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Identification of *Taenia* Metacestodes from Mongolian Mammals Using Multivariate Morphometrics of the Rostellar Hooks

Danielle M. Tufts
Columbia University, dmtufts11@yahoo.com

Nyamsuren Batsaikhan
Mongolian National University, batsaikhan@num.edu.mn

Michael Pitner
University of Nebraska Medical Center, michael.pitner@unmc.edu

Gábor R. Rácz
University of Nebraska - Lincoln, gracz2@unl.edu

Altangerel Tsogtsaikhan Dursahinhan
University of Nebraska - Lincoln, stylodipusats@gmail.com

See next page for additional authors

Authors
Danielle M. Tufts, Nyamsuren Batsaikhan, Michael Pitner, Gábor R. Rácz, Altangerel Tsogtsaikhan Dursahinhan, and Scott Lyell Gardner

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Identification of *Taenia* Metacestodes from Mongolian mammals using multivariate morphometrics of the rostellar hooks

D.M. Tufts, N. Batsaikhan, M. Pitner, G.R. Racz, A.T. Dursahinhan & S.L. Gardner

Abstract

Parasite diversity in and among various species of mammals within Mongolia is still poorly understood. The current paper focusses on a small part of the results of the Mongolian Vertebrate Parasite Project (MVPP), which entailed a broad-scale biodiversity survey of the vertebrates and their parasites of the Gobi and Altai regions of Mongolia. We report on the prevalence and morphological variation of larval cestodes of the family Taeniidae that occurred in small mammals that were collected from 2009-2012 from various locations in southern Mongolia. From these metacestodes, we studied both large and small rostellar hooks and analyzed both size and shape using univariate and multivariate morphometric statistical methods, specifically principal components analysis (PCA). Of species representing the four mammalian orders collected and examined for parasites, less than 1% of animals examined were infected with larval cestodes of the family Taeniidae. We identified nine species of larval cestodes from this work, including seven species of *Taenia*, one species of *Versteria*, and one species of *Echinococcus*. Principal component analysis of eight characters of hooks from both rows 1 and 2 (large and small hooks, respectively) showed that loadings on PC-I appeared to be mostly a size component with PC-II potentially indicating shape; furthermore, our results indicate that, as blade-length increased guard-width and handle-length decreased leading to observed differences in overall hook-shape. This work provides an update to the previous studies by GANZORIG (1996-1997) and other mammalogists and parasitologists in Mongolia.

Key words: *Taenia*, Gobi, Altai, Rodentia, Biodiversity, Multivariate Statistics, Mongolia, rostellum, rostellar hooks

Introduction

Mongolia is a landlocked country of around 1.56 million km^2 located in the heart of central Asia, bordered by Russia to the north and China to the south (fig. 1). Mongolia contains all the major biomes, ecosystems, and life zones of Eurasia, including Taiga, alpine, forest steppe, steppe, desert steppe, and desert (MALLON 1985). Elevations range from a minimum of 560 m in the farthest south-eastern part of the country, the eastern steppe region to 4,374 m at Khuiten peak in the Altai Mountains in the far west. In many parts of Mongolia, extreme geographic relief creates myriad pockets of fragmented alpine and riparian zones in island-like, geographically distinct areas separated from each other by desert or desert-steppe habitats, many as recently as late Pleistocene-time (TINNIN et al. 2002, JARGAL 2003, OPGENOORTH et al. 2005). Diverse mammal/faunal assemblages occur in this region not only because of the convergence of these biomes, but also because of the presence of discontinuous/isolated mountain alpine and larch forests on the sides and tops of mountains. Even as research projects increase our knowledge of the number of known species in the area of southern Mongolia, natural areas are under extreme threat from overgrazing and other poor agricultural practices. These threats are leading directly to severe erosion, desertification, and deforestation. Mining, oil, and gas exploration has been steadily increasing and are now the leading causes of ecological disturbances throughout Mongolia. From 1992 through 1999, it is estimated the number of domestic grazing animals in the steppe regions of Mongolia increased from 20 million to 33 million head (mh), and in 2006 to 34 mh, while forested lands decreased by 1.4 million hectares, and total prime pasture land declined by 6.9 million hectares (JARGAL 2003, STÜRMER et al. 2004, HILBIG & OPGENOORTH 2005).

Beginning in 1999, we worked with biologists at the National University of Mongolia who were investigating the biodiversity of vertebrates and their parasite faunas in the area of southern Mongolia. To fully implement a survey of the vertebrates of the area, we received funding from the US National Science Foundation to conduct expeditionary research in the Gobi and Altai region of south-central and southwestern Mongolia. From 2009 through 2012, the Mongolian Vertebrate Parasite Project (MVPP) was conducted and consisted of a collaboration among Mongolian, Mexican, American, Japanese, and French institutions. The objective of the MVPP was to conduct a survey of southern Mongolia to discover, describe, and document vertebrates and their parasites, which had been historically understudied (TINNIN 2008). In addition, there is a growing body of literature that recognizes the importance of parasite faunas as indicators of biodiversity through knowledge of historical constancy and diversity of the fauna (GARDNER & CAMPBELL 1992, BROOKS et al. 2014). The current paper reports the results of detailed and extensive collecting efforts that eventually will be useable to make science based recommendations to establish protected areas, assess current conservation efforts, and determine the preservation status of species studied in areas of interest: "Document-Assess- Monitor-Act [DAMA]" of BROOKS et al. (2014). Detailed and published information of the fauna of a region is also necessary to enable discovery and documentation of the presence or existence of new zoonoses or monitoring of previously identified zoonotic agents (GARDNER et al. 1988, BROOKS & HOBERG 2000, HOBERG 2002, HOBERG et al. 2003, DOWLING et al. 2003). This point is emphasized by the recent definitive first-report of metacestodes of *Echinococcus multilocularis* LEUCKART, 1863 from the lacustrine vole, *Microtus limnophilus* BÜCHNER, 1889 collected in the grassland meadows of Har Us Nuur northeast of Hovd (GARDNER et al. 2014). With accurate small mammal host–parasite data, and a comprehensive database on the presence/absence and distribution of the species, it is now becoming possible to establish the level of anthropogenic threat to at–risk species, and to classify regions or faunas for conservation efforts (GARDNER & CAMPBELL 1992, BROOKS & HOBERG 2000, HOBERG 2002, HOBERG et al. 2003, BROOKS et al. 2014).

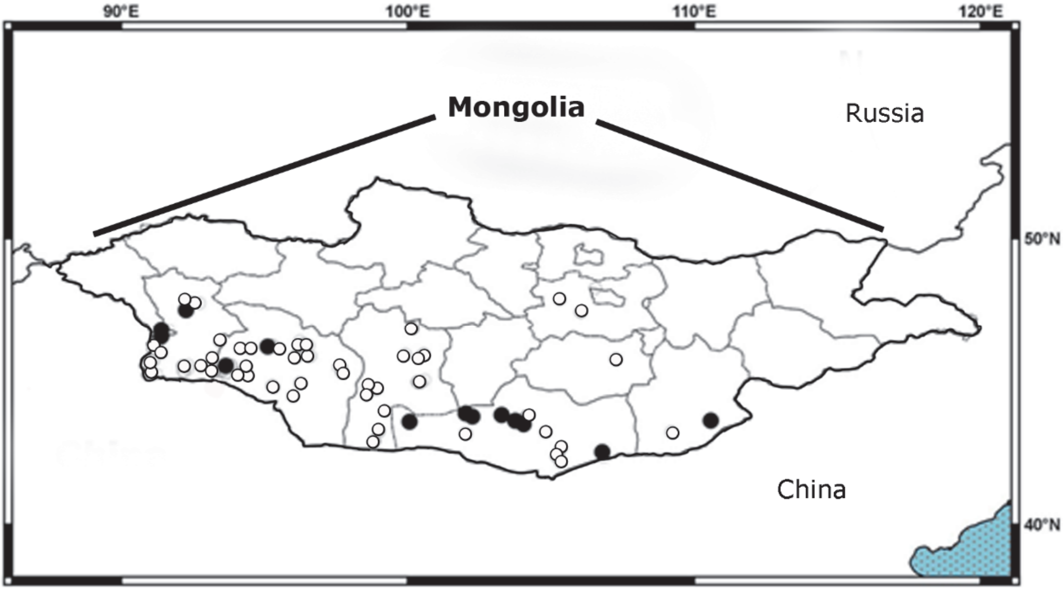

Fig. 1: Approximate localities of specimens of both infected and uninfected mammals collected from 2009-2012 extending through central, south-central, and south-western Mongolia. Localities where metacestodes of the family Taeniidae were collected are shown by solid black circles. Sites where mammals were examined but found to be negative for infection are shown by the open circles.

During the course of our biodiversity survey it became evident that, in some of the small mammals sampled, larval cestodes of the genus *Taenia* LINNAEUS, 1758 (Cyclophyllidea: Taeniidae LUDWIG, 1886) had some of the highest numerical densities (per individual mammals examined) of all helminth parasites that were collected. Cestodes of the family Taeniidae are a monophyletic group of species that use herbivorous mammals as intermediate hosts and carnivorous mammals as final or definitive hosts, [see Life-Cycle Figure] (ABULADZE 1964, HOBERG et al. 2000).

Species of *Taenia*, especially those occurring in humans and synanthropic mammals, have been studied intensively from the initial speculative/descriptive work by the Greeks and Romans (see summary in GROVE 1990) through the beginnings of modern systematics (LINNAEUS 1758, ZEDER 1803) and summarized by RAUSCH (1997) up to the present (TEREFE et al. 2014). Because of the importance of this group of cestodes to health of both human and domestic animals and because of the relative commonness of larvae of species of *Taenia* in mammals that we collected from throughout south-central to southwestern Mongolia, we focused our efforts on the collections of larval forms of *Taenia* in Mongolia.

Some species of *Taenia* are known pathogens of both humans and domestic animals (ABULADZE 1964, RAUSCH 1997) and they have previously been reported from Mongolia in a scattered literature base (for a summary of helminths reported in and around Mongolia, see TINNIN et al. 2013). An understanding of the in-country geographic distribution, range of species of intermediate hosts they infect, and general morphological characters useful for identification is necessary to make informed decisions relative to preservation/conservation, green-development, and health of both humans and domestic animals (*sensu* BROOKS et al. 2014).

Relatively little has been published on uses of multivariate statistical methods as applied to understanding morphological diversity among species in the genus *Taenia.* A notable exception is the work of GUBÁNYI (1999) where he used landmark morphometrics to describe and evaluate diversity in rostellar hooks from larval taeniids from Eastern Europe and Asia. In the current study, we focus on identification of larval forms of cestodes of the family Taeniidae using standard morphology of the hooks combined with multivariate morphometric techniques to explore the diversity of species of *Taenia* collected during the MVPP throughout the southern part of Mongolia starting first in 1999 followed by intensive, yearly expeditionary fieldwork from 2009 - 2012. For examples of studies on non-armed cestodes of the genus *Hymenolepis* Weinland, 1858 see GARDNER et al. (2014).

Materials and Methods

Specimen collection

From throughout southern Mongolia, mammals were collected from many separate localities extending from near the border of China in the central part of the Gobi, southwest through the steppes and Gobi region, to the far western part of the country into the Mongolian Altai mountains (fig. 1). Small mammals were collected using Sherman® live traps, Museum Special® snap traps, shooting, or by hand capture using spotlights and hand-held insect nets (table 1). All mammals captured were euthanized and necropsied as soon as possible following the methods outlined by GARDNER (1996) and GARDNER & JIMÉNEZ-RUIZ (2009) under guidelines of the University of Nebraska Animal Care and Use Committee (protocol IACUC No. 07-06-028D). In the field-laboratory, peritoneal and pleural cavities, and viscera were examined for the presence of metazoan parasites with a magnification at or greater than 20x. For the current study, specimens of any larvae or other parasites found were initially placed in physiological saline, observed, photographed, and preserved in vials of 70 % or 95 % ethanol or 10 % buffered formalin (v/v) solution.

Larval and hook morphology

In the Manter Laboratory, larvae were initially removed from a vial of preservative solution or from a cryotube and placed in a solution of 70 % ethanol and distilled water (v/v). Scolexes from specimens included in the study were carefully dissected in this solution and imaged at low power (10x or 20x with a Leica^{TM} MZ-8 dissecting microscope). The rostellum of each specimen was dissected using a new scalpel, insect pins, or a sharp hypodermic needle from a syringe and placed on a microscope slide in lactophenol. Because the acidic nature of lactophenol causes carbon dioxide to form as it reacts with the calcium in the tissues of larval taeniids, the preparation was allowed to sit on the microscope slide overnight in a covered, glass petri dish. After about 24 hours, a No. “1” coverslip was then applied to the specimen and the hooks were imaged and then spread by application of pressure to the coverslip using a slightly worn pencil eraser. Specimens prepared in this way were imaged using a Zeiss Axioplan microscope with 20x, 40x, and 63x (oil) objective lenses. The total number of hooks for each specimen was recorded and at least 10 large hooks and 10 small hooks were measured from each specimen and univariate statistics were calculated.

Table 1: Species of mammals collected, examined, and found to be infected with larval Taeniidae

(All mammals were examined in the field by members of the Mongolian Vertebrate Parasite Project in Mongolia from 2009 - 2012. Data given in table include accepted species name of host mammal, common name, order, number of individuals examined, the number of individuals infected with taeniid larvae, and the host species preferred habitat in Mongolia. List of mammals examined but not infected is given in the text.)

species name	common name	order	number examined	number infected	preferred habitat
Allactaga balikunica	Balikun jerboa	Rodentia	77	1*	arid deserts and semi-desert Gobi with clay-based soil
Allactaga bullata	Gobi Jerboa	Rodentia	62	2	shrubby arid desert and semi-desert with gravely soil
Alticola cf barakshin	Gobi Altai Mountain vole	Rodentia	308	2	grassy meadows interspersed with dry shrub and steppe
Capra hircus	Domestic goat	Artiodactyla	2	1	domestic goat occurs all throughout the country
Meriones meridianus	Midday jird; gerbil	Rodentia	284	7	favours small hills, lowlands and valleys with sandy soil
Meriones unguiculatus	Mongolian gerbil	Rodentia	161	2	main range in the Gobi and dry-steppe with grass, pea shrubs (*Caragana* spp.) and thistles (*Salsola* spp.) but penetrates into the forest steppe along river valleys; occurs in mountain steppe habitats; also occurs near towns and settled areas
Microtus limnophilus	Lacustrine vole	Rodentia	8	1†	moist grassy areas, especially around lakes; prefers areas with salty soil, such as lowland wet meadows and pea shrubs.
Mus musculus	House mouse	Rodentia	46	1	often associated with human dwellings; inhabits urban areas, oases, steppe habits, and river valleys with dense shrubs and grass
Ochotona pallasi	Pallas’s Pika	Lagomorpha	218	11	steep rocky or grassy slopes in dry steppe, mountain steppe, semi-desert, and high mountains

* - infected with larvae of *Versteria mustelae* † - infected with *Echinococcus multilocularis*

Life Cycle of Cestodes of the genus *Taenia*

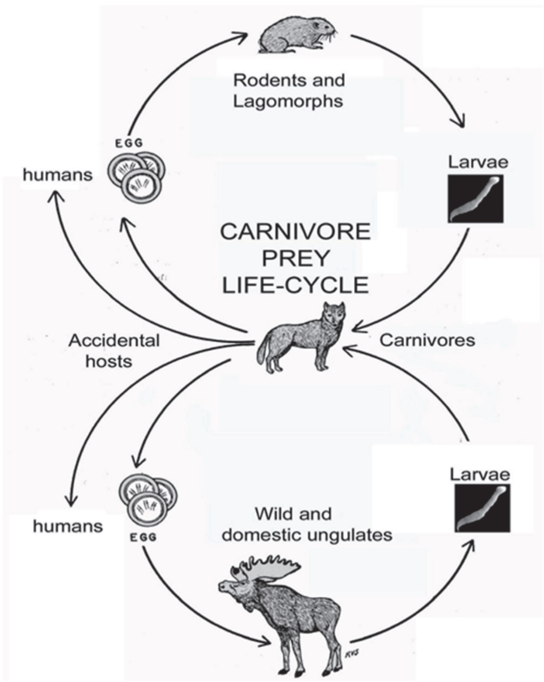

Fig. 2: Typical life-cycle of cestodes of the family Taeniidae. All known species of *Taenia* utilize mammals as both definitive and intermediate hosts.

Species identification

Hooks from the rostellum were used to identify metacestodes to species using example images, drawings, and measurements from VERSTER (1969), GUBÁNYI (1995), GANZORIG et al. (1996-1997) and LOOS-FRANK (2000). Measurements of each set of hooks were then subjected to both univariate and multivariate statistical analysis (as shown below) to test the identifications we made based on qualitative and general quantitative characters. A subset of the identified larvae were sequenced using multiple genes (CO1, 18S, nad1, and nad5) to confirm morphological identification, these will be reported elsewhere (TUFTS et al. in preparation).

All images included in this paper were archived in the Arctos database of the Harold W. Manter Laboratory of Parasitology Parasite Collection image database, University of Nebraska-Lincoln, Lincoln, Nebraska (http://arctos.museum.edu).

Statistical analyses

Univariate statistics including measures of central tendency (mean, standard deviation, coefficient of variation, range, and variance) were calculated for all characters measured. The coefficient of variation is defined as the standard deviation expressed as a percentage of the mean of each character (SOKAL & ROHLF 1995). To decrease the effect of size, in general, and to standardize the measurements to an approximately normal distribution, all variables were $\log_{10}$ transformed before multivariate analysis was employed (SOKAL & ROHLF 1995).

Multivariate morphometrics

To explore and investigate the morphological diversity of the various species of taeniids collected during this study from throughout the Gobi of south-central and southwestern Mongolia, we used both principal component analysis (PCA) and canonical discriminant analysis (CDA). These multivariate techniques were used to summarize data from measurements of both the large and small rostellar hooks of individuals from each species of Taeniidae studied. Before running multivariate statistics on the data-set, and based on comparative data taken from the literature, we allocated each individual metacestode to a species based on univariate comparisons of sizes and shapes of rostellar hooks, metacestode aspect, and number of hooks in each row on the rostellum. Our final data-set was restricted to individual metacestodes representing species of both *Taenia* and *Versteria*; data on *Echinococcus multilocularis* (LEUCKART, 1863) collected in 2012 are presented elsewhere (GARDNER et al. 2014 and GARDNER et al. 2016 - in review). To view the dataset in an inclusive way and in order to decrease chances of biases in our analysis, data on both large (row 1) and small (row 2) hooks were combined for analysis [see introduction in ABULADZE (1964) for a compelling discussion on why to designate hooks from row 1 or 2 instead of only large or small].

Table 2: Measurements of hooks from both rows 1 and 2 - large hooks and small hooks, respectively) (data given here includes host species, total number of hooks on rostellum of specimens examined, mean measurement lengths for large and small hooks (all measurements are in µm), and *Taenia* species identification based on measurements and shape of hooks)

host species name	no. hooks	***Large Hooks***			***Small Hooks***			species ID
		total length	**guard length**	**handle length**	**total length**	**guard length**	**handle length**	
Allactaga bullata	68	188.54	48.33	88.99	117.10	40.54	24.73	*T. polyacantha*
Allactaga balikunica	46	44.19	13.85	20.00	33.64	13.63	16.03	*Versteria* cf. *mustelae*
A. bullata	54	167.58	41.93	69.46	111.70	31.93	26.15	*T. polyacantha*
Alticola sp.	40	476.11	178.71	216.45	302.43	124.42	118.38	*T. taeniaeformis*
Alticola cf. *barakshin*	**38**	**446.86**	**149.57**	**192.25**	**281.55**	**96.37**	**81.53**	**undescribed species ??**
Capra hircus	42	212.88	66.34	105.39	147.72	63.66	62.77	*T. hydatigena*
Meriones meridianus	66	319.85	104.45	132.94	215.55	66.43	65.30	*T. krepkogorski*
M. meridianus	64	360.45	84.03	146.13	214.90	62.70	64.55	*T. endothoracicus*
M. meridianus	64	349.27	74.59	148.81	214.53	56.67	74.22	*T. endothoracicus*
M. meridianus	60	328.88	71.29	150.62	206.66	56.94	67.27	*T. endothoracicus*
M. meridianus	56	191.31	52.90	90.22	122.14	41.89	24.62	*T. polyacantha*
M. unguiculatus	59	353.97	76.17	147.00	220.50	58.05	70.81	*T. endothoracicus*
M. unguiculatus	58	352.89	68.16	155.01	217.08	58.51	75.47	*T. endothoracicus*
M. unguiculatus	58	360.43	73.42	153.92	232.05	66.85	78.88	*T. endothoracicus*
Mus musculus	70	186.06	51.12	83.26	119.93	47.88	32.95	*T. polyacantha*
Ochotona pallasi	40	300.99	66.62	103.10	204.44	52.59	70.27	*T. retracta*
O. pallasi	40	307.38	64.21	104.92	201.77	52.13	75.69	*T. retracta*
O. pallasi	46	289.71	60.12	111.96	179.32	47.66	55.65	*T. retracta*
O. pallasi	42	289.86	61.82	110.64	189.52	47.63	62.98	*T. retracta*
O. pallasi	42	266.51	50.60	82.69	181.04	51.92	50.82	*T. retracta*
O. pallasi	38	317.14	62.46	117.25	210.22	58.87	77.41	*T. retracta*
O. pallasi	38	316.06	66.23	111.65	210.03	55.17	74.08	*T. retracta*
O. pallasi	38	310.56	70.08	118.27	203.26	58.78	74.46	*T. retracta*
O. pallasi	38	309.12	59.10	115.23	187.41	49.28	61.72	*T. retracta*
O. pallasi	34	306.30	63.79	102.60	188.68	52.66	62.97	*T. retracta*
O. pallasi	38	303.67	64.19	108.00	206.67	54.68	70.16	*T. retracta*

Results

All mammals collected during our four years (2009-2012) of fieldwork in Mongolia were examined for both ecto- and endoparasites. During our expeditionary research in Mongolia, 3,624 individual mammal specimens were collected, necropsied, examined, and preserved as museum specimens. Note that not all specimens were fully necropsied as this total number could include skulls, individuals found dead, or other skeletal or tissue material from which no parasite specimens could be taken.

Of species representing mammals of five orders (Artiodactyla, Erinaceomorpha, Lagomorpha, Rodentia, and Soricomorpha) that were potentially or actually infected with larval individuals of species of Taeniidae, 28 of 3,329 (<1%) were found to be infected with larval cestodes of the family Taeniidae (table 1). From these mammals, nine species of Taeniidae were collected, including seven species of *Taenia,* which is the subject of this report, one species of *Versteria,* and one species of *Echinococcus* (see GARDNER et al. 2014). An additional 36 samples of larval cestodes were allocated to the families Mesocestoididae, Anoplocephalidae, or Dilepididae. For example, of three *Hemiechinus auritus* GMELIN, 1770 found to harbor larval cestodes, all were infected with metacestodes of the genus *Mesocestoides* while of the 11 individual *Ochotona* spp. (GRAY 1867) examined and discovered to be infected with metacestodes in the mesenteries all harbored *Taenia retracta* LINSTOW, 1903 (table 2).

Species of mammals examined by us, but were not found to be infected with larval cestodes of the family Taeniidae include *Allactaga sibirica* (Forster, 1778), *Allocricetulus curtatus* (Allen, 1925), *A. eversmanni* (Brandt, 1859), *Apodemus peninsulae* (Thomas, 1907), *A. uralensis* (Pallas, 1811), *Cardiocranius paradoxus* Satunin, 1903, *Cricetulus barabensis* (Pallas, 1773), *C. longicaudatus* (Milne-Edwards, 1876), *C. migratorius* (Pallas, 1773), *C. sokolovi* Orlov & Malygin, 1988, *Crocidura suaveolens* Pallas, 1811, *Dipus sagitta* (Pallas, 1773), *Dryomys nitedula* (Pallas, 1778), *Ellobius tancrei* Blasius, 1884, *Eolagurus luteus* (Eversmann, 1840), *Euchoreutes naso* Sclater, 1891, *Hemiechinus auritus* (Gmelin 1770), *Lasiopodomys brandtii* (Radde, 1861), *Lepus tolai* Pallas, 1778, *Marmota baibacina* Kastschenko, 1899, *M. sibirica* (Radde, 1862), *Meriones tamariscinus* Pallas, 1773, *Microtus gregalis* (Pallas, 1779), *M. oeconomus* (Pallas, 1776), *Clethrionomys rufocanus* (Sundevall, 1846), *C. rutilus* (Pallas, 1779), *Ochotona alpina* (Pallas, 1773), *O. dauurica* (Pallas, 1776), *O. hyperborea* (Pallas, 1811), *Phodopus campbelli* (Thomas, 1905), *P. roborovskii* (Satunin, 1903), *P. sungorus* (Pallas, 1773), *Pygeretmus pumilio* (Kerr, 1792), *Rhombomys opimus* (Lichtenstein, 1823), *Salpingotus crassicauda* Vinogradov, 1924, *S. kozlovi* Vinogradov, 1922, *Sciurus vulgaris* Linnaeus, 1758, *Sorex minutissimus* Zimmermann, 1780, *Spermophilus alashanicus* Büchner, 1888, *S. pallidicauda* (Satunin, 1903), *S. undulatus* (Pallas, 1778), *Stylodipus andrewsi* Allen, 1925, *S. sungorus* Sokolov & Shenbrot, 1987, *Tamias sibiricus* (Laxmann, 1769).

Six known species of *Taenia* were identified from the mammals collected during our fieldwork in addition to a species that we could not identify using either morphology or via comparisons of similarity of the DNA sequences of available species of *Taenia*. The sequence and the morphological characteristics of the hooks of these individual larvae indicate that it is probably an undescribed species (TUFTS et al., unpublished data). Species that we collected with confirmed identifications included *T. krepkogorski* (Schulz & Landa 1934) [fig. 3, 1 & 2], *T. endothoracicus* (Kirschenblatt 1948) [fig. 3, 3 & 4], *T. retracta* (Linstow 1903), [fig. 3, 5 & 6], *T. polyacantha* (Leuckart 1856) [fig. 4, 7 & 8], undescribed species of *Taenia* [fig. 4, 9 & 10], *T. taeniaeformis* (Batsch 1786) [fig. 4, 11 & 12], *T. hydatigena* (Pallas 1766) [fig. 5, 13 & 14], and *Versteria* cf. *mustelae* [fig. 5, 15 & 16]. Our measures of central tendency showed considerable variation in the means and ranges of large and small hooks among and within each of the seven Taeniid species (table 3).

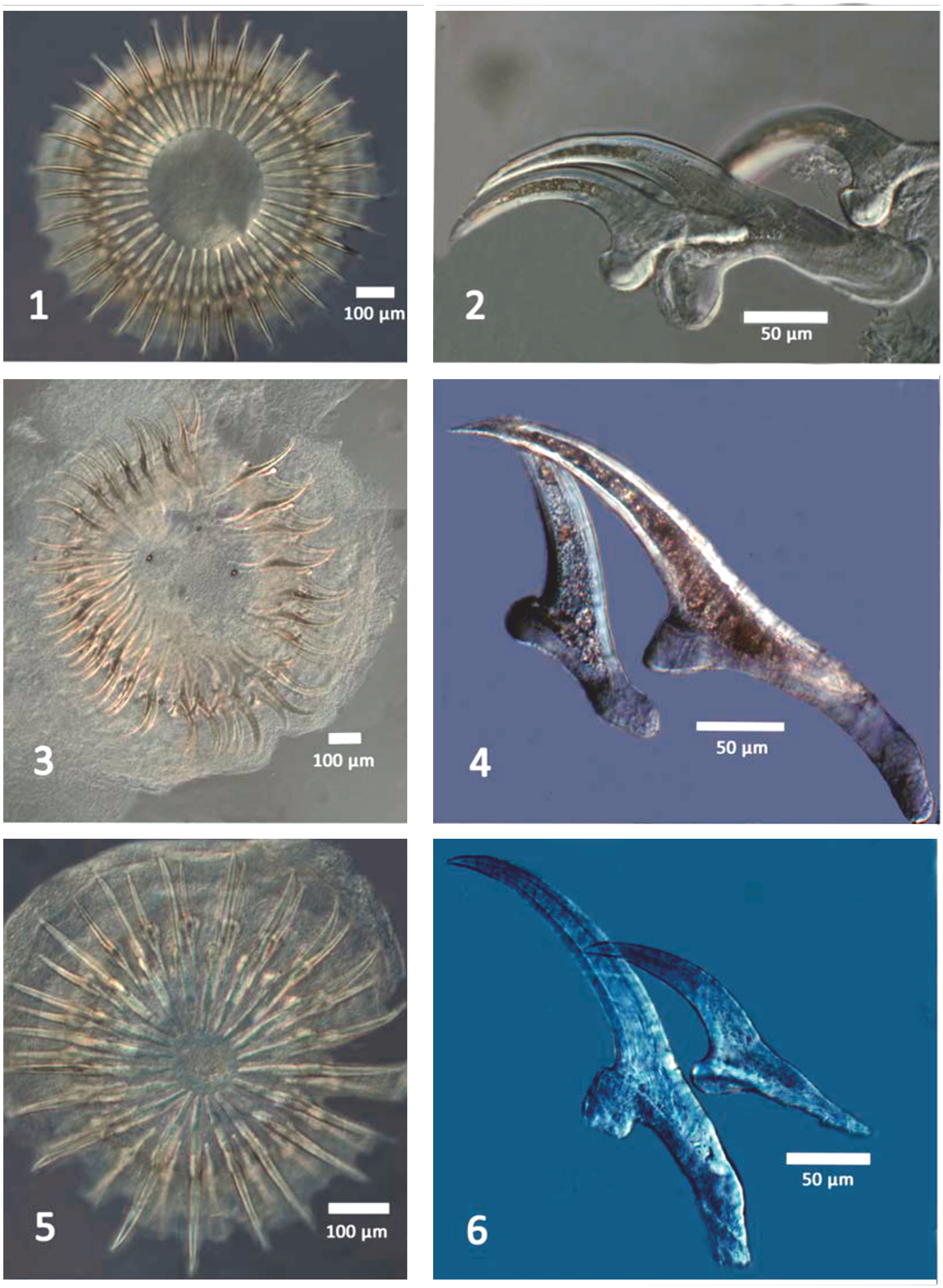

Fig. 3: Rostellum and hooks extracted from larvae collected from metacestodes representing species of *Taenia* from small mammals obtained during our biodiversity survey of south and southwestern Mongolia - collected from 2009 - 2012; *Taenia krepkogorski* - 1 & 2 *,* *T. endothoracicus* 3 & 4*, T. retracta* 5 & 6.

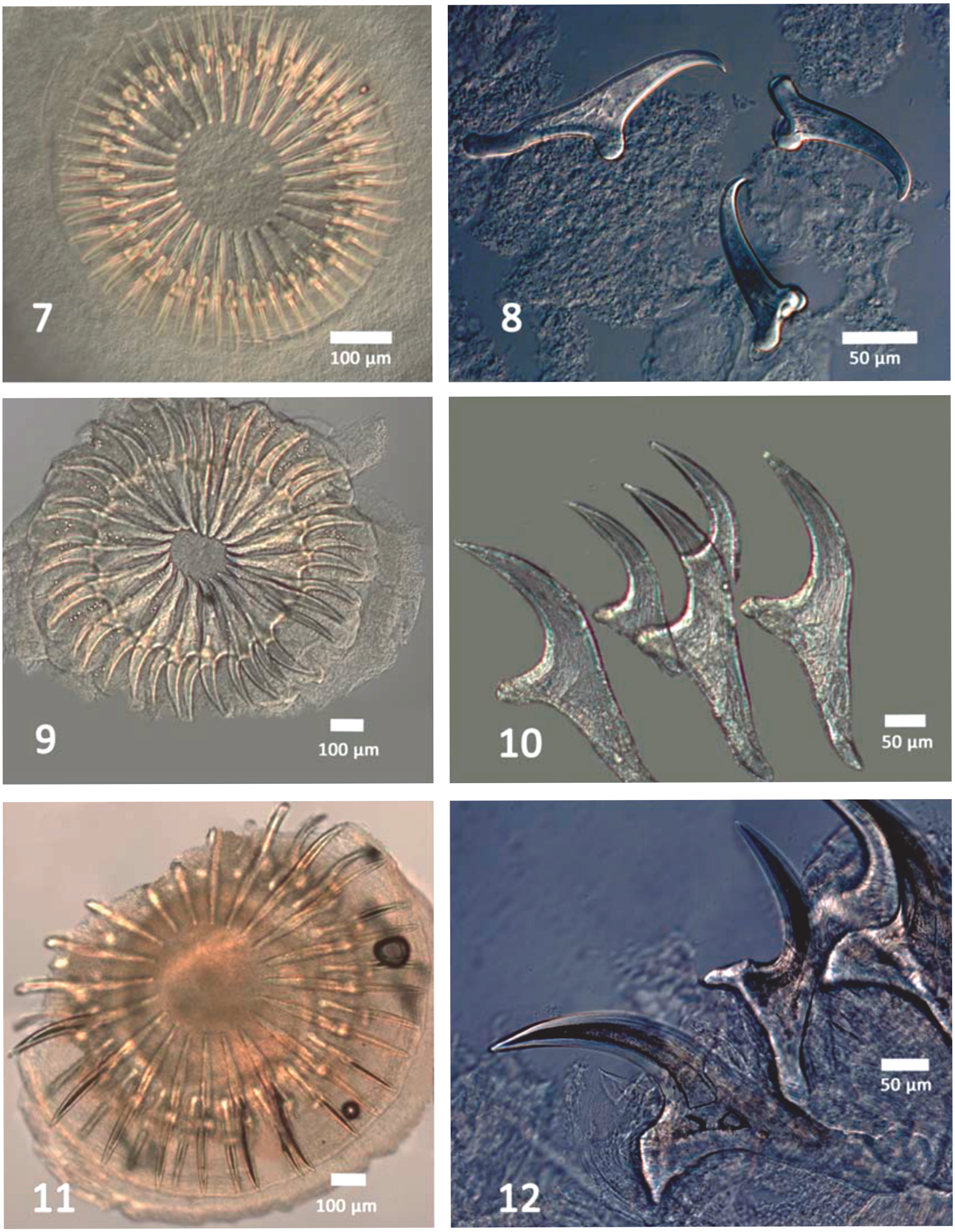

Fig. 4: Rostellum and hooks extracted from larvae collected from metacestodes representing species of *Taenia* from small mammals obtained during our biodiversity survey of south and southwestern Mongolia - collected from 2009 - 2012; *Taenia polyacantha* - 7 & 8, undescribed species of *Taenia* - 9 & 10, *T. taeniaeformis* - 11 & 12.

Table 3: Univariate statistics showing measures of mean, standard deviation (Stdv), coefficient of variation (CV), the range of hook sizes, and the variance (var) for both large and small hooks for each of the seven species of *Taenia* found in mammals from Mongolia

Taeniid spp.	*large hooks*					*small hooks*				
	mean	**Stdv**	**CV**	**range**	**var**	**mean**	**Stdv**	**CV**	**range**	**var**
	total length					***total length***				
T. endothoracicus	356.66	7.17	2.01	344-367	51.47	224.56	8.49	3.78	212-237	72.02
T. hydatigena	213.07	3.57	1.68	206-217	12.77	148.58	4.41	2.97	140-156	19.46
T. krepkogorski	321.33	4.18	1.30	316-327	17.47	217.86	2.11	0.97	214-222	4.44
T. polyacantha	179.45	12.59	7.02	166-193	158.57	116.92	5.76	4.93	109-123	33.20
T. retracta	316.60	2.66	0.84	314-323	7.10	210.12	2.36	1.12	206-214	5.58
T. taeniaeformis	479.10	4.19	0.87	471-484	17.56	300.97	11.21	3.72	280-315	125.65
undescribed	448.56	2.95	0.66	444-454	8.68	291.90	4.57	1.57	285-299	20.90
	guard length					***guard length***				
T. endothoracicus	70.79	5.06	7.15	62-75	25.65	62.68	4.74	7.56	58-70	22.44
T. hydatigena	65.38	1.95	2.98	62-69	3.81	66.24	4.15	6.27	60-72	17.25
T. krepkogorski	107.41	4.71	4.39	101-115	22.23	67.06	4.59	6.84	57-71	21.08
T. polyacantha	47.41	7.07	14.91	34-55	49.93	36.91	5.58	15.12	30-44	31.13
T. retracta	64.35	3.36	5.22	56-68	11.30	57.02	2.94	5.16	52-62	8.66
T. taeniaeformis	176.26	8.54	4.85	162-189	72.95	121.33	7.39	6.09	105-131	54.58
undescribed	151.86	5.70	3.75	143-163	32.49	101.46	5.20	5.13	88-105	27.04
	handle length					***handle length***				
T. endothoracicus	154.47	6.28	4.07	142-162	39.50	77.17	3.64	4.72	73-83	13.24
T. hydatigena	105.04	3.54	3.37	101-111	12.55	63.52	3.05	4.8	59-67	9.33
T. krepkogorski	135.23	1.46	1.08	133-138	2.14	67.08	2.47	3.68	63-71	6.08
T. polyacantha	79.46	10.66	13.42	66-90	113.62	25.39	2.29	9.02	20-29	5.25
T. retracta	114.45	3.48	3.04	109-119	12.09	75.75	3.89	5.14	70-81	15.15
T. taeniaeformis	222.34	5.07	2.28	214-229	25.69	116.44	8.19	7.03	96-125	67.06
undescribed	199.55	2.30	1.15	196-203	5.28	91.33	3.13	3.43	87-99	9.83

Multivariate Morphometrics

Principal Component Analysis

In the principal component analysis (PCA), most of the variance in the dataset was accounted for by the first three components with contribution of eigenvalues of 85 %, 8.5 %, and 4.1 % for components 1, 2, and 3, respectively. Size was the most important in the first component and all eight characters showed positive loadings on PC-1. The loadings of the characters on PC-2 showed more variability and this can probably be considered more of a shape component with handle length of the large hooks and especially guard width of both small and large hooks showing negative loadings on PC-2 (fig. 6). For both large and small hooks among all species considered, negative loadings show that as length of the blade increases, the size of the guard-width and handle-length decreases. The plot of individual eigenvalues on both PC-1 and PC-2 suggest good separation among individuals with only *T. endothoracicus* and *T. retracta* clustering relatively closely together (fig. 6). The same pattern of discrimination among species was achieved using canonical discriminant analysis (CDA) providing no additional resolution, therefore results of the CDA are not shown.

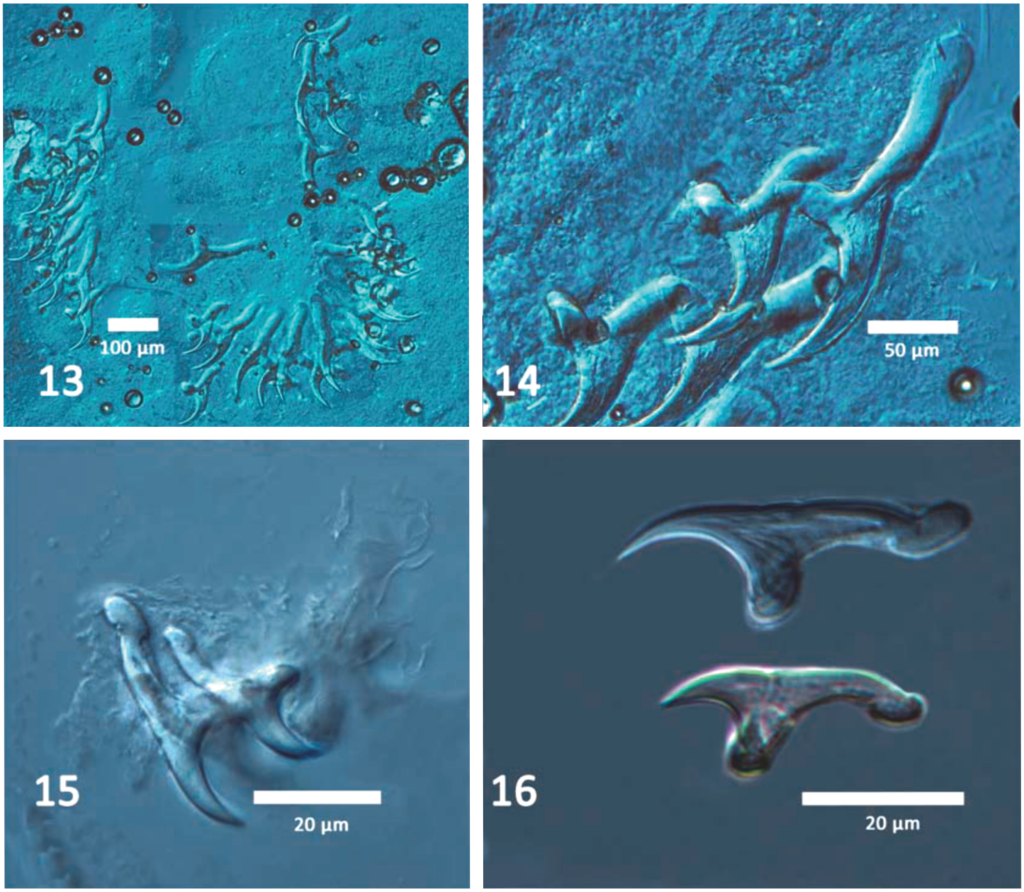

Fig. 4: Rostellum and hooks extracted from larvae collected from metacestodes representing species of *Taenia* from small mammals obtained during our biodiversity survey of south and southwestern Mongolia - collected from 2009 - 2012]; *Taenia hydatigena* - 13 & 14, and *Versteria* cf. *mustelae* – 15 & 16.

Discussion

Much of the initial impetus of work on the helminth fauna of the mammals of Mongolia came from the study by BANNIKOV (1954) who published the first large-scale monographic and scientific treatment of the mammal fauna there. The current paper reports on the results of only a small part of our survey of small mammals and their parasites from south-central and south-west Mongolia. For recent reviews of parasitology of mammals from in and around Mongolia (see TINNIN et al., 2011a; TINNIN et al., 2011b; GANZORIG et al., 1996-1997; and GANZORIG et al., 1998 [for helminths]; TINNIN et al., 2012a, 2012b; JENSEN et al., 2015 [for coccidia]; GARDNER et al., 2013 [for *Echinococcus*]; KIEFER et al., 2012 [for Siphonaptera]; and SCHEFFLER et al., 2012 [for ectoparasites of bats]).

Larval cestodes of the family Taeniidae have previously been reported from wild mammals from Mongolia by several researchers (see summaries in: DANZAN 1978; GANZORIG et al., 1996-1997; GANZORIG et al., 1998; and TINNIN et al., 2011).

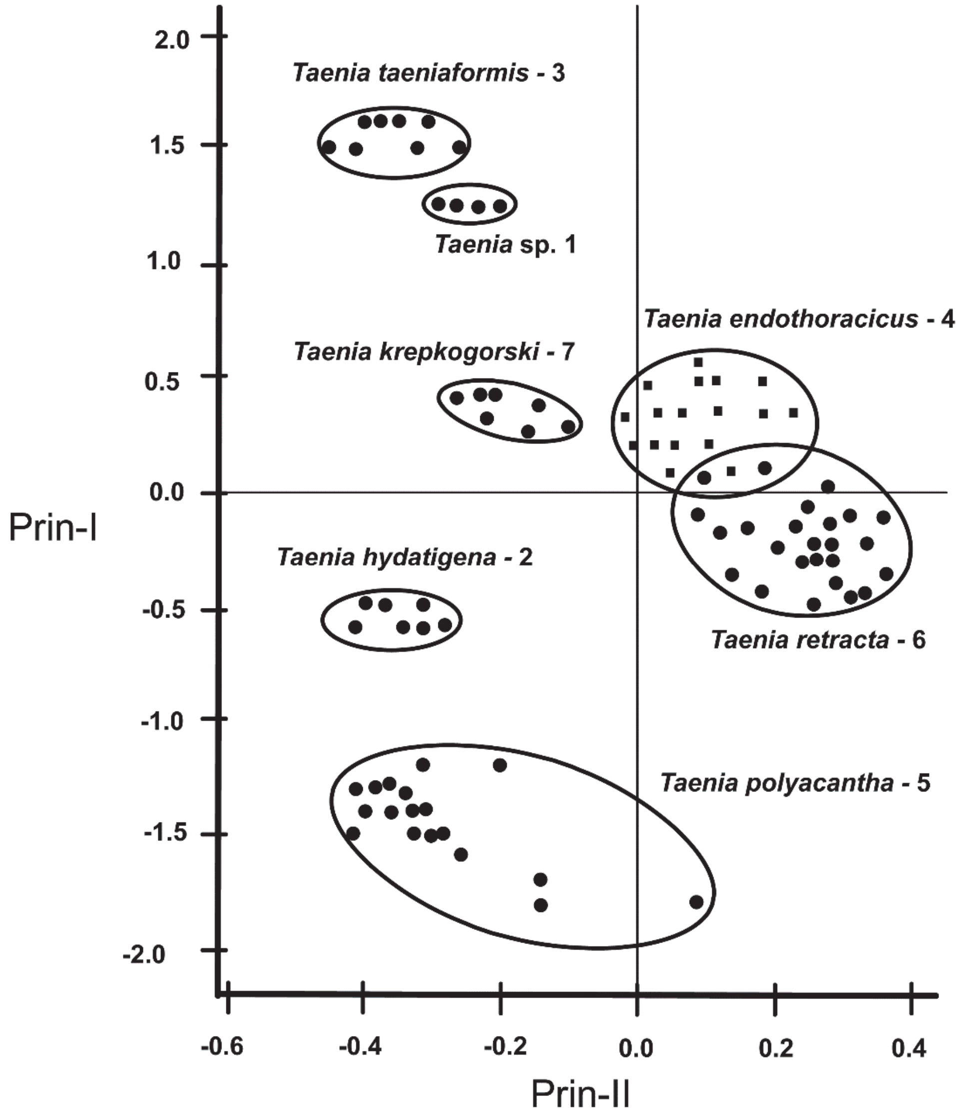

Fig. 6: Plot of eigenvalues of matrix of principal components I and II of all hooks of *Taenia* measured in this study. Good separation of all species except *T. edothoracicus* (TE) (fig. 3, 3 & 4) and *T. retracta* (TR) (fig. 3, 5 & 6) is evident. The species TE and TR have similar shapes and are closer in multivariate space than the other species analyzed.

Interestingly, of the six species of parasitic helminths reported by GANZORIG et al. (1998) only three of the same species of the family Taeniidae were collected during our work; these included *T. polyacantha*, *T. endothoracicus*, and *Versteria cf. mustelae* (GMELIN, 1790). The diversity of species of the larval forms we collected from both rodents and lagomorphs was highest in the area south of Ulaanbaatar at the Gurvan Saikhan National Park. A combination of environmental factors contributed to this high diversity: the area encompasses many habitat-types including steppe, steppe desert, grassland, deciduous forest, and alpine meadows. The protected status of the park-area also ensures the perpetuation of a greater diversity of both potential definitive and intermediate hosts. In this general area, we obtained five species of *Taenia* with three different species alone from a single locality (Baruun Saikhan Uul).

Our multivariate morphometric analyses indicate that hook length and shape were important characteristics for identification of larvae of Taeniide. Hook shape and size varied greatly among the seven *Taenia* species in our study and seemed to be relatively species specific. For example, *T. retracta* and *T. endothoracicus* were more specialized and only found within *Ochotona* and *Meriones* spp., respectively, but *T. polyacantha* was more of a generalist and occurs in species of rodents in the genera *Allactaga*, *Meriones*, and *Mus*. Another interesting characteristic of hook morphometrics was that as the length of the blade increased in size, the guard-width and handle-length decreased. This might be a result of stabilizing selection preventing hook sizes from getting too big for the rostellum. The undescribed species in our analysis was different from any of the other *Taenia* in our study or other previously described species. This Taeniid species showed measurements that were similar to *T. taeniaeformis*, however the shape and structure of the large and small hooks looked much different (*T. taeniaeformis* [fig. 4, 11 & 12] and undescribed sp. [fig. 2, 9 & 10]). Unfortunately, only one individual mammal in our study was infected with this undescribed species of *Taenia* and we would need additional specimen to make any further conclusions.

Our analysis of hooks of the larval stages of cestodes and the use of multivariate statistics to quickly classify specimens of *Taenia* to various species is an obvious rapid and inexpensive way to assist ecologists and systematists with identifications. Diagnosis of species can be done with DNA technology, but for many laboratories, this can still be time consuming and expensive.

While our work focussed on the diversity of small mammals and their parasites that in this case includes only larval Taeniidae, we had some opportunity to collect carnivores that serve as definitive hosts for the species of cestodes identified during our study. Unfortunately, our collection of carnivores was limited to only a few individuals of the family Mustelidae. In addition, the conservation status of other canid and felid species (CLARKE et al. 2006) made it impossible to examine fresh specimens for parasites. Additional work on this group of cestodes using the same species is just being completed and will include DNA sequence and genetic analysis along with ecological niche modeling of the cestode species collected.

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Addresses:

S.L. Gardner[1]*
D. M. Tufts[2]
M. Pitner
G. R. Racz
Harold W Manter Laboratory of Parasitology
University of Nebraska State Museum
University of Nebraska-Lincoln
Lincoln, NE 68588-0514
U.S.A.

N. Batsaikhan
Department of Biology
School of Arts and Sciences
National University of Mongolia
P.O.Box 348,
Ulaanbaatar 210646
Mongolia
e-mail: batsaikhan@num.edu.mn

[1] Harold W Manter Laboratory of Parasitology, e-mail: slg@unl.edu
[2] Present address: Postdoctoral Researcher, Columbia University, Department of Ecology, Evolution & Environmental Biology, 1200 Amsterdam Ave, New York, NY 10027
e-mail: dmtufts11@yahoo.com

* Corresponding author