962). Permanent museum voucher preparations of all gregarine taxa were made with standard methods (Clopton, 2006; Clopton and Hays, 2006; Clopton et al., 2007; Hays et al., 2007; Clopton et al., 2008a, 2008b.) In addition, individual gregarines were collected by taxon, washed, and preserved by transfer through 3 changes of TMS and 3 changes of 90% ethanol. Specimens were stored in 90% ethanol for later DNA extraction. Prior to extraction, individual trophozoites and/or gamonts were identified and transferred to a sterile microcentrifuge tube for rehydration and DNA extraction. Individual gregarines were pooled by species (30–90 individuals per sample) for DNA extraction. Individual gametocysts were washed by transfer through 3 changes of TMS and 3 changes of distilled water and transferred to individual microcentrifuge tubes. A hypodermic needle was used to rupture each gametocyst and the liberated contents allowed to dry before the microcentrifuge tube was capped. All samples were stored at −20°C prior to DNA extraction.

DNA extraction, amplification, and sequencing

The DNA from each pooled sample of trophozoites and/or gamonts was isolated either with a protocol similar to that reported by Laird et al. (1991) or with a PureLink genomic DNA mini kit (Invitrogen, Carlsbad, California, U.S.A.). Dried gametocyst samples were extracted with the use of the PureLink genomic DNA mini kit's accompanying FTA protocol. Isolated DNA samples were resuspended in NE (Tris, no EDTA) buffer (USB Corporation, Cleveland, Ohio, U.S.A.) and stored by aliquot at 4 °C for subsequent analysis.

Polymerase chain reaction (PCR) was used to amplify ssu rDNA genes for all taxa with the use of the forward outside primer, Lssu5-5′ (5″-CGAATTCAACCTGGTTGATCCTGCCAGT) and reverse outside primer, Lssu6-3′ (5′- CCGGATCCTGATCCTTCTGCAGGTTCACCTAC) reported by Leander, Clopton, and Keeling (2003). The resulting products were separated and isolated by gel electrophoresis. Bands of interest were cut from the gel and PCR products isolated and purified with the use of the PrepEase gel extraction kit (USB Corporation, Cleveland, Ohio, U.S.A.) according to the manufacturer's protocols and the resuspended PCR products used to seed a reamplification PCR reaction to produce products for sequencing. Reamplified PCR products were purified with the use of the PrepEase DNA clean up kit (USB Corporation, Cleveland, Ohio, U.S.A.) according to the manufacturer's protocols and resuspended in NE buffer. Initial reactions were performed in a total volume of 25 µl using 12.5 µl of Taq PCR Master Mix 2X as provided by USB Corporation (20 mM Tris-HCl [pH 8.6], 100 mM KCl, 3 mM $MgCl_2$, 0.4 mM dNTPs [dATP, dCTP, dGTP, dTTP], 50 units/ml Taq DNA Polymerase), 1.25 µl each Lssu5 and Lssu6 primers (10 mM), and 10 µl of template, normalized with NE buffer to 2–4 ng/µl. Reamplification reactions were scaled up to 100 µl total volume. Amplifications performed with the use of a Perkin Elmer GeneAmp 2400 thermocycler under the following conditions: 95°C for 90 sec, followed by 20 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec, followed by a 72°C soak for 3 min produced double-stranded products of ca. 1,600 bases. Products were direct sequenced using Lssu5, Lssu6, and 6 internal primers described by White et al. (1990) (Wssu2-3′, 5′- GGCTGCTGGCACCAGACTTGC; Wssu3-5′, 5′-GCAAGTCTGGTGCCAGCAGCC; Wssu4-3′, 5′-CTTCCGTCAATTCCTTTAAG; Wssu5-5′, 5′-AACTTAAAGGAATTGACGGAAG; Wssu6-3′, 5′-GCATCACAGACCTGTTATTGCCTC; Wssu8-3′, 5′- TCCGCAGGTTCACCTACGGA) by the Nevada Genomics Center (University of Reno, Reno, Nevada, U.S.A.) with the use of the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, California, U.S.A.) run on the ABI3730 DNA Analyzer. Direct sequencing using both external and extensive internal primer sets produced a minimum 3× overread for all base positions in each product. Base sequences of ssu rDNA for each taxon were constructed with the use of ContigExpress (Vector NTI Advanced V10, Invitrogen, Carlsbad, California, U.S.A.) with consensus positions among primer products confirmed

visually with the use of ABI3730 chromatographic trace data.

Sequence analysis

Previous phylogenetic analyses based on ssu rDNA indicate that *Cryptosporidium* and the "gregarines" are more closely related to each other than to any other major alveolate group (Carreno et al., 1999; Barta, 2001; Barta et al., 2001). Thus 5 species of *Cryptosporidium* used by Carreno et al. (1999) were selected as the outgroup for the current analysis. Species chosen, their source host, and GenBank accession number for sequences used herein are as follows: *Cryptosporidium baileyi* ex *Gallus domesticus*, L19068; *Cryptosporidium wrairi* ex *Cavia porcellus*, U11440; *Cryptosporidium parvum* ex *Homo sapiens*, AF093489; *Cryptosporidium serpentis* ex *Elaphe guttata*, AF093499; and *Cryptosporidium muris* ex *Mus musculus*, L19069. Sequence alignments were provisionally constructed with the use of ClustalX V2.0 (Larkin et al., 2007). Outgroup sequences were provisionally aligned to form an outgroup profile, and sequence data from taxa collected for the current study were provisionally aligned to form an ingroup profile. The ingroup profile was aligned to the outgroup profile with the use of ClustalX V2.0 and the subsequent secondary alignment iteratively optimized in sub-blocks by eye and using MUSCLE V3.5.2 (Edgar, 2004) to approximate a "staggered alignment" as proposed by Barta (1997). The resulting alignment comprised 1,573 positions in the final data set, including 201 (13%) unique sites, 857 (54%) parsimony-informative sites of 1,061 (67%) variable sites, and 493 (31%) fully conserved sites.

Evolutionary model and analysis parameters were estimated with Akaike's Information Criterion in bootstrapped tests with the use of MrAIC.pl and ModelTest (Posada and Buckley, 2004). Subsequent phylogenetic analyses assumed a general time reversible model with gamma distribution (6 rate categories, $\gamma = 0.6$), proportion of invariant sites = 0.2, and transitions weighted twice transversions, as appropriate.

Phylogenetic hypotheses were constructed with the use of 3 methods: maximum likelihood with a 2,500 replicate bootstrap test (PHYML; Guindon and Gascuel [2003]), Bayesian analysis (MRBAYES; Ronquist and Huelsenbeck [2003]; 2,200,000 generations; 400 generation sample frequency, 4 hot, 1 cold chain; MCMC; 20% burn in) and maximum parsimony with a 5,000 replicate bootstrap test (PAUP 4.0b8 BETA; Swofford [2002]; heuristic search using AIC parameters, gaps treated as missing characters, random-taxon addition, 100 tree-bisection reconnection branch swaps/replicate). The resulting hypotheses were compared and overall composite strength of nodes and clades used to postulate a final topology.

RESULTS

Gregarine taxa sampled accompanied by their host taxa, HWML accession numbers of voucher specimens, and GenBank accession numbers are presented in Table 1.

Maximum parsimony analysis of the ssu rDNA sequence data produced a single most parsimonious tree (tree length = 4,790; consistency index = 0.972) with the topology shown in Figure 1. Bayesian inference and maximum likelihood analyses of the ssu rDNA sequence data produced single identical trees with the topology shown in Figure 1 and log likelihoods of −27,387.7 and −25,557.6, respectively. Overall nodal support for the individual hypotheses is good. In the Bayesian topology 50% (13/26) of nodes have support exceeding 90%; 77% (20/26) have support exceeding 70% and only 2 nodes (8%) have support of 50% or less. In the maximum likelihood topology 92% (24/26) of nodes have support exceeding 90% and 100% (26/26) have support exceeding 70%. In the maximum parsimony topology 50% (13/26) of nodes have support exceeding 90%; 69% (18/26) have support exceeding 70%; and 5 nodes (19%) have support of 50% or less.

Tree topologies produced by maximum parsimony, Bayesian inference, and maximum likelihood analyses are congruent with exception of 2 nodes. Bayesian inference and maximum likelihood methods place *X. triplogemmatus* and *X. ellisi* as the interior group of a larger clade including *Sty. giganteus*. In contrast, maximum parsimony methods place *Sty. giganteus* and *X. ellisi* as the interior group of a larger clade including *X. triplogemmatus*. The Bayesian inference/maximum likelihood arrangement is accepted as the consensus topology herein. Covariance of nodal support is apparent among phylogenetic methods (Fig. 1) and overall nodal support for the consensus hypothesis is excellent. In the consensus topology 96% (25/34) of nodes have support exceeding 90% and 100% (26/26) of nodes have support exceeding 70% by at least 1 method.

DISCUSSION

Nomenclatural emendations

The following nomenclatural emendations are required to bring the current gregarine classification into agreement with the International Code of Zoological Nomenclature, 4th edition, Article 29.2.

Stenophoroidea (=Stenophoricae) emendation.

Gregarinoidea (=Gregarinicae) emendation.

Definition of cardinal character transitions

To facilitate the systematic analysis herein it is necessary to define the cardinal character transitions precisely at suprageneric levels, primarily the character transitions for association form, association timing, syzygy, gametocyst dehiscence, and oocyst liberation.

The timing and form of "association" and "syzygy" are cardinal superfamily- and family-level

Table 1. Gregarine taxa accompanied by HWML and GenBank accession numbers and host taxa.

Gregarine species	Accession number		Host species (order: family)
	HWML	GenBank	
Amoebogregarina nigra	100000	FJ459737	*Melanoplus differentialis* (Orthoptera: Acrididae)
Blabericola cubensis (=*Gregarina cubensis*)	100001	FJ459751	*Blaberus discoidalis* (Blattaria: Blaberidae)
Blabericola haasi (=*Leidyana hassi*)	100002	FJ459753	*Nauphoeta cinerea* (Blattaria: Blaberidae)
Blabericola migratory (=*Leidyana migrator*)	100003	FJ459754	*Gromphadorhina portentosa* (Blattaria: Blaberidae)
Colepismatophila watsonae	100004	FJ459738	*Lepisma saccharina* (Thysanura: Lepismatidae)
Geneiorhynchus manifestus	48465–48468	FJ459739	*Anax junius* (Odonata: Aeshnidae)
Gregarina basiconstrictonea	100005	FJ459740	*Tribolium castaneum* (Coleoptera: Tenebrionidae)
Gregarina blattarum	100006	FJ459741	*Blattella germanica* (Blattaria: Blattellidae)
	100007		
Gregarina cloptoni	100008	FJ459742	*Tribolium freemani* (Coleoptera: Tenebrionidae)
Gregarina coronata	100009	FJ459743	*Diabrotica undecimpunctata* (Coleoptera: Chryosomelidae)
Gregarina cuneata	100010	FJ459744	*Tenebrio molitor* (Coleoptera: Tenebrionidae)
Gregarina diabrotica	100011	FJ459745	*Acalymma vitattum* (Coleoptera: Chryosomelidae)
Gregarina kingi	100012	FJ459746	*Gryllus pennsylvanicus* (Orthoptera: Gryllidae)
Gregarina niphandrodes	100013	FJ459747	*Tenebrio molitor* (Coleoptera: Tenebrionidae)
Gregarina polymorpha	100014	FJ459748	*Tenebrio molitor* (Coleoptera: Tenebrionidae)
Gregarina tropica	48797	FJ459749	*Vostox brunneipennis* (Dermaptera: Labiidae)
	48798		
Hoplorhynchus acanthatholius	100015	FJ459750	*Enallagma civile* (Odonata: Coenagrionidae)
Leidyana erratica	100016	FJ459752	*Gryllus pennsylvanicus* (Orthoptera: Gryllidae)
Paraschneideria metamorphosa	100017	FJ459755	*Sciara coprophila* (Diptera: Sciadophoridae)
Prismatospora evansi	45592	FJ459756	*Anax junius* (Odonata: Aeshnidae)
	45593		
Protomaghalensia granulosae	100018	FJ459757	*Blaberus discoidalis* (Blattaria: Blaberidae)
Protomaghalensia wolfi	100019	FJ459758	*Nauphoeta cinerea* (Blattaria: Blaberidae)
Pyxinia crystalligera	100020	FJ459759	*Dermestes maculata* (Coleoptera: Dermestidae)
Stenophora robusta	100021	FJ459760	*Oxidus gracilis* (Diplopoda: Paradoxosomatidae)
Stylocephalus giganteus	100022	FJ459761	*Eleodes obscura* (Coleoptera: Tenebrionidae)
Xiphocephalus ellisi	48105–48108	FJ459762	*Eleodes opaca* (Coleoptera: Tenebrionidae)
Xiphocephalus triplogemmatus	48109–48116	FJ459763	*Eleodes tricostata* (Coleoptera: Tenebrionidae)

characters within Septatorina, but their use is often confusing because these terms have not been clearly defined for gregarines and they are used, often interchangeably, with a variety of meanings for apicomplexans in general (Levine, 1971, 1973; Barta, 1989). When used in reference to gregarines, "syzygy" should be restricted to the actual rotation of associated gamonts for the purpose of forming the gametocyst wall. "Association" should be used to refer to the form and timing of gregarine pairing. Association may be frontal, lateral, frontolateral, laterocaudalfrontal, or caudofrontal in form. Timing of association varies by family and is categorized by concomitant life-cycle stages or events. Association may be trophozoic while trophozoites are still attached to the epithelium; gamontic after trophozoites have released their epimerites from the epithelium but significantly before the onset of syzygy; or syzygial, immediately prior to syzygy.

Gametocyst dehiscence and oocyst liberation are tightly associated with the physical form of the gregarine gametocyst. Clopton et al. (2008b) discussed the phylogenetically variable form and function of the "gametocyst envelope" or epicyst. The epicyst has been described variously as either hyaline or gelatinous, and although the thickness and consistency vary among taxa the epicyst appears to be present in all newly formed gregarine gametocysts. There are at least 3 distinct forms of the epicyst: the hyaline epicyst typical of Gregarinoidea, the gelatinous epicyst typical of the Actinocephalidae, and the papyriform epicyst of the Stylocephalidae. Epicyst form limits methods of gametocyst dehiscence and oocyst liberation. Among the Stylocephalidae the epicyst dries to form a papery mammelated shell that may serve to prevent desiccation in terrestrial or xeric environments (Clopton et al., 2008b). Among the Actinocephalidae the epicyst remains gelatinous throughout gametocyst development, apparently isolating the gametocyst and protecting it from bacterial and fungal colonization (Clopton et al., 2008b). Among the Gregarinoidea the epicyst dehydrates,

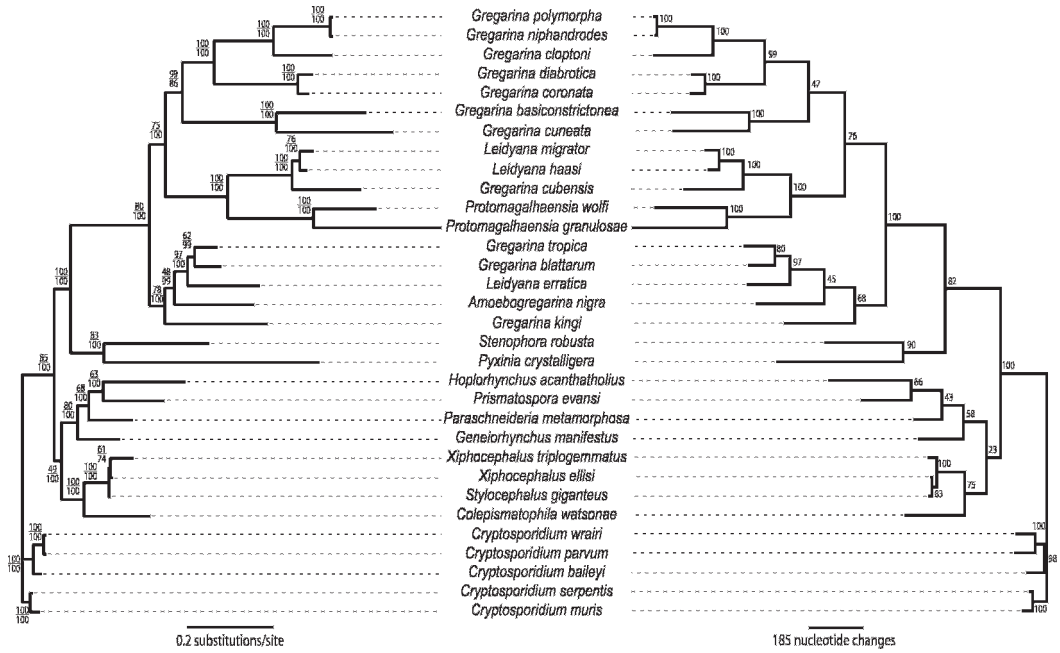

Figure 1. Topology of maximum-likelihood 2,500 replicate bootstrap consensus tree (left), Bayesian majority rules consensus tree of 5,000 trees (left), and maximum parsimony 5,000 replicate bootstrap consensus tree (right) based on DNA sequence of the genomic ssu rDNA gene from 27 taxa of septatorinid gregarines. Numbers represent bootstrap support by node (left, Bayesian analysis/maximum likelihood bootstrap values).

shrinking to bind the gametocyst proper during development tightly and then absorbing water internally to swell and pressurize the mature gametocyst for dehiscence and oocyst liberation (Peregrine, 1970; Clopton et al., 2008b).

Gametocysts with papyriform and gelatinous epicysts apparently have no mechanism to pressurize the gametocyst internally and dehisce by simple rupture of the gametocyst; that is, the epicyst and gametocyst walls break down, either by cracking (papyriform gametocysts) or by rupture and dissolution (gelatinous gametocysts). Their oocysts are neither expulsed or extruded: rupture of the epicyst and gametocyst walls simply exposes the oocysts for dispersal in the environment. The oocysts themselves may be liberated singly or in chains. Single oocysts are unattached one to another and simply disseminate in response to local currents or mechanical agitation. Oocysts linked in chains expand and disperse in response to humidity, air movement, and mechanical disturbance (Ellis, 1912, 1913; Adams and Travis, 1935; Crusz, 1960; Clopton, 1999, 2006).

Gametocysts with hyaline epicysts dehisce either by expulsion or extrusion due to internal pressurization of the gametocyst produced either by expansion of the gametocyst residuum (Tuzet et al., 1957) or constriction of the epicyst itself (Peregrine, 1970). Expulsion is the forceful liberation and dispersal of oocysts en masse or in polyete chains through a single rupture or structure in the gametocyst wall. Extrusion is the forceful liberation and dispersal of monete chains through 1 or more structures ("spore tubes") breaching the gametocyst wall. Some systematists have assigned considerable significance to the number, length, and arrangement of spore tubes on the gametocyst, but the taxonomic value of these characters is doubtful. Although there is no doubt that spore tube structures are real, they are variable in size, number, and structure depending upon environmental conditions (Filipponi, 1948; Watwood et al., 1997), as are gametocyst morphometrics themselves (Filipponi, 1950). Given the environmental variability of spore tube structures, the extrusion of monete oocyst chains is a reasonable proxy for the presence of spore tube structures and has predictable taxonomic significance. With the possible exception of the massive, well-formed spore tubes of the Monoductidae, spore tube number is not stable and probably has no taxonomic character value.

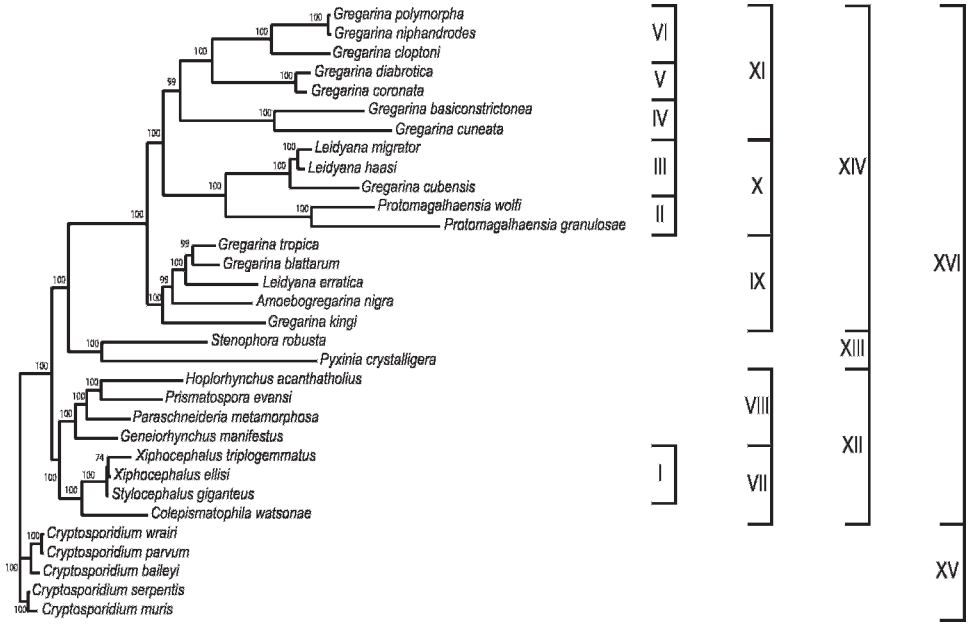

Figure 2. Relevant clades recovered from the consensus molecular phylogeny of 27 taxa of septatorinid gregarines. Roman numerals identify clades for text reference.

Clade recognition and implications for the existing systematic arrangement

Clopton (2002) presents the most recent comprehensive systematic arrangement of the gregarines. Clades recovered by the molecular phylogenetic analysis presented herein are delineated in Figure 2 and denoted by Roman numeral for purposes of the following discussion. Systematic acts committed herein are reflected in the revised phylogeny depicted in Figure 3.

Clade I (Generic level)*:* This clade includes *X. triplogemmatus* and *X. ellisi* but excludes the sister taxon, *Sty. giganteus*. This clade was recovered by both Bayesian inference and maximum likelihood analyses, but maximum parsimony analysis grouped *Sty. giganteus* and *X. ellisi* to the exclusion of *X. triplogemmatus*. Théodoridès (1963) established *Xiphocephalus* as 1 of 3 subgenera comprising *Stylocephalus*. Corbel (1971) elevated the taxon to the generic level, and to date 11 species have been recognized as members of the genus (Tuzet and Ormières, 1955; Théodoridès et al., 1965; Corbel, 1971; Devdhar and Amoji, 1977; Levine, 1984; Patil and Amoji, 1985; Clopton, 1999, 2006). The consensus phylogeny supports this notion of *Xiphocephalus* and the sister relationship between *Stylocephalus* and *Xiphocephalus* but additional taxon sampling within both genera is required to confirm the rank status relationship of these genera.

Clade II (Generic level)*:* This clade includes *Pro. granulosae* and *Pro. wolfi*, each parasitic in a single species of blaberid cockroach. The clade is monophyletic and supports the generic validity of *Protomagalhaensia*.

Clade III (Generic level)*:* This clade includes *Lei. migrator*, *Lei. haasi*, and *Gre. cubensis* Based on the current classification, Clade III is polyphyletic in grouping members of 2 genera but paraphyletic in excluding members of both *Leidyana* and *Gregarina*. However, there is good morphological and life history evidence to support Clade III, signifying the need for systematic rearrangement and the recognition of a new genus.

Watson (1915) erected *Leidyana* to comprise gregarine species united by the following characters: "sporonts solitary, epimerite a simple globular knob, dehiscence by spores ducts, spores dolioform." *Leidyana erratica* (=*Leidyana solitaria*), parasitic in the field cricket, *Gry. pennsylvanicus*, is the type species. The genus *Leidyana* currently comprises 33 species reported from 5 insect orders: Orthoptera (Dufour, 1837; Watson, 1915; Narain, 1961; Théodoridès and Echard, 1962; Corbel, 1967a, 1967b, 1968a; Issi and Lipa, 1968; Geus, 1969;

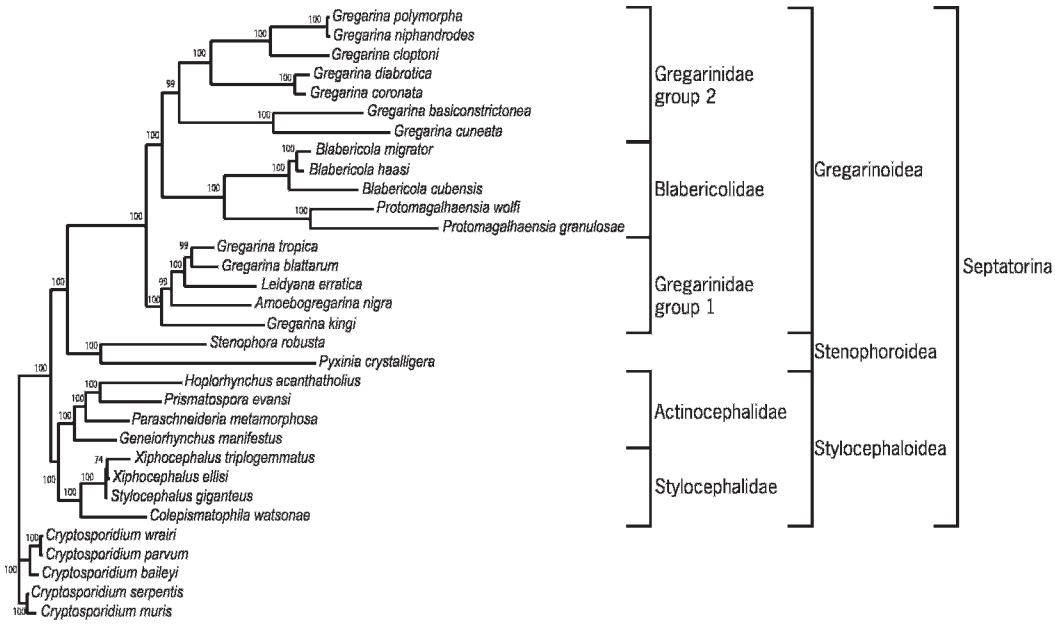

Figure 3. Revised gregarine classification juxtaposed with the consensus molecular phylogeny of 27 taxa of septatorinid gregarines.

Hoshide, 1973a, 1973b, 1978; Haldar and Sarkar, 1979; Hooger and Amoji, 1986; Sarkar, 1988; Pushkala and Muralirangan, 1998; Johny et al., 1999; Haldar and Patra, 2000; Johny et al., 2000; Lange and Cigliano, 2004), Lepidoptera (Keilin, 1918; Tuzet and Ormières, 1956; Hoshide, 1958; Geus, 1966; Ormières, 1967; Rabindra and Jayaraj, 1981; Lipa and Martignoni, 1984; Ghosh and Choudhury, 1992; Clopton and Lucarotti, 1997), Coleoptera (Braune, 1930; Finlayson, 1950; Geus, 1969; Patil and Amoji, 1979; Golemansky and Duhlinska, 1982; Roy, 1989), Trichoptera (Baudoin, 1966, 1967), Hymenoptera (Bhatia and Setna, 1924), and Blattodea (Peregrine, 1970; Ball et al., 1995; Clopton, 1995; Clopton and Hays, 2006). The breadth of host utilization reported for *Leidyana* crosses both host orders and host metabolic groups, a catholic approach to host utilization quite atypical of most gregarine genera.

The analysis presented herein associates all putative members of *Leidyana* sensu lato with the Gregarinoidea (Clade XIV), but indicates that at least 2 distinct lineages are included in the genus (Clades III, IX). *Leidyana erratica* is the type species of *Leidyana* and is placed in Clade IX, thus *Lei. migrator* and *Lei. haasi* are not members of *Leidyana* sensu stricto. *Leidyana migrator* and *Lei. haasi* are both described from blaberid cockroaches, *Gro. portentosa* and *Na. cinerea*, respectively. *Gregarina cubensis* is misplaced in the current classification but bears significant morphological and life history allegiance to *Lei. migrator* and *Lei. haasi*. Peregrine (1970) described *Gre. cubensis* from another blaberid cockroach, *B. discoidalis* (Blattodea: Blaberidae: Blaberinae) and placed the parasite in the genus *Gregarina* despite documenting the "solitary sporonts" that are the single character traditionally distinguishing *Leidyana* from *Gregarina*: "free sporonts … they do not associate until immediately prior to encystations." Thus *Gre. cubensis* falls within *Leidyana* sensu lato rather than *Gregarina*. *Leidyana migrator*, *Lei. haasi*, and *Gre. cubensis* form a cohesive, generic-level clade in the current analysis. In addition, all 3 gregarines parasitize cockroaches of the family Blaberidae. The distinct obpanduriform deutomerite of the gamont in these species distinguishes them from all other described species of *Leidyana* and warrants recognition of a new genus.

Blabericola n. gen.

Epimerite simple, globular to pyriform, without diamerite; deutomerite of gamont obpanduriform; association gamontic, presyzygial, caudofrontal; gametocysts with spore tubes, dehiscing by extrusion of

monete oocyst chains; oocysts elliptoid to dolioform; parasites of blaberid cockroaches.

Type species: Blabericola migrator n. comb. (=*Lei. migrator* Clopton, 1995).

Described species: Blabericola migrator n. comb. (=*Lei. migrator* Clopton, 1995); *Blabericola haasi* n. comb. (=*Lei. haasi* Clopton and Hays, 2006; =*Gregarina haasi* Geus, 1969); *Blabericola cubensis* n. comb. (=*Gregarina cubensis* Peregrine, 1970); *Blabericola princisi* n. comb. (=*Gregarina princisi* Peregrine, 1970).

Etymology: "Blaberid cockroach dweller": G. (m.) "*Blaberus*," noxious; modern: a member of the cockroach genus *Blaberus* + L. "*cola*," to inhabit or dwell within.

Clades IV, V, VI (generic level)*:* These are small internal clades comprised of species that all parasitize beetles (Insecta: Coleoptera) and are all putatively members of *Gregarina*. The analysis presented herein associates all putative members of *Gregarina* sensu lato with the Gregarinoidea (Clade XIV), but at least 2 distinct lineages (Clade IX and Clade XI) are included in the genus as it is currently defined. Further systematic analysis requires a priori exclusion of 1 of these lineages from *Gregarina* sensu stricto.

That our notion of *Gregarina* is polyphyletic is no surprise. Levine (1979) noted the tendency to use *Gregarina* as "a collective group name to stand for any gregarine, no matter what its characteristics." *Gregarina* has become a taxonomic lumber room of unrelated forms comprising over 300 described species, a majority of which are poorly or incompletely known and a plurality of which have not been reported since their original description (Levine, 1988; Clopton, 2002). There is little evidence to suggest that the host specificity of gregarines justifies the maxim of "different host-different gregarine" (Levine, 1979, 1988), but host specificity is usually fairly rigid and can extend to developmental stages of a single host species (Clopton et al., 1991, 1992b). Rarely does host utilization extend beyond a host family for members of a single gregarine genus (Corbel, 1968b, 1971, 1973; Levine, 1988; Clopton and Gold, 1996). Nonetheless, putative species of *Gregarina* are reported from hosts ranging across all major arthropod lineages, from acarine mites and crustaceans to members of a least 11 orders (Blattodea, Coleoptera, Collembola, Dermaptera, Diptera, Embioptera, Ephemeroptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanura) of insects (Levine, 1988; Clopton, 2002). Given general trends of gregarine host specificity, the reported host range of *Gregarina* is a klaxon warning of polyphyly.

When an existing generic taxon is polyphyletic, a sensu stricto definition of the taxon should be based on lineal placement of the type species. Admittedly pragmatic, this strategy reduces the number and complexity of subsequent nomenclature acts, increasing overall nomenclatural stability. In this case, *Gregarina ovata*, a parasite of the European earwig, *Forficula auricularia* (Insecta: Dermaptera), is the type species of *Gregarina* but is not included in this analysis. The analysis does include *Gregarina tropica*, a congeneric species that infects a different species of earwig, *V. brunneipennis*. *Gregarina ovata* and *Gre. tropica* share similar life history and morphological traits (Clopton et al., 2008a) and both are parasites of earwigs, an indication that these species share a closer relationship with each other than either shares with another species in the current analysis. Thus *Gre. tropica* serves as a proxy for placing the type of *Gregarina* within Clade IX and the members of Clades IV–VI cannot belong to *Gregarina* sensu stricto.

Clade IV includes 2 species, *Gre. cuneata* and *Gre. basiconstrictonea*, that parasitize the grain-infesting beetles, *Te. molitor* and *Trib. castaneum*, respectively (Ghose, Sengupta, and Haldar 1986; Clopton et al., 1991, 1992). Clade VI includes 3 additional species that also infect beetles infesting stored grain products (Clopton et al., 1991, 1992; Janovy et al., 2007): *Gre. niphandrodes* and *Gre. polymorpha* from adult and larval *T. molitor*, respectively, and *Gre. cloptoni* from *Trib. freemani*. More than a dozen gregarine species representing at least 4 genera are reported from tenebrionid beetles infesting stored grain products (e.g., Ishii, 1914; Watson, 1916; Hoshide, 1951; Geus, 1969; Lipa, 1967; Golemansky and Duhlinska, 1982; Ghose, Ray, and Haldar, 1986, Ghose, Sengupta, and Haldar, 1986, Ghose et al., 1987; Ghose and Haldar, 1987, 1989; Roy, 1989; Clopton et al., 1991; Sengupta, 1991; Clopton et al., 1992; Watwood et al., 1997; Janovy et al., 2007). Of those ascribed to the genus *Gregarina* only the 2 species forming Clade IV are characterized by a spatulate protomerite. They are distinguished by differences in deutomerite and oocyst size and morphology. Similarly, those species forming Clade VI differ within their group primarily in overall size, deutomerite morphology, and oocyst size and morphology. No member of Clade V or VI has a spatulate protomerite. The species comprising Clade V, *Gre. coronata* and *Gre. diabrotica*, are parasites of adult chrysomelid

beetles. Although morphologically similar to each other and united by the presence of an apical hyaline crown (Watson, 1915; Clopton et al., 1992a) absent in members of Clades IV and VI, *Gre. coronata* and *Gre. diabrotica* are readily distinguished one from the other by differences in oocyst size. Gregarines in Clade V are also united by a persistent epimerite that remains apparent and functional until the onset of syzygy (Théodoridès, 1988), whereas gregarines in Clades IV and VI shed the epimerite much earlier in development. Although identified with the use of molecular characters alone, all 3 clades are supported by morphological characters, primarily gamont or trophozoite morphology. Within each group, species are most readily distinguished with the use of oocyst morphometrics; thus oocysts appear to provide the most reliable diagnostic characters at the species level within these clades, whereas gamont/trophozoite morphology provides diagnostic characters at the clade level. Clades IV–VI probably constitute unique genera, but existing literature data are insufficient to recognize and separate the mix of genera comprising *Gregarina* sensu lato and such a task is well beyond the scope of the current work. To facilitate the systematic analysis herein, I will refer to the species of *Gregarina* in Clade IX as *Gregarina* group 1 (*Gregarina* sensu stricto) and refer to those in Clades IV–VI as *Gregarina* group 2.

Clade VII (family level)*:* This clade unites interior generic Clade I (*X. triplogemmatus*, *X. ellisi*, and *Sty. giganteus*) with *Co. watsonae*, a parasite of the ametabolic thysanuran, *Lep. saccharina*. This clade is consistent with the Stylocephalidae: All species in the analysis are currently placed within Stylocephalidae and demonstrate the diagnostic characters of the family (Clopton, 2002). This is a monophyletic group united by oocyst and gametocyst characters. Gametocysts darken from white to dark brown or black as they mature and carry a papyriform epicyst or secondary gametocyst wall that is uniquely dry and paperlike with a mammelated surface. Their gametocysts dehisce by simple rupture of the gametocyst and epicyst walls. Oocysts are uniquely gibbous (obtuse tetrahedriods; axially asymmetric, broadly deltoid in lateral aspect), dark brown in color, and released in monete chains. These chains are neither expulsed nor extruded: rupture of the epicyst and gametocyst walls simply exposes the oocyst chains, which expand and disperse in response to humidity, air movement, and mechanical disturbance (Ellis, 1912, 1913; Adams and Travis, 1935; Crusz, 1960; Clopton, 1999, 2006). Current definitions of the family include epimeritic characters (Chakravarty, 1959, Levine, 1988, Clopton, 2002) but gametocyst, dehiscence, and oocyst characters are the diagnostic characters of the family. The generalized epimeritic characters of the current family definition are of little use in distinguishing the family but significant use in distinguishing genera and species within the group.

Clade VIII (family level)*:* This is a monophyletic clade consistent with the current family Actinocephalidae (Clopton, 2002). All species within the clade share the gametocyst and oocyst characters of Actinocephalidae: association syzygial, frontal; gametocysts with a persistent hyaline epicyst; gametocyst dehiscence by simple rupture; oocysts released singly by simple exposure. The clade includes 3 species currently placed within Actinocephalidae: *Hop. acanthatholius*, *Pri. evansi*, and *Ge. manifestus*, all parasites of aquatic odonates. Clade VII also includes *Paraschneideria metamorphosa*, a parasite of the fungus gnat, *Sci. coprophila*, whose larvae live in water films on decaying vegetation (Oldroyd, 1965). *Paraschneideria metamorphosa* was placed in the Actinocephalidae both on original description (Nowlin, 1922) and first revision (Nieschulz, 1924) but was later placed in the Sphaerocystidae based on the ephemeral nature of the protomerite and superficial resemblance of the trophozoites and gamonts of *Schneideria* and *Paraschneideria* (Chakravarty, 1959; Levine, 1985, 1988). Sphaerocystidae was erected to include a series of genera clearly constituents of the Stenophoroidea on the basis of gametocyst structure, oocyst dehiscence, and form of the association, but possessing trophozoites and gamonts with no obvious affinities to other known genera (Chakravarty, 1959). As defined, the family is not valid because no member genus reflects the cardinal characters of Stenophoroidea. Placement of genera within the family based on secondary loss of the protomerite–deutomerite septum is invalid because this is not a unique character. Secondary loss of the protomerite–deutomerite septum occurs in various other members of the Stenophoroidea and several members of Gregarinoidea. Sphaerocystidae currently comprises 4 genera: *Sphaerocystis*, *Neoschneideria*, *Schneideria*, and *Paraschneideria*. *Neoschneideria*, *Schneideria*, and *Paraschneideria* all share the diagnostic gametocyst and oocyst characters of Actinocephalidae: association syzygial, frontal; gametocysts with a persistent hyaline epicyst; gametocyst dehiscence by simple rupture; oocysts released singly by simple exposure (cf. Nowlin, 1922; Hoshide, 1959; Ormières et al., 1965). None is

consistent with an existing actinocephalid genus. Not entering into generic synonymy within Actinocephalidae, *Neoschneideria*, *Schneideria*, and *Paraschneideria* are hereby removed from Sphaerocystidae and placed within the family Actinocephalidae.

Sphaerocystis is the type genus of Sphaerocystidae and comprises 4 species described from termites. The morphology of member species (e.g., Desai and Uttangi, 1962; Kalavati, 1979; Kalavati and Narasimhamurti, 1980) is unique within Stenophoroidea and serves to delimit the family as follows.

Sphaerocystidae (revised diagnosis)

Stenophoroidea. Epimerite simple, globular, labile; trophozoites and gamonts subspherical to spherical; limits of protomerite and deutomerite septum marked by distinct differences in cytoplasmic density and granularity, protomerite deutomerite septum ephemeral; oocysts ovoid. Monotypic. Type genus: *Sphaerocystis*, 4 described species.

Clade VIII confirms the monophyletic nature of the Actinocephalidae and the signature gametocyst and oocyst characters of the family: association syzygial, frontal; gametocysts with a persistent gelatinous epicyst; gametocyst dehiscence by simple rupture or dissolution; oocysts released singly by simple exposure. There is little internal resolution for the clade, but all members are parasites of aquatic insects. Additional taxon sampling is required to test the existing subfamilial classification of the Actinocephalidae.

Clade IX (family level)*:* This clade comprises gregarines that all parasitize paurometabolic insect hosts. Molecular sequence data demonstrate a monophyletic group, but there is little internal resolution, suggesting that taxon sampling in this clade is too sparse to resolve internal relationships. The clade is not consistent with the current gregarine classification in that it includes representatives of 3 genera: *Gregarina*, *Leidyana*, and *Amoebogregarina*, which all are either the type species or near-type proxy. This clade probably represents a mix of genera whose individual monophyly and relationship cannot be resolved without additional taxon sampling. All members of the clade demonstrate the diagnostic characters of Gregarinoidea (see Clade XIV, below) and includes members of both families Gregarinidae and Leidyanidae, but the presence of Clades X and XI suggest the existence of heretofore unrecognized families within Gregarinoidea. Clade IX includes *Gre. tropica* and *Lei. erratica*, the type species and proxy type species for their respective genera in this analysis (see discussion of Clades IV, V, VI, above), thus I will refer to the genera in Clade IX as Gregarinidae–Leidyanidae and to those in Clade XI as Gregarinidae group 2. Clade X represents a distinct family, recognized below.

Clade X (family level)*:* This is a monophyletic clade that includes 2 monophyletic genera whose members parasitize blaberid cockroaches: *Protomagalhaensia* and *Blabericola*. This clade is united by gamontic association. Species comprising *Protomagalhaensia* and *Blabericola* exhibit caudofrontal gamontic association, character states present in no other genus within Gregarinoidea, and warrant recognition of a new family as follows.

Blabericolidae n. fam.

Gregarinoidea with caudofrontal gamontic association. Type genus: *Blabericola*, 5 described species. Other genera: *Protomagalhaensia*, 4 described species.

Clade XI (family level)*:* This clade comprises the 3 internal clades (Clades IV–VI) of Gregarinidae group 2 (see Clade IX, above). They probably represent a series of unrecognized genera comprising an unrecognized family, but existing literature data are insufficient to recognize and delineate these taxa here within. Additional taxon sampling for morphological, life cycle, and molecular characters is required to resolve the generic and familial status of this clade.

Clade XII (superfamily level)*:* This clade is a monophyletic group comprising 2 internal clades representing the families Stylocephalidae and Actinocephalidae. Both families are currently placed within the superfamily Stenophoroidea but are unique among Stenophoroidea in that only members of Stylocephalidae and Actinocephalidae possess gametocysts that dehisce by simple rupture to liberate their spores by simple exposure. Although "simple rupture" is reported for other families comprising Stenophoroidea, a review of the literature suggests that genera comprising these families are primarily composed of species for which the method of gametocyst dehiscence is either expulsion of oocysts in polyete chains by an internal gametocyst residuum (Monoductidae: *Monoductus*, Ray and Chakravarty [1933]; *Stenoductus*; Ramachandran [1976a, 1976b]), rupture of a gametocyst valve and expulsion of oocysts in monete chains by an internal gametocyst

residuum (Trichorhynchidae, *Trichorhynchus*, Ormières et al. [1977]; Dactylophoridae, *Dactylophorus*, Ormières et al. [1977]), or expulsion of unchained oocysts en masse in response to hyaline epicyst constriction or an internal gametocyst residuum (Stenophoridae, *Stenophora* [personal observation]; Sphaerocystidae, *Sphaerocystis*, Desai and Uttangi [1962], Kalavati and Narasimhamurti [1980]; Amphiplatysporidae, *Amphiplatyspora*, Kundu and Haldar [1984]; Cnemidiosporidae, *Cnemidiospora*, Schneider [1882]). Actinocephalidae and Stylocephalidae form a monophyletic clade at the level of the superfamily as follows.

Stylocephaloidea new superfamily

Association syzygial; frontal or frontolateral. Gametocysts with gelatinous or papyriform epicyst, dehiscence by simple rupture or dissolution; oocysts liberated singly or in monete chains by simple exposure.

Recognition of Stylocephaloidea begs redefinition of Stenophoroidea and recognition of 2 additional superfamilies based on method of gametocyst dehiscence. A revised definition of Stenophoroidea follows (see Clade XIII, below), but recognition of additional superfamilies within Stenophoroidea requires additional taxon sampling to provide the necessary morphological and molecular data and is beyond the scope of the work presented herein.

Clade XIII (superfamily level)*:* This clade includes 2 species, *Stenop. robusta* and *Pyxinia crystalligera*, placed within the current families Stenophoridae and Actinocephalidae (Clopton, 2002). There is little overlap in host utilization among these genera: Species comprising *Stenophora* are parasites of centipedes (Myriapoda: Chilopoda) and millipedes (Myriapoda: Diplopoda) whereas species of *Pyxinia* are parasites of beetles, primarily members of the family Dermestidae. Nonetheless, *Stenophora* and *Pyxinia* form a well-supported monophyletic clade corresponding to the superfamily Stenophoroidea sensu stricto. *Stenophora* is the nominate genus of both the family and superfamily, demonstrating the following diagnostic characters of the Stenophoridae: association syzygial, laterocaudalfrontal (Rodgi and Ball, 1961; Amoji, 1977); gametocysts with hyaline epicyst, dehiscence by expulsion of single oocysts en masse by internal pressure of gametocyst residuum (Tuzet et al., 1957). In the current gregarine classification, *Pyxinia* is placed within the Actinocephalidae but demonstrates superfamily-level diagnostic characters consistent with Stenophoroidea rather than Stylocephaloidea as follows: association syzygial, frontal, gametocyst with hyaline epicyst, dehiscence by expulsion of oocysts in tetrete chains by internal pressure of gametocyst residuum (Vincent, 1922; Kozloff, 1953, 1958; Dunkel and Boush, 1968; Geus, 1969; Hall et al., 1971). Thus *Pyxinia* is removed from Stylocephaloidea: Actinocephalidae and placed provisionally within Stenophoroidea: Monoductidae because of similarities in gametocyst structure, dehiscence, and oocyst liberation. Clade XIII is monophyletic and represents the superfamily Stenophoroidea, revised as follows:

Stenophoroidea (revised diagnosis)

Association syzygial; frontal or laterocaudofrontal. Gametocysts with hyaline epicyst, dehiscence by expulsion of single oocysts en masse by internal pressure of gametocyst residuum, expulsion of oocysts in polyete chains by an internal gametocyst residuum; rupture of a gametocyst valve and expulsion of oocysts in monete chains by an internal gametocyst residuum, or expulsion of unchained oocysts en masse in response to hyaline epicyst constriction or an internal gametocyst residuum.

Clade XIV (superfamily level)*:* A monophyletic group comprising 3 major clades that together correspond to the Gregarinoidea, revised as follows.

Gregarinoidea (revised diagnosis)

Association presyzygial (trophic or gamontic); caudofrontal. Gametocysts with hyaline epicyst, dehiscence by extrusion through spore tubes; oocysts liberated in monete chains.

Clades XV and XVI represent the in-group and outgroup taxa of the analysis, respectively. Clade XV comprises the monophyletic Septatorina. Nomenclatural acts committed herein are summarized in Table 2.

Correlation of molecular and cardinal morphological characters

The revised gregarine systematic arrangement presented in Figure 3 is based on correlation of molecular and morphological characters to facilitate clade recognition at meaningful ranks. Morphological characters are mapped on the phylogenetic topology reduced to a cladogram in Figure 4.

The Septatorina are a monophyletic group characterized by syzygial association and gametocysts with a distinct, functional epicyst. The Septatorina com-

Table 2. Summary of nomenclatural and taxonomic acts.

Taxon	Act
Stenophoroidea	Emendation, revised diagnosis
Gregarinoidea	Emendation, revised diagnosis
Stylocephaloidea n. supfam.	New superfamily
Sphaerocystidae	Revised diagnosis
Blabericolidae n. fam.	New family
Blabericola n. gen.	New genus
Blabericola princisi n. comb. (=*Gregarina princisi* Peregrine, 1970)	New combination
Blabericola cubensis n. comb. (=*Gregarina cubensis* Peregrine, 1970)	New combination
Blabericola haasi n. comb. (=*Leidyana haasi* Clopton and Hays, 2006; =*Gregarina haasi* Geus, 1969)	New combination
Blabericola migrator n. comb. (=*Leidyana migrator* Clopton, 1995)	New combination
Neoschneideria	Removed from Sphaeorcystidae
Neoschneideria	Placed in Actinocephalidae
Paraschneideria	Removed from Sphaeorcystidae
Paraschneideria	Placed in Actinocephalidae
Protomagalhaensia	Removed from Hirmocystidae
Protomagalhaensia	Placed in Blabericolidae
Pyxinia	Removed from Stylocephaloidea: Actinocephalidae
Pyxinia	Placed in Stenophoroidea: Monoductidae
Schneideria	Removed from Sphaeorcystidae
Schneideria	Placed in Actinocephalidae

prise at least 3 superfamilies distinguished by their method of gametocyst dehiscence.

The Stylocephaloidea are a basal monophyletic group within Septatorina characterized by gametocysts that dehisce by simple rupture, that is, by simple splitting or dissolution of the gametocyst and epicyst wall. Oocysts are liberated by simple exposure: They are not forcibly expelled or extruded from the gametocyst proper. The Stylocephaloidea comprises 2 families distinguished by the form of the epicyst, the form of association, and the formation of oocyst chains. The Stylocephalidae are characterized by a papyriform, mammelated epicyst that ruptures with the gametocyst by cracking or splitting. Gamonts associate frontally just prior to syzygy. They have uniquely gibbous oocysts that are released in monete chains. Monete chains are not a unique character of the Stylocephalidae, having arisen separately among the Gregarinoidea. The Actinocephalidae are characterized by a gelatinous epicyst that ruptures with the gametocyst by dissolution. Gamonts associate frontolaterally just prior to syzygy. Their oocysts are released singly rather than in monete chains.

The Stenophoroidea comprise those septate gregarines whose gametocysts dehisce by expulsion of oocysts through a break in the gametocyst and epicyst walls. Their gametocysts possess a hyaline epicyst that contributes to the internal pressurization of the gametocyst for expulsion. Oocysts may be released in polyete chains or singly, en masse. Gamonts associate frontally or laterocaudofrontally just prior to syzygy. Stenophoroidea are not well represented in the current analysis and additional taxon sampling may resolve additional families within the existing group. They form a larger clade with the Gregarinoidea, apparently based on the hyaline nature of the epicyst, but their relationship to both Stylocephaloidea and Gregarinoidea cannot be ascertained without additional taxon sampling.

The Gregarinoidea comprise a monophyletic group whose gametocysts possess hyaline epicyst walls and dehisce by extrusion of oocysts in monete chains because of internal pressurization of the gametocyst. Extrusion is through spore tubes, although the number and size of the spore tubes themselves appears to be of limited taxonomic value. The group is characterized by presyzygial association that is caudofrontal in form. Gregarinoidea includes at least 3 family level groups: Gregarinidae groups 1 and 2, and Blabericolidae. Blabericolidae are characterized by gamontic association. Gregarinidae itself is polyphyletic, including 2 lineages characterized by trophozoitic association. Gregarinidae group 1 comprises *Leidyana* sensu stricto, *Gregarina* sensu stricto, and perhaps at least 1 unrecognized genus, but the relationship between these taxa cannot be resolved without additional taxon sampling. Gregarinidae group 2 probably comprises a series of heretofore unrecognized genera, but again, additional taxon sampling is required to sort out relationships

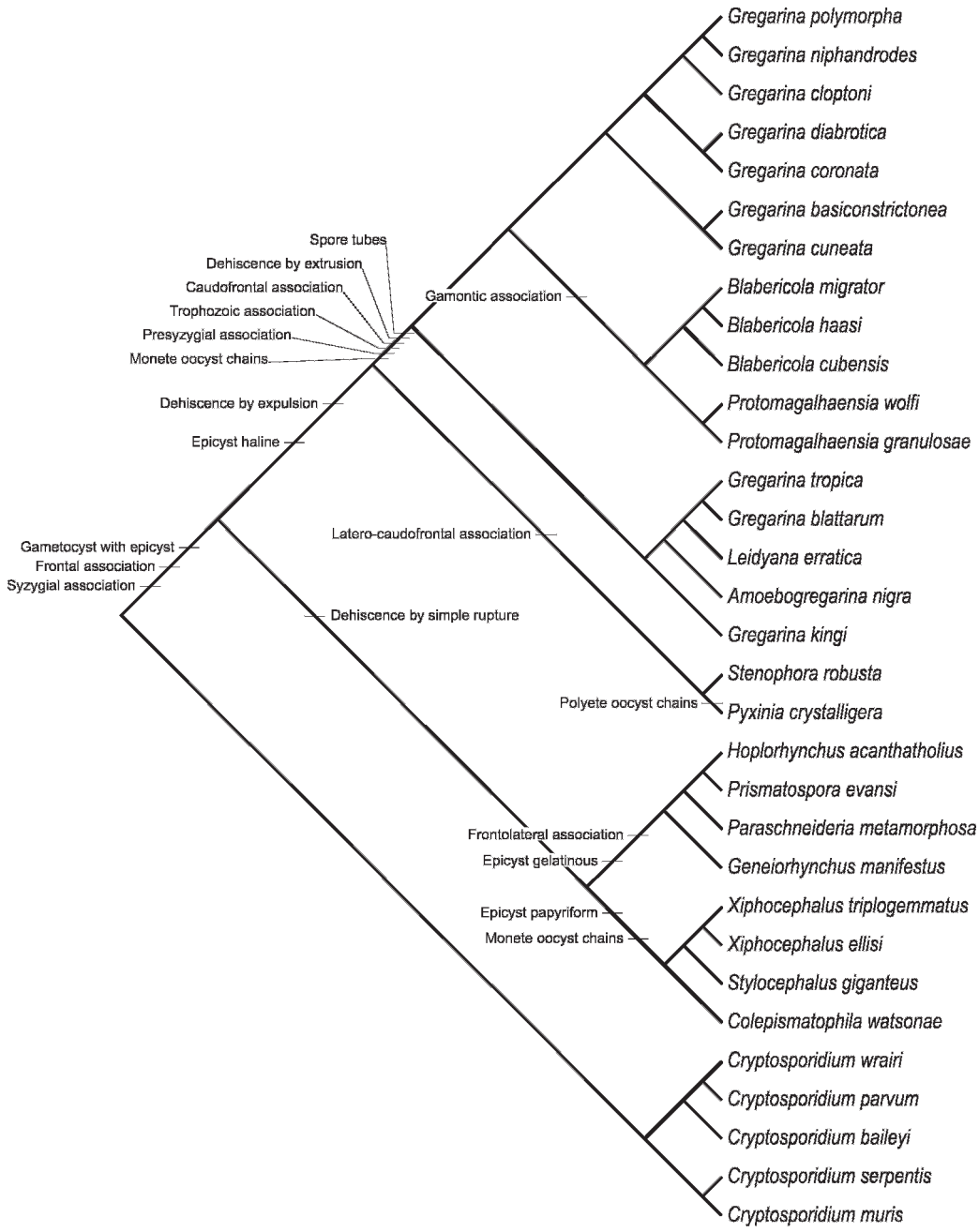

Figure 4. Suprageneric morphological characters correlating with the consensus molecular phylogeny of 27 taxa of septatorinid gregarines.

within the clade. The exact nature of the relationship between Gregarinidae group 1 and Gregarinidae group 2 also remains unclear. Given the diagnostic value of oocyst-derived characters in other clades, morphometric support for groups within Gregarinidae groups 1 and 2 likely will require new comparative studies of oocyst morphology with a standard shape nomenclature (Clopton, 2004).

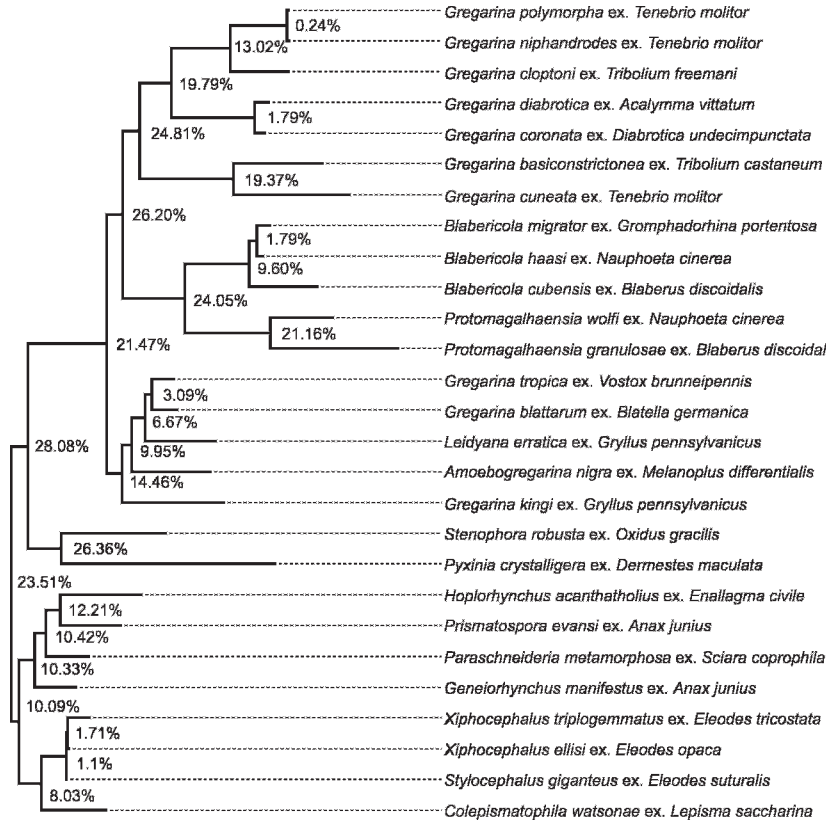

Figure 5. Consensus phylogeny of 27 taxa of septatorinid gregarines showing average pair wise sequence variation within nodes.

Sequence analysis

Figure 5 presents average pairwise sequence variation for all nodes of the consensus topology. The taxon sample size of the current analysis is small relative to the number of described species, genera, and families of Septatorina, yet some general trends in ssu rDNA pairwise sequence variation are worth noting. Sibling species seem to vary by less than 2% in most cases. Variation among *Pro. wolfi* and *Pro. granulosae* is much higher at 21%, but their hosts are Ethiopian and Neotropical in origin, respectively, and thus their allopatry is probably measured on tectonic time scales. As a general trend, average pairwise sequence variation between 10 and 20% seems to correlate with differences among clades at a generic level, whereas average pairwise sequence variation exceeding 20% appears to correlate with differences among clades at the family level.

Leander, Clopton, and Keeling (2003) reported ssu rDNA sequence variation of as much as 6% among isolates of *Monocystis agilis* and suggested that low pairwise sequence variation points to a problem with the underlying taxonomy of *Gre. niphandrodes*. As with Leander, Clopton, and Keeling (2003), the sequence variation observed herein among *Gre. polymorpha* and *Gre. niphandrodes* is very low (0.24%). However, sequence variation is low (<2%) for all cases of potential sibling species in the current analysis (Fig. 5). The analysis presented herein suggests that the Leander, Clopton, and Keeling (2003) benchmark for variation among isolates of *Monoc. agilis* is unusually high and probably reflects congeneric rather than conspecific sequence variation among *Monoc. agilis* isolates in GenBank. Morphological methods underlying aseptatorinid taxonomy are not as well developed as those in use for Septatorina. A taxonomic problem almost certainly exists, but it involves identification of *Monoc. agilis* rather than *Gre. niphandrodes*.

Patterns of gregarine radiation

Figure 6 presents patterns of host habitat use, host metamorphic pattern, and host-stadium specificity

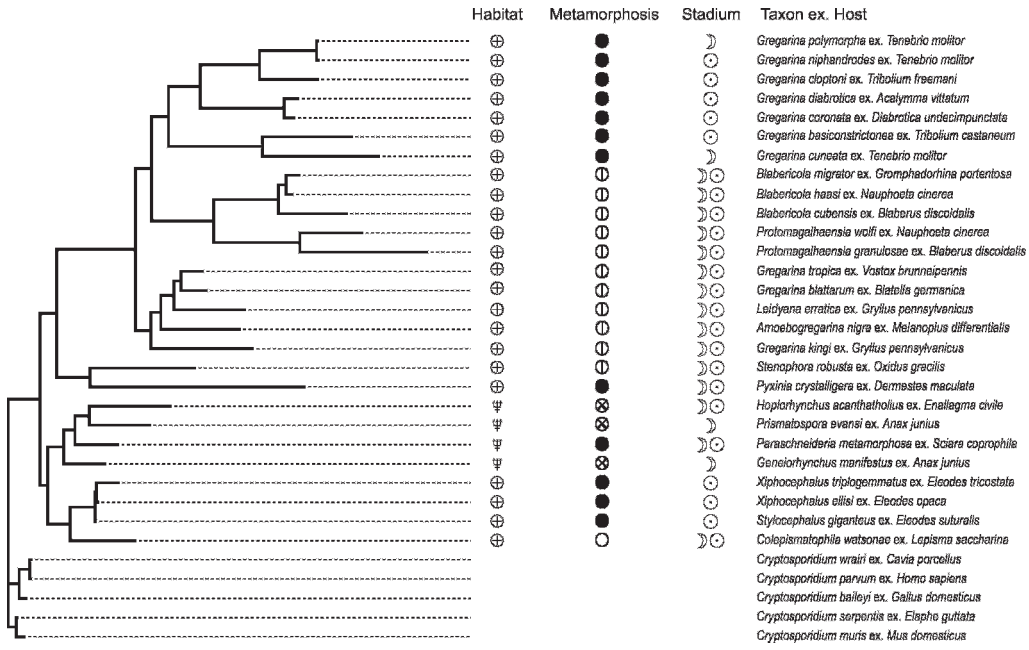

Figure 6. Patterns of host habitat use, host metamorphic pattern, and host stadium specificity juxtaposed against the consensus phylogenetic topology for 27 taxa of septatorinid gregarines (terrestrial, ⊕; freshwater, Ψ; ametabolic, ○; paurometabolic, ◑; hemimetabolic, ⊗; holometabolic, ●; adult, ⊙; juvenile, ☽).

juxtaposed against the consensus phylogenetic topology for the 27 gregarine taxa included in this analysis.

Host habitat and gregarine radiation: As basal apicomplexans in a larger Alveolate clade (Barta et al., 1991; Carreno et al., 1999; Wolters, 1991), the eugregarines are of marine origin (Perkins et al., 2002) although the exact phylogenetic nature of their relationship to marine aseptate gregarines and archigregarines remains unclear (Leander, Clopton, and Keeling, 2003; Leander, Harper, and Keeling, 2003; Leander and Keeling, 2004; Leander, 2007). Given a marine origin within the alveolates, the initial radiation of septatorinids was driven by differential functional responses of the gregarine epicyst to environmental conditions in the host habitat (cf. Figs. 3–6). Stylocephaloidea includes lineages in which the epicyst functionally mediates the environment, either by protecting the gametocyst from colonization by other microorganisms (Actinocephalidae) or retaining moisture in a xeric environment (Stylocephalidae). In contrast, the epicysts of Stenophoricae and Gregarinoidea do not appear to buffer environmental conditions, but functionally mediate oocyst dispersal in terrestrial environments. The older stylocephaloidid radiation reflects both an initial colonization of terrestrial ametabolic hosts such as thysanurans followed by a radiation in coleopterans (Stylocephalidae) and the origin of the single sweet-water lineage (Actinocephalidae) within Septatorina, but the order of sweet-water and terrestrial invasion is not resolved by the current analysis. This initial septatorinid colonization was followed by a general radiation of Stenophoroidea and Gregarinoidea through terrestrial hosts.

Host metamorphosis and gregarine radiation: There is a striking correlation between host metamorphic pattern, host-stadium specificity, and the radiation of associated gregarine groups. Radiation within family-level clades correlates with particular modes of host metamorphosis and observed levels of host-stadium specificity, the restriction of a parasite not only to a single host but to a single metamorphic life-cycle stage of a single host species (Clopton et al., 1992b). Evolutionarily, gregarine species track niche resources along lines of transmission: They do not necessarily track host species in evolutionary time.

In the current analysis gregarines parasitizing holometabolic hosts comprise the entirety of Gregarinidae group 2 and the plurality of Stylocephalidae. These clades are comprised entirely of coleopteran

hosts, which have been colonized multiple times, reflecting the hyperdiversity of beetles in the insect world. Given the numerical and taxonomic ubiquity of beetles, a host capture attempt probabilistically is more likely to be in a beetle than in all other insect orders combined. All of these gregarines are host-stadium specific, infecting exclusively either adults or larvae of a single host species. As a general trend, adult and larval stages in a holometabolic life cycle exploit different niches or at least different resource bases within a niche. Adults and larvae generally do not compete for resources and thus holometabolic life histories provide separate transmission routes for gregarine parasites. Niche or resource base specialization based on host stadium appears to provide a mechanism for gene pool isolation and gregarine diversification.

In contrast to gregarines parasitizing holometabolic hosts, Gregarinidae group 1 and Blabericolidae comprise gregarines that parasitize paurometabolic hosts. As a general trend, adult and larval stages in a paurometabolic life cycle exploit the same niche and resource base. All life-cycle stages of a paurometabolic host are subject to the same gregarine transmission mechanisms and routes, thus these gregarines exploit both larval and adult stages in the host life cycle.

Hemimetabolic insect life cycles are found only in truly aquatic insect orders. In the analysis presented herein, hemimetabolic hosts are exploited only by the Actinocephalidae. Patterns of host-stadium specificity are more complex in this group. Host-stadium specificity is observed in species of *Prismatospora* and *Geneiorhynchus*, which infect dragonfly naiads (Odonata: Anisoptera) but not in species of *Hoplorhynchus*, which infect damselfly naiads and adults (Odonata: Zygoptera). As a general trend, anisopterans are strong fliers with comparatively large home ranges, and zygopterans are weak fliers with small home ranges. Adult anisopterans are rarely infected, probably because they are exploiting a different, perhaps nonaquatic, resource base than are their naiads. Although physiologically hemimetabolic, from a gregarine's point of view anisopteran hosts are holometabolic ecological actors, whereas zygopteran hosts are hemimetabolic ecological actors.

The notable exception to these patterns is *Paraschneideria metamorphosa*, an actinocephalid gregarine parasitizing a holometabolic host, the nematoceran fungus gnat *Sci. coprophila*. The life cycle of *P. metamorphosa* is described in some detail by Nowlin (1922) and is synchronized to host metamorphosis in a profound way unknown in any other gregarine. Briefly, trophozoites occur in larval gnats but do not associate: they mature, migrate to the posterior midgut, and await pupation. When the larvae enters pupation, the gamonts associate but do not form gametocysts until pupation is almost complete. Gametocysts occur and mature in the intestine of the adult gnat. This life cycle synchronization makes a physiologically holometabolic host act as a ecologically paurometabolic host. *Paraschneideria metamorphosa* is also notable because the species represents a secondary terrestrial invasion by Actinocephalidae. Fungus gnat larvae are not truly aquatic, but are restricted to substantial water films on decaying terrestrial vegetation. No gregarine life cycle from another nematoceran host is known. Synchronization may be the normal mode for actinocephalid gregarines infecting nematocerans rather than a special adaptation for secondary terrestrial invasion.

Vicariant and ecotypic assemblages: Parasitology has explored the fundamental nature and organizing principles of parasite communities and parasite community structure for almost 40 years. Controversy has revolved around the degree to which coevolutionary phylogeny and ecological fitting determine the members of a parasitic assemblage (see Zelmer and Platt, 2008 and references therein), but few systems amenable to experimental manipulation are available to test these ideas (see Janovy [2002] and references therein for a review of major issues). The septatorinid phylogeny presented herein suggests that gregarine assemblages within a single host species may have their origins either as vicariant assemblages, (i.e., as products of co-evolutionary, phylogenetic effects), or ecotypic assemblages, (i.e., as products of ecological fitting and host switching).

The Blabericolidae appear to include a series of vicariant assemblages. The clade includes gregarines infecting 3 cockroach species. The Madagascar hissing cockroach, *Gro. portentosa*, is native to Madagascar and hosts *Blaberi. migrator* but no protomagalhaensid gregarine. The lobster cockroach, *Na. cinerea*, originated in tropical sub-Saharan Africa and subsequently dispersed via trade routes to Madagascar, the Mascarenes, the Near East, Asia, Mexico, and subtropical portions of the eastern United States. *Nauphoeta cinerea* hosts *Pro. wolfi* and *Blaberi. haasi*. The discoid cockroach, *Blaberu. discoidalis*, is Central American in origin and hosts another protomagalhaensid–blabericolid pair: *Pro. granulosae* and *Blaberi. cubensis*. The phylogeny

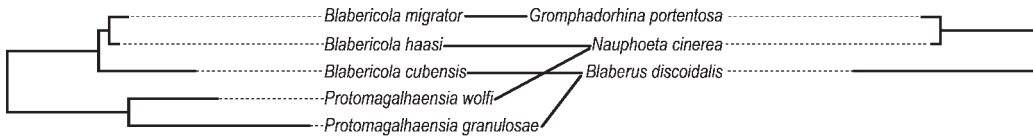

Figure 7. Phylogenetic congruence of the Blabericolidae (left) with their blaberid cockroach hosts (right; cockroach phylogeny based on Kambhampati [1995] and Inward et al. [2007]).

of *Blabericola* species is congruent with the phylogeny of their hosts (Fig. 7), as postulated with the use of sequence data from 6 different mitochondrial and genomic gene loci in 2 separate analyses (Kambhampati, 1995; Inward et al., 2007). Assemblages within each cockroach species appear to be products of a vicariant process, namely, parallel, sequential speciation, and radiation subsequent to radiation and speciation in the host lineage. Peregrine (1970) reports another protomagalhaensid–blabericolid pair: from the Bolivian cockroach, *Blaberus boliviensis*, *Protomagalhaensia blaberae* and *Blaberi. princisi*, offering the opportunity for an additional test of the vicariant assemblage pattern.

The gregarine parasites of the yellow mealworm, *Te. molitor*, are an ecotypic assemblage that probably arose as a result of common habitat utilization rather than host diversification. *Gregarina niphandrodes*, *Gre. cuneata* and *Gre. polymorpha* all parasitize *Te. molitor*, but with strict host-stadium specificity (Clopton et al., 1992b). *Gregarina niphandrodes* is restricted to adult beetles, and *Gre. cuneata* and *Gre. polymorpha* are restricted to beetle larvae. If the assemblage were vicariant in origin, all 3 gregarine species would appear as siblings in the phylogeny. Instead, these gregarines are members of 2 different clades and probably represent 2 distinct genera (Fig. 6). *Gregarina niphandrodes* and *Gre. polymorpha* are siblings, separated by less than 1% sequence variation even though they parasitize difference ontogenetic stadia of *Te. molitor*. Based on branch lengths, *Gre. polymorpha* is the likely descendant sibling, its gene pool having isolated by colonization of larval *Te. molitor*. *Gregarina cuneata* also parasitizes larvae of *Te. molitor*, but belongs to an interior clade with *Gre. basiconstrictonea*, a parasite in the Red Flour Beetle, *Trib. castaneum*. The phylogeny suggests that *Te. molitor* larvae acquired *Gre. cuneata* ecotypically, through common habitat use with other grain beetle species rather than through speciation subsequent to host diversification. Again, gregarine parasites track resources and isolate gene pools along lines of transmission. They do not necessarily track hosts in evolutionary time and often appear to treat holometabolic host species as multiple hosts based on differences in habitat and resource use among host stadia.

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