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Identification of prey of *Myotis septentrionalis* using DNA-based techniques

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Identifying and characterizing trophic linkages is fundamental to understanding how prey availability affects predator behavior, distribution, and density, and to elucidating the extent to which predators regulate prey populations. Vertebrate insectivores, such as bats, are a pervasive top-down force on insect populations in forest ecosystems. Bats are predators sensitive to habitat perturbations, whose prey selection behavior warrants further study. We identified trophic connections between a forest bat (*Myotis septentrionalis*) and its prey by isolating and sequencing cytochrome c oxidase subunit I gene (*COI*) fragments of insect prey obtained from bat fecal samples. Prey identities were inferred using GenBank and the Barcode of Life Data System (BOLD). We also identified prey remains morphologically from the same fecal samples, allowing comparisons across novel (GenBank and BOLD) and traditional (morphological) techniques. Lepidopterans were the most frequently detected prey in all approaches. Detection of common insect orders varied across procedures ($P < 0.001$), suggesting methodological bias at coarse taxonomic resolutions. Of the DNA-based approaches, GenBank provided the more diverse assessment of prey. We reveal trophic linkages for northern bats by identifying 20 prey genera and species using BOLD, more than one-half of which were smaller lepidopterans. The disproportionate number of smaller lepidopteran prey (mean [$\pm SE$] wingspan 24.2 $\pm$ 2.05 mm) exceeded expectations based on field-based assessments. *M. septentrionalis* is known to use both aerial-hawking and gleaning behaviors. Lepidopteran prey consumed by *M. septentrionalis* were smaller than reported for conspecfic genera, suggesting this species differentiates itself from sympatric insectivorous bats with the prey it selects.

Key words: diet, foraging theory, insects, Lepidoptera, molecular scatology, predator–prey interactions, trophic interactions

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Delineation of trophic linkages between predators and prey is fundamental to understanding how species interact at the population level. In particular, understanding foraging ecology of predators is integral to elucidating their role in regulating prey populations and how prey availability potentially affects predator density and distribution (Holling 1961). Predators are often cryptic, so direct observation of predation events may not be possible (Sheppard and Harwood 2005). Even if direct observations are possible, frequency with which predation events are observed is often extremely low (e.g., Jackson 1977; Reddy and Fenton 2003). Aerial foraging activity and nocturnal habits make insectivorous bats an elusive group in which to characterize trophic linkages. Current knowledge of prey selection by these predators and, thus, a framework for testing hypotheses of optimal foraging is largely limited to a coarse resolution because most studies rely on morphological identification of undigested, chitinous fragments of exoskeleton present in feces or the digestive tract (Jones and Rydell 2003; Lacki et al. 2007a; Whitaker 1988). Given global ubiquity of insects and their ecological significance, as well as the primary role that predators such as bats and birds play in depredating insects, these trophic linkages are important and in need of increased depth and breadth of study (Brigham 2007; Gonzalez-Gomez et al. 2006; Williams-Guillén et al. 2008).

Relative consumption of different insect taxa varies geographically across bat species (Brigham 2007; Jones and Rydell 2003; Lacki et al. 2007a). Despite evidence of specialization and variation, knowledge of prey selection by insectivorous bats is largely limited to the ordinal level (Jones and Rydell 2003; Lacki et al. 2007a; Whitaker 1988). Given

that the prey available to insectivorous bats varies within and among landscapes (Burford et al. 1999; Dodd et al. 2008; Morris et al. 2010; Wickramasinghe et al. 2004), and that foraging behavior and habitat use are correlated with bat morphology (Freeman 1981; Norberg and Raynor 1987), it is likely that many bat species follow predictions of optimal foraging theory and select prey in relation to size, nutritional content, abundance, and ease of capture (Barclay 1991; Charnov 1976; Lacki et al. 2007a).

The DNA-based techniques used to detect prey provide valuable insights into cryptic trophic linkages between predators and prey in terrestrial (Harwood et al. 2007; Lundgren et al. 2009; Read et al. 2006) and marine (Deagle et al. 2007, 2009) systems. A foundation for use of DNA-based techniques has been laid for both sanguinivorous (Carter et al. 2006) and insectivorous (Clare et al. 2009, 2011; Zeale et al. 2011) bat species, but further efforts to integrate these techniques into the discipline are warranted, because traditional and DNA-based means have varied resolution and inherent biases (Clare et al. 2011; Lacki et al. 2007a; Whitaker 1988; Zeale et al. 2011).

We used DNA-based techniques to identify specific trophic linkages of the northern bat (*Myotis septentrionalis*; Chiroptera: Vespertilionidae). The objectives of our study were 3-fold. First, we demonstrate a means of extracting and amplifying mitochondrial DNA of prey from fecal samples of field-captured *M. septentrionalis* suitable for food habits analysis, and using web-based searches, identify prey taxa to the genus and species level in the diet of these bats. Species-level identifications may sometimes be made for bats using morphological means either directly (e.g., the spotted cucumber beetle [*Diabrotica undecimpunctata*], heavily eaten by many species of bats—Whitaker 2004) or indirectly (e.g., the golden dung fly [*Scatophaga stercoraria*], eaten by both myotine and plecotine species in Europe—Shiel et al. 1991). However, DNA-based techniques offer direct identification at a resolution greater than that attainable with morphological identification (Brigham 2007), particularly for soft-bodied prey items such as Lepidoptera (J. O. Whitaker, Jr., pers. comm.). Given this, our 2nd research objective was to assess consumption patterns of forest Lepidoptera by *M. septentrionalis* as related to prey abundance and selection. Although Lepidoptera are widely and heavily consumed, consumption rates and patterns of various Lepidoptera are unknown for most bats and birds (Barbaro and Battisti 2010; Lacki et al. 2007a; Wilson et al. 1999). Thus, our null hypothesis was that consumption across available Lepidoptera would be in proportion to abundance (i.e., no prey selection would occur). Third, by sequencing prey DNA from the same fecal samples that we evaluated concurrently using morphological dissection, we compared prey inferences generated using different web-based database searches and that of commonly used microscopic analyses. We investigated methodological bias of approaches to analysis of food habits in insectivorous bats. We expected that in the absence of bias our analyses of food habits would yield comparable results, albeit of varied taxonomic resolution.

Materials and Methods

Study areas and sample collection.—Fecal samples of bats were collected regionally across the central Appalachians of eastern North America, including the Daniel Boone National Forest, Kentucky (38°2′N, 83°35′W), the Raccoon Ecological Management Area, Ohio (39°11′N, 82°22′W), and the Royal Blue Wildlife Management Area, Tennessee (39°11′N, 82°23′W). Sites in Kentucky and Ohio are part of the western Allegheny Plateau, whereas the site in Tennessee is part of the central Appalachians (Omernik's Level III Ecoregions—Griffith et al. 1998; Woods et al. 1998, 1999, 2002). Field collections took place in a matrix of upland forestland actively managed for timber production and used for scientific research.

Bats were captured throughout their active period from March through September of 2007 and 2008 in monofilament nylon mist nets (2.6, 6, and 9 m in length; Avinet, Dryden, New York) placed throughout study areas over flyways formed by roads and road ruts with pooled water, small streams, trails, and ridgelines (i.e., <3 m above ground). Captured bats were handled in accordance with the University of Kentucky Institutional Animal Care and Use Committee (01019A2006), guidelines of the American Society of Mammalogists (Sikes et al. 2011), and state and federal collection permits. Bats were held separately in single-use, disposable cotton bags (20×30.5 cm; Avinet) for approximately 20 min to allow defecation. Fecal samples from individuals were collected into 1.5-ml microcentrifuge tubes, placed on ice or in a mobile freezer ($\sim$0°C, MT17; Engel, Jupiter, Florida), and transferred to long-term freezer storage (–80°C) immediately upon return to the laboratory. At no time were fecal samples allowed to contact any surface other than the single-use cotton bag or the microcentrifuge tube.

Concurrent with mist-net captures of bats, we surveyed forest Lepidoptera across study areas to assess prey abundance during growing seasons of 2007 and 2008. Because *M. septentrionalis* is known to depredate Lepidoptera by both aerial hawking and gleaning (Caceres and Barclay 2000; Faure et al. 1993; Ratcliffe and Dawson 2003), we made no discernment of availability of prey either in flight or at rest. The "true availability" of insect prey to bats is unknown, so we assessed catch of Lepidoptera over entire survey nights as a relative index of abundance (Whitaker 1994). Surveys of prey abundance in each study area were $\leq$2.5 km from mist-net locations. Lepidoptera were surveyed in upland deciduous forests across a continuum of silvicultural disturbance (i.e., unharvested, uneven-aged forest and 3 increasing levels of harvest established at each study site during the dormant season of 2006–2007), emphasizing slope positions known to be used as foraging habitat by *M. septentrionalis* in the central Appalachians (Lacki et al. 2009).

Lepidoptera were trapped using 10-W blacklight traps (Universal Light Trap; Bioquip Products, Rancho Dominguez, California) suspended 2.5 m above ground (Burford et al. 1999; Dodd et al. 2008). Although light traps are biased toward phototactic lepidopteran taxa, they are widely considered the standard technique for sampling lepidopteran assemblages (Covell 2005; Dodd et al. 2008). Consequently, Lepidoptera that were diurnal, not phototactic, or only attracted to bait were not sampled in this study. Fixed survey locations were established for blacklight traps across disturbance gradients, and were chosen to represent habitats under study, habitats potentially used by predator and prey (i.e., flyways and corridors), and accessibility. See Dodd et al. (2012) for additional explanation. Traps were operated through the night and a cotton wad soaked in ethyl acetate was used to kill trapped insects. Lepidoptera were removed the following day, frozen, and returned to the laboratory for identification. Lepidoptera with wingspans $\geq$ 20 mm were identified using available guides (Covell 2005; Holland 1903) and reference collections at the University of Kentucky. Lepidoptera with wingspans $<$ 20 mm were not identified or enumerated due to damage to specimens from the blacklighting technique (Burford et al. 1999; Dodd et al. 2008, 2012).

Screening fecal samples.—Fecal pellets of collected bats were dissected microscopically and prey remains were identified to the most-specific taxon possible (Whitaker 1988). Individual fecal pellets were placed in a sterile pour boat (4.1 × 3.2 × 0.8 cm; Fisher Scientific, Pittsburgh, Pennsylvania), diluted with 100% ethanol, and teased apart using a disposable pestle (Fisher Scientific) for microscopic dissection. We estimated percent frequency of prey items in the diet of individual bats, and estimated the percent volume of prey items in pellets from each bat to the nearest 5%. Up to 3 pellets were dissected from each bat, and values were averaged across pellets to determine percent volumes of prey in the diet (Lacki et al. 2007b). Individual fecal pellets were then preserved in approximately 1.5 ml of 95% ethanol and placed in freezer storage (–80°C) for subsequent DNA-based analysis.

Molecular remains of prey are not homogenous within feces (Deagle et al. 2005). Considering the number of fecal pellets generally yielded by each bat, up to 3 fecal pellets from each individual bat were used for DNA-based analysis to increase the likelihood of accurate and reliable identification of all prey items consumed. The entire pellets used for morphological identification were then used for each individual DNA extraction. Prior to DNA extraction, each fecal sample was homogenized for approximately 1 min in 2.0-ml mortar-and-pestle microcentrifuge tubes, vortexed approximately 1 min, then centrifuged at 20,000 × *g* for 3 min, discarding the resulting supernatant. Afterward, 1 ml of tris-ethylenediamine-tetraacetic acid (TE) buffer was added to samples, which were then vortexed approximately 1 min and centrifuged at 20,000 × *g* for 3 min, and the supernatant was discarded. DNA was then extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California). Protocol was followed according to the manufacturer's instructions for the isolation of DNA from stool for pathogen detection carrying out lysis with the ASL buffer at 70°C, and using all applicable extra centrifugation steps.

Polymerase chain reactions (total volume = 50 μl) for nucleotide sequencing of the cytochrome c oxidase subunit I gene (*COI*) were carried out with C1-J-1859 with C1-N-2191 primers, resulting in a 333-base amplicon (Simon et al. 1994). The polymerase chain reaction cocktail contained 2 μl of template DNA solution of unknown concentration, 1.25 U of Qiagen HotStar Taq polymerase, 0.2 mM of deoxynucleoside triphosphates, 0.25 mM of each primer, 1.5 mM of 10× reaction buffer, and 1 mM of $MgCl_2$ (for a 2.5-mM total concentration of $MgCl_2$). Cycling conditions were 15 min at 95°C, 50 cycles of 50 s at 94°C, 45 s at 45°C, 45 s at 72°C, and a final elongation for 5 min at 72°C. Reaction success was confirmed by electrophoresis of 10 μl of polymerase chain reaction product in 1.0% agarose (Sigma-Aldrich Co., St. Louis, Missouri) in 1× TAE (Promega Corp., Madison, Wisconsin). Sequencing (University of Kentucky Advanced Genetic Technologies Center, Lexington, Kentucky) was carried out for those reactions that yielded strong polymerase chain reaction bands of expected size, using BigDye terminator kits (version 3.1) and the previously mentioned primer set on an ABI3100 sequencer (Applied Biosystems, Foster City, California). Reactions were sequenced bidirectionally to reduce the possibility of chimeric sequences consisting of multiple prey DNA fragments; overlapping forward and reverse sequences were edited and assembled using Vector NTI (version 10.3; Invitrogen Corp., Carlsbad, California). If strong, corresponding signals were not present in forward and reverse chromatographs, such portions of sequences were marked as unidentifiable or discarded if the bulk of a sequence was unknown. We generated a single sequence per fecal pellet.

Prey identification and statistical comparison across techniques.—Prey identities were inferred using web-based searches to compare unknown DNA sequences with the Barcode of Life Data System (BOLD) and GenBank. Using BOLD, species-level identification of unknown sequences was carried out using methods of Clare et al. (2009). We compared our sequences to reference sequences from arthropods present with species-level barcodes in BOLD (Ratnasingham and Hebert 2007) in April 2011. Matches of $\geq$99% similarity between our unknown sequence and a single species in the database were considered close enough to warrant species identification (Clare et al. 2009). Coarser taxonomic identifications of unknown sequences were made in the absence of species-level matches if there was a 100% "probability of placement" within the broader phylogeny indexed by BOLD (and $\geq$98% similarity—Clare et al. 2011). Using GenBank, similarity of unknown sequences was considered using a basic local alignment and search tool (Altschul et al. 1990); the megablast variant was used with the default settings. Identity of prey at the ordinal level was inferred by the closest match generated by this search; ranking

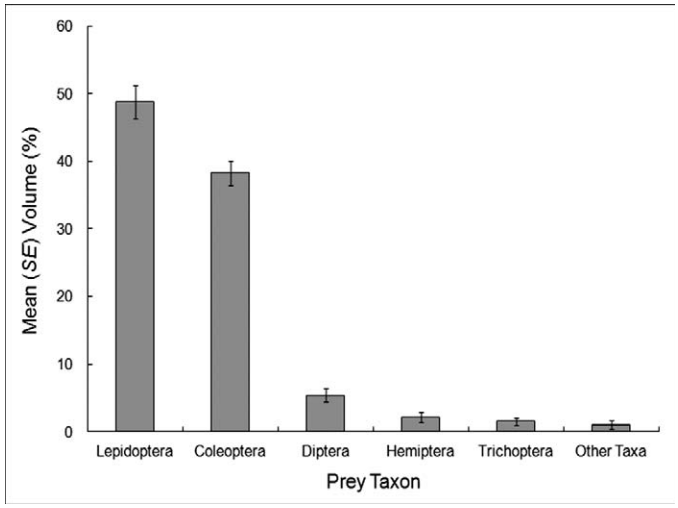

FIG. 1.—Volumes of prey identified in fecal samples of *Myotis septentrionalis* using morphological identification. "Other Taxa" include all taxa with mean volumes < 1% and include Hymenoptera, Neuroptera, and Arachnida.

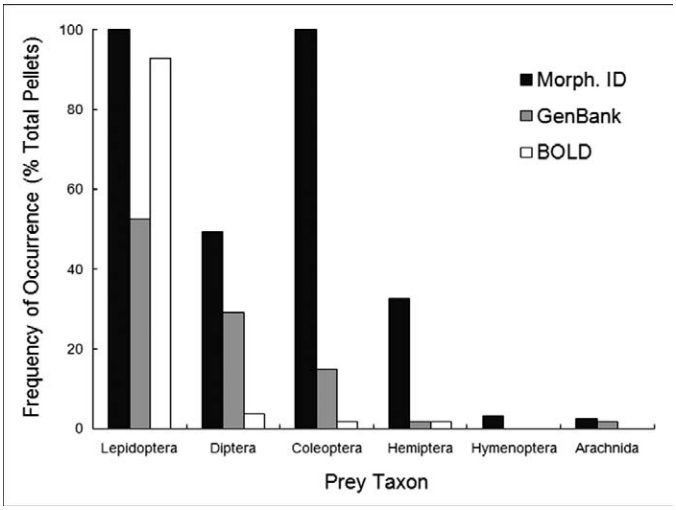

FIG. 2.—Frequency of occurrence of prey taxa in fecal pellets of *Myotis septentrionalis* by identification procedure.

was according to maximum similarity (all were ≥75%) and maximum score parameters.

Order-level data were compared using a 2 × 3 contingency table with a chi-square test of independence (Triola 1986) across the 3 methods of identification (morphological, GenBank, and BOLD) using presence–absence counts across fecal pellets. Separate chi-square tests of independence were conducted for each of the most frequently identified orders of prey (Coleoptera, Diptera, Hemiptera, and Lepidoptera). Calculated expected values were defined as: observations within category (i.e., insect order) × sum of observations across categories/total observations (Triola 1986). Following a significant test value, individual variation of each identification method from the calculated expected value was assessed in terms of contribution (%) toward the overall chi-square test score. This allowed assessment of which identification technique(s) deviated the most from the null hypothesis (observed = expected), permitting evaluation of differences in the frequency of occurrence of prey orders across identification procedures.

At a more-specific resolution, we calculated the mean wingspan for all genera and species of Lepidoptera identified in fecal samples using BOLD. Prey inferences generated with BOLD were used to calculate wingspan values (as opposed to GenBank) because of the precedence for species-level prey inferences reported by Clare et al. (2009). Wingspan values were taken from Covell (2005) and in March 2011 from the *Bug Guide* web-based database hosted by Iowa State University (www.bugguide.net). For taxa for which wingspan values could not be determined (i.e., species and genus not indexed in the source), a wingspan value at a coarser level of taxonomic resolution was used (i.e., family level). As a comparison with the data collected in this study for a myotine species, a similarly calculated wingspan for prey of plecotine species was taken from Lacki and Dodd (2011) and a mean wingspan was calculated for a lasiurine species from the lepidopteran species reported by Clare et al. (2009).

RESULTS

A total of 139 fecal pellets from 62 bats showed evidence of consumption of 7 insect orders, as well as Arachnida, using the morphological identification technique (Fig. 1). Lepidoptera and Coleoptera constituted the greatest volume within fecal samples (mean ± *SE*: 48.8% ± 2.5% and 38.2% ± 1.8 %, respectively), and were identified in all fecal pellets using morphological identification. Data gathered by morphological identification were then converted to presence–absence of prey orders per fecal pellet for comparison with DNA-based identification procedures (Fig. 2).

From the 139 fecal pellets, we extracted and amplified DNA from 123 (88% success); 120 pellets were sequenced (86% success). Web-based identification procedures using DNA sequences identified 4 prey orders with BOLD ($n = 60$) and 5 prey orders with GenBank ($n = 120$), respectively (Fig. 2). Overwhelmingly, 93% of the pellet contents that we identified using BOLD were identified as Lepidoptera. The majority of the pellets that could not be identified using BOLD (86%) were identified as non-Lepidoptera using GenBank. With GenBank, 53% of all sequences were identified as Lepidoptera and other prey orders (e.g., Coleoptera, Diptera, and Hemiptera) were identified more frequently than with BOLD.

Detection of the most commonly recorded orders of prey varied across identification procedures; results of chi-square tests of independence conducted for each order of prey were significant ($P < 0.001$; Table 1). Individual chi-square contributions to the overall test score ($\chi^2 = 240.5$) indicate that morphological identification of Coleoptera varied most from expected values. Individual chi-square contributions for Diptera ($\chi^2 = 39.8$) indicated identification using BOLD, and the presence of Diptera using morphological identification varied most from the expected value. Individual chi-square

Table 1.—Contribution of percent variation to overall chi-square scores among methods of identification. Separate tests were conducted for each order of prey; critical chi-square $\alpha = 0.001$, 4 *d.f.* = 18.47. Percentages in boldface type indicate values exceeding equitable variation. ID = identification; BOLD = Barcode of Life Data System.

Prey order (chi-square score)	Occurrence	Variation from expected chi-square score (%)		
		Morphological ID	GenBank	BOLD
Coleoptera (240.5)	Presence	**29.5**	11.3	11.2
	Absence	**27.2**	10.5	10.3
Diptera (39.8)	Presence	**26.1**	1.2	**39.9**
	Absence	12.7	0.6	**19.5**
Hemiptera (56.8)	Presence	**49.2**	**24.2**	12.1
	Absence	8.4	4.1	2.1
Lepidoptera (94.1)	Presence	6.8	11.9	1.5
	Absence	**27.0**	**47.0**	5.8

contributions to the overall test score for Hemiptera ($\chi^2 = 56.8$) indicated that presence of this prey order within fecal pellets contributed the most variation to the overall test score, with the exception of BOLD. Finally, individual chi-square contributions to the overall test score for Lepidoptera ($\chi^2 = 94.1$) indicated that absence in morphological identification and GenBank procedures varied most substantially from expected values.

At a finer resolution, BOLD allowed identification of 20 distinct species or genera ($n = 29$; Table 2). All sequences for which these inferences were generated were identified as Lepidoptera (Fig. 3), with a dipteran exception ($n = 2$). Sequences were uploaded to GenBank. The majority of Lepidoptera were Tortricidae ($n = 10$) and Noctuidae ($n = 7$). Other Lepidoptera identified included 2 each of Acrolophidae and Arctiidae, and one each of Coleophoridae, Epipyropidae, Gelechiliidae, Geometridae, Lasiocampidae, and Tineidae. The mean ($\pm SE$) wingspan of all Lepidoptera identified using BOLD was 24.2 ± 2.05 mm, in contrast with 34.1 ± 1.6 mm calculated from Lepidoptera previously documented in the diet of the eastern red bat (*Lasiurus borealis*—Clare et al. 2009).

Our survey of Lepidoptera served as an index of prey availability and yielded 20,256 moths representing 23 families from 184 blacklight trap samples (Fig. 3). Noctuids were the most abundant, with 6,273 individuals captured. Other common families included Geometridae ($n = 3,800$), Arctiidae ($n = 3,334$), Notodontidae ($n = 2,291$), Pyralidae ($n = 1,553$), Lasiocampidae ($n = 765$), Saturniidae ($n = 724$), Oecophoridae ($n = 325$), Limacodidae ($n = 171$), Tortricidae ($n = 166$), and Lymantriidae ($n = 118$). Families classified as "Uncommon" ($n < 100$) included Apatelodidae, Cossidae, Drepanidae, Epiplemidae, Megalopygidae, Mimallonidae, Pterophoridae, Sesiidae, Sphingidae, Yponomeutidae, and Zygaenidae (Fig. 3).

Discussion

Our study demonstrates the importance of microlepidoptera as a prey group of *M. septentrionalis*. In total, 55% of species-level observations fell within the paraphyletic group of moths

Table 2.—List of insect prey taxa identified by comparing cytochrome c oxidase subunit I gene (*COI*) sequences from the fecal samples of *Myotis septentrionalis* to the Barcode of Life Data System (BOLD).

Order	Family	Taxon	Mean wingspan (mm)	GenBank accession no.
Diptera	Tipulidae	*Tipula submaculata*	—	JQ396891, JQ396899
Lepidoptera	Acrolophidae	*Acrolophus propinqua*	21.5	JQ396898
	Arctiidae	*Halysidota tessellaris*	42.5	JQ396903, JQ396904
	Coleophoridae	*Blastobasis* sp.	12.0	JQ396909
	Epipyropidae	*Fulgoraecia exigua*	10.5	JQ396911
	Gelechiliidae	*Chionodes adamas*	13.0	JQ396907
	Geometridae	*Hypagyrtis* sp.	32.3	JQ396908
		Macaria sp.	26.5	JQ407047, JQ407048
	Lasiocampidae	*Malacasoma americanum*	33.0	JQ396886
	Noctuidae	*Abagrotis alternata*	40.5	JQ396885, JQ396900, JQ396901, JQ396902
		Idia julia	23.7	JQ396887
		Noctua pronuba	32.5	JQ396896
	Tineidae	*Isocorypha mediostriatella*	21.5	JQ396894
	Tortricidae	*Choristoneura fractivittana*	22.0	JQ396890
		Clepsis peritana	12.5	JQ396893, JQ396906
		Clepsis virescana	12.5	JQ396888, JQ396889, JQ396895
		Eucosma derelecta	21.5	JQ396910
		Paralobesia liriodendrana	21.5	JQ396905
		Phaecasiophora confixana	21.5	JQ396892
		Pseudexentera sp.	18.5	JQ407046

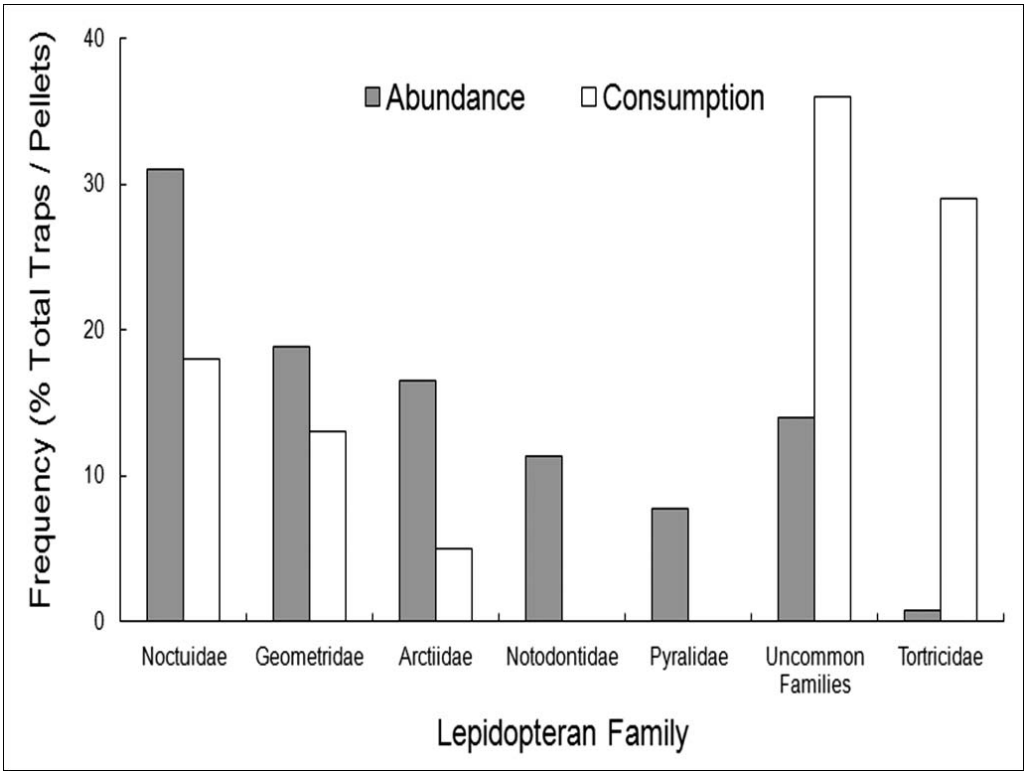

Fig. 3.—Abundance of lepidopteran prey as assessed by blacklight traps, compared with lepidopteran consumption, as assessed using the Barcode of Life Data System (BOLD). "Uncommon families" include all families $< 5\%$ of total catch in blacklight traps, with the exception of the Tortricidae (0.8%).

historically designated as microlepidoptera (Covell 2005). These data deepen our understanding of the role that prey selection can play within the framework of optimal foraging theory; although the prey consumed by *M. septentrionalis* were not uncommon across the landscape, they often were not the most abundant recorded, and presumably not the most available. Both DNA-based identification procedures that we employed indicated that the majority of prey sequences belonged to Lepidoptera, corroborating data from morphological identification, and providing evidence that our DNA-based results are congruent with previous studies of *M. septentrionalis* in our region (Griffith and Gates 1985; Lacki et al. 2009).

For our 1st objective, examination of our data demonstrated that *M. septentrionalis* consumed Lepidoptera that were smaller (24.2 ± 2.05 mm) than those documented for either lasiurine or plecotine bats (Clare et al. 2009; Lacki and Dodd 2011). This may reflect a predator constrained to handling smaller prey because of its size (Aldridge and Rautenbach 1987; Caceres and Barclay 2000; Lacki et al. 2007a). Even so, this species may have evolved within the bat community to feed on smaller prey. The capacity for *M. septentrionalis* and other myotine species to echolocate at higher peak frequencies than other sympatric insectivorous bats increases their potential to better locate and capture smaller-sized prey (Fenton 1990). Examinaiton of data from discarded wings of Lepidoptera suggests that plecotine bats in the genus *Corynorhinus* consume taxa with a wingspan of 47 ± 1.3 mm (Lacki and Dodd 2011). However, assessment of such prey remnants only allows identification of prey from parts that are culled, thus smaller prey items that may be eaten in their entirety or those with little chitin may not be recorded (Lacki et al. 2007a). Even so, examination of the data that do exist for plecotine species (gleaners) suggests that these bats consume larger prey relative to the myotine species we evaluated. Similarly, Lepidoptera consumed by *L. borealis*, a common lasiurine species (aerial hawker), have wingspans of 34.1 ± 1.6 mm (Clare et al. 2009). Based on existing data sets, we hypothesize that *M. septentrionalis* and likely other similar-sized myotine bats that both hawk and glean prey (Ratcliffe and Dawson 2003; Whitaker 2004) occupy a prey selection niche distinct from other sympatric groups of insectivorous bats.

Our results help further elucidate the extent of prey consumption in the context of prey availability, addressing our 2nd objective. Microlepidoptera are difficult to identify and enumerate in assessments of prey availability; consequently, identification efforts of Lepidoptera have focused on larger specimens (Burford et al. 1999; Dodd et al. 2008, 2012). The Lepidoptera consumed by *M. septentrionalis* in our study generally corresponded to the size class of prey (i.e., wingspans ≥ 20 mm) identified in our assessment of prey abundance, but some prey species evident from our molecular analysis fell below this threshold (i.e., mean wingspans < 20 mm, including *Blastobasis* sp., *Chionodes adamas*, *Clepsis* spp., and *Fulgoraecia exigua*). Thus, the importance of these smaller

Table 3.—Comparison of total cytochrome c oxidase subunit I gene (*COI*) sequences from the Barcode of Life Data System (BOLD) and GenBank databases (accessed March 2011). Search phrases for GenBank consisted of "<taxon of interest> AND cytochrome oxidase subunit I." For BOLD, all taxa are as indexed by BOLD, with the exception of "Pyralidae," which is the sum of data indexed as Pyralidae and Crambidae.

Taxon	BOLD		GenBank
	Specimens	Species	total hits
Hemiptera	16,622	2,286	14,739
Coleoptera	22,931	5,228	26,001
Diptera	74,684	6,594	46,635
Lepidoptera	466,340	53,112	216,731
Notodontidae	18,439	1,334	4,182
Tortricidae	20,464	1,959	2,129
Arctiidae	22,104	2,417	3,312
Pyralidae	40,233	4,645	1,090
Geometridae	74,556	11,942	6,187
Noctuidae	84,880	8,569	7,652

prey items to foraging bats, and consequently to food habits studies, should not be overlooked and should be closely considered in subsequent studies. As web-based DNA databases grow in taxonomic and regional representation, DNA-based prey identification procedures are becoming increasingly powerful. Furthermore, using web-based search tools to identify sequences from microlepidoptera and other taxa that are difficult to identify will allow ecologists to assess consumption of taxa that would otherwise be difficult to identify and permit new insights into foraging behavior of cryptic trophic linkages that previously were impractical or impossible to untangle.

Our results illustrated differences in the sequence composition between BOLD and GenBank databases (3rd objective). Although the preponderance of sequence matches of smaller Lepidoptera in the diet of *M. septentrionalis* could be due to primer bias, we suggest this is unlikely given that our primer set has been used to target a wide breadth of insect taxa (Harper et al. 2006; Jourdie et al. 2008; Simon et al. 1994). Further, the diet breadth as identified using GenBank suggests DNA amplification across a broad cross section of arthropod taxa. The total number of BOLD sequences across Lepidoptera generally corresponded with our data on prey abundance. Despite their high frequency (33%) within fecal samples of *M. septentrionalis*, Tortricidae ($\geq$20 mm) were not a major component of our blacklight trap catches, suggesting that frequent selection of Tortricidae and other microlepidoptera occurs. Thus, we reject the null hypothesis for our 2nd objective; consumption was disproportionate across Lepidoptera.

To further investigate potential primer bias between macrolepidoptera and microlepidoptera, we aligned the primers to a *COI* sequence database of 89 macrolepidopteran species that occurred at our collecting sites (153 sequences—Dodd 2010), as well as the microlepidopteran prey documented in our analysis (all but *E. derelecta* and *Pseudexentera* spp.; 295 public sequences in BOLD). Although mismatches were common across the forward primer region (C1-J-1859) for both macrolepidopteran and microlepidopteran sequences (81% and 90% of sequences, respectively), we found no sequence mismatched at the 2 final bases of the 3′ end, which is integral for effective priming (Erlandson and Gariepy 2005). Additionally, the low annealing temperature (45°C) used in the polymerase chain reactions could compensate for primer mismatches. Furthermore, Simon et al. (1994:690) stated that this primer "works well in thysanopterans, coleopterans, homopterans, hemipterans, lepidopterans and psocoptrans", indicating its utility as a general primer. Our reverse primer (C1-N-2191) is outside the range of the sequences available for comparison (i.e., those amplified using LCO1490 and HCO2198—Folmer et al. 1994). However, Simon et al. (1994:690) stated that this primer "works well with virtually all insects tested including lepidopterans, dipterans, coleopterans, thysanopterans, hemipterans and homopterans." Therefore, data for our forward primer, the low annealing temperature, and the findings of Simon et al. (1994) support the notion that our primer set was effective across a broad array of prey taxa.

We suggest that bias exists for our DNA-based technique at a broader taxonomic resolution, and we reject the hypothesis of equivocal methodological bias within DNA-based approaches. If only considering data generated with BOLD, we would have reached the conclusion that *M. septentrionalis* is a highly specialized predator of Lepidoptera (>80% of diet), consistent with observations for plecotine bats in eastern North America (Lacki et al. 2007a). This is likely not the case for *M. septentrionalis*, considering data generated on prey consumption using GenBank and the morphological technique, as well as results from other studies (Brack and Whitaker 2001; Caceres and Barclay 2000; Carter et al. 2003; Feldhamer et al. 2009; Griffith and Gates 1985; Lacki et al. 2009; Lee and McCracken 2004; Whitaker 2004). The distribution of *COI* sequences in both databases offers a more likely explanation. Lepidoptera are the most amply represented prey order, although more so in BOLD. It is logical that our lepidopteran sequence matches were more numerous when using BOLD than GenBank; BOLD may better facilitate species-level identifications of Lepidoptera but is not as powerful for species-level identifications of Coleoptera or Hemiptera. BOLD and GenBank contain less than one-twentieth and one-tenth of the *COI* sequences for Coleoptera than they do for Lepidoptera, which is likely the primary reason for the discrepancy between the large number of morphological identifications of Coleoptera in feces of *M. septentrionalis* compared to the much smaller number of molecular identifications. We suggest that the species-level data we have generated is correct, albeit an incomplete view of the food habits of *M. septentrionalis*. Considering this, a priori knowledge of diet breadth may dictate which identification algorithm and database provides the optimal basis for analysis of a given predator species.

Trends in the sources of variation in chi-square test scores suggest bias across prey identification procedures, and we reject the hypothesis of equivocal methodological bias between

DNA-based and morphological approaches (3rd objective). Previous studies suggest that morphological identification overrepresents hard-bodied prey (Lacki et al. 2007a); our data corroborate this. Notably, the insect taxa for which we most commonly achieve genus- or species-level identifications (i.e., Lepidoptera) are those precluded from such identifications using traditional techniques. Therefore, DNA-based prey identification techniques lend insight where it is most needed for bat food identification (J. O. Whitaker, Jr., pers. comm.). Even so, quantitative assessment of prey consumption is difficult with current DNA-based techniques (Harwood and Greenstone 2008). Until DNA-based methods evolve further (e.g., real-time polymerase chain reaction—Durbin et al. 2007; Harwood and Greenstone 2008; Weber and Lundgren 2009), a union between DNA-based and morphological identification will best allow high-resolution prey identification and quantitative estimates of prey consumption (Zeale et al. 2011).

Unlike DNA-based work evaluating food habits of *L. borealis* that suggested a much broader diet breadth than previously reported (Clare et al. 2009), the sample units in our study (individual fecal pellets) provided a narrower perspective of prey items consumed by individual bats. Our perspective is narrower because we generated single prey inferences generated per pellet. In the absence of primer bias, we have amplified the most common DNA products within fecal pellets and, thus, provide an indication of the most common items by volume within the diet of *M. septentrionalis*.

We documented predation of Lepidoptera that are important agricultural and forest pests, many of which demonstrate outbreak behavior (Covell 2005). Tortricidae larvae are leaf rollers and tiers, and root, stem, and fruit borers with broad economic importance (Covell 2005). The eastern tent caterpillar (*Malacosoma americanum* (Fabricius); Lasiocampidae) is a serious defoliator of rosaceous trees (Covell 2005) and sporadically impacts equine reproductive health (Webb et al. 2004). Our study provides data suggesting that forest bats may play a role in depredating pest Lepidoptera species; future research should consider the role that forest bats may play in regulating these populations (Kalka et al. 2008; Williams-Guillén et al. 2008). Given the diversity of Lepidoptera consumed across bat species, conservation goals should promote land management and forest stewardship practices that contribute to a diverse prey base for these ecologically sensitive predators.

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