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ABSTRACT: The Document, Assess, Monitoring, Act (DAMA) protocol details an approach to integrating information about parasites into large-scale studies of biodiversity, climate change, and emerging diseases. This study represents an effort to put the DAMA protocol into practice. We collected 101 individuals of protected ranid frogs belonging to the *Pelophylax esculentus* complex during 2012 and 2013 in the Hortobágy National Park (HNP) in eastern Hungary in an area where an inventory of amphibian helminths had been conducted 40 yr previously. Collecting sites included flowing water, a fish pond system, and a wetland marsh system. We found the following helminth species: Digeneans: *Diplodiscus subclavatus*, *Haematoloechus variegatus*, *Opisthioglyphe ranae*, *Pleurogenes claviger*, *Pleurogenoides medians*; Nematodes: *Oswaldocruzia filiformis*, *Rhabdias esculentarum*; and Acanthocephala: *Acanthocephalus ranae*. *Rhabdais esculentarum* is a new species for the Hungarian fauna and *P. ridibundus* represents a new host record for *R. esculentarum* while *D. subclavatus*, *P. claviger*, and *P. medians* are new species for the helminthofauna of the HNP. Our findings showed a significant discrepancy from the results of baseline inventories carried out 40 yr ago, although the reasons for this discrepancy are not clear. We suspect that the previously reported helminth species that we did not encounter are restricted to *Pelophylax lessonae*, a host we have not yet collected at this location, but factors associated with climate change or anthropogenic impacts cannot be ruled out.

KEY WORDS: Helminths of amphibians, *Pelophylax ridibundus*, *Pelophylax esculentus*, Digenea, Nematoda, Acanthocephala, *Rhabdias esculentarum*, Hungary.

For more than a decade, researchers have made the case that parasites should occupy a central role in our efforts to monitor changes in ecosystem structure emerging from global climate change, for detecting the potential for emerging diseases, and for changes in the colonization of human, livestock, or wildlife hosts (Brooks and Hoberg, 2000, 2006, 2013; Daszak et al., 2000; Agosta et al., 2010; Hoberg and Brooks, 2013; Brooks et al., 2014). To accomplish this it is thought that traditional faunal surveys of parasites must be brought back to center stage, but within a novel conceptual framework (Brooks et al., 2014). Traditional parasitological inventories of helminths aim to collect as many parasites from as many hosts as possible—especially hosts that have not been examined for parasites previously—gathering both taxonomic, ecologic, and population data from that extensive destructive sampling. In many cases today, however, the rationale for any such inventory encompasses host species of interest from the perspective of emerging diseases and biodiversity loss. Such hosts, however, often comprise vulnerable populations. Laws and regulations minimizing destructive sampling are aimed at maintaining populations of threatened or endangered animal and plant species in their natural habitats in the face of the current accelerated loss of biodiversity. However, collecting for such a scientific purpose is by no means the greatest threat to such populations (Minteer et al., 2014), and rapidly expanding urbanization, environmental pollution, habitat loss, fragmentation, and emerging infectious diseases pose greater threats (Blaunstein et al., 1994; Berill et al., 1997; Berger et al., 1998; Daszak et al., 1999; Corn, 2000; Cushman, 2006). Nonetheless, the paradoxical notion that parasitologists are "killing animals to save them" is deeply entrenched, leading to the paradox that many researchers desire information about the parasites of their hosts yet do not support inventory activities aimed at obtaining such data. If parasitologists believe that their inventory activities are justified, they need

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to advocate an approach that obviates such knee-jerk reactions by both the public and the scientific community in order to maximize and most efficiently obtain information pertaining to biodiversity.

Brooks et al. (2014) summarized a protocol (Document, Assess, Monitor, Act) with the acronym DAMA in reference to the above-noted need to sample threatened host species, suggesting that their integrated approach provides a proactive capacity to understand, anticipate, and respond to the outcomes of accelerating environmental change and its impact on parasitic and other diseases. While such an approach involves destructive sampling, the DAMA approach suggests that it is not necessary to kill large numbers of hosts to obtain important information about parasite biodiversity. In short, much more important is having and maximizing information available about each species collected (Brooks and Hoberg, 2000). For example, information about parasite transmission dynamics provides critical information about trophic structure in ecosystems (Marcogliese and Cone, 1997; Marcogliese, 2005), and much of that information can now be obtained using nondestructive sampling.

Within the DAMA approach Brooks (1998), and Brooks and Hoberg (2000), suggest that if a host is rare in the inventory site, then fewer hosts should be sampled. If a host is rare and endemic over it is entire geographic range, the option exists to not perform destructive sampling; in such cases, information can still be obtained based upon what is found in phylogenetically or ecologically (or both) related host species in the same area (Brooks and McLennan, 2002).

Such a destructively minimalist approach involves collecting voucher specimens of each species of parasite encountered, along with their deposition in properly curated museum collections to maximize biodiversity information (Davis, 1996; Global Taxonomy Initiative, 1999), in order to provide important historical data and predictive baselines for understanding the patterns and distribution of organisms. This implies that the infrastructure for collections must be regarded as an integral component of any developing program to survey, inventory, and document biodiversity resources, and such voucher specimens become essential references for future nondestructive sampling within the context of the assessment and monitoring elements of DAMA.

Much of the previous work on anuran helminths has focused on faunistic inventories based upon collections taken during a single season. In this study we compare our faunistical findings with historical baselines set up 4 decades ago (Edelényi, 1972; Murai et al., 1983). While information derived from such long-term study allows the determination of the persistence and stability of parasite diversity (Bursey et al., 2010), the DAMA protocol departs from traditional surveys by suggesting that destructive sampling ends with this initial documentation phase. In this light we collected parasite material not just for classical morphology-based taxonomic indentification, but the majority of the helminth specimens were preserved in ethanol for the development of DNA barcodes (unpublished data). Methods such as DNA barcoding have the potential to provide quick and cost-effective ways to monitor parasite species and their transmission dynamics (Moszczynska et al., 2009; Locke et al., 2010, 2011; Prosser et al., 2013) and thus can be used as a baseline for future nondestructive study within the monitoring phase of DAMA using, for example, host feces, urine, or both. While such an approach precludes the possibility of obtaining direct documentation of statistically robust population estimates (noting that such data are part of the assessment phase in the DAMA protocol), such data can be obtained using noninvasive methods—with a caveat. At present, nondestructive sampling of parasites allows only the determination that at least 1 specimen of a given species of parasite is present in a host; hence, there is a critical need to develop better quantitative assessments of parasite loads based on data obtained from noninvasive methods.

The Hortobágy National Park (HNP) was founded in 1973 and is the largest contiguous alkaline steppe in Europe, covering 80,000 ha, and is the largest and oldest national park in Hungary. Approximately 30% of HNP is covered by wetlands including alkaline marshes, wet grasslands, and 75 fish ponds that cover 6,000 ha. The importance of HNP in sustaining wetland wildlife is recognized by the Ramsar Convention, designating 27,000 ha of the national park as Ramsar sites (Ecsedi, 2004).

Inventory data for helminths inhabiting amphibians in the HNP had been reported earlier by Edelényi (1972) and Murai et al. (1983). As in earlier studies of helminths of European amphibians (e.g., Buchvarov, 1962; Kuc and Sulgostowska, 1988a; Düsen and Öz, 2006; Popiolek et al., 2011), the hosts for most of the helminths previously reported from the amphibians of HNP are water frogs of the *Pelophylax esculentus* complex. Therefore we focused on those hosts in this study.

Systematists have begun untangling the complex evolutionary history of the *P. esculentus* complex. In

the process, they have cast doubt on many host identifications in older studies for which no voucher specimens (of hosts or parasites) exist, thus making comparisons with recent studies difficult. For example, Dely (1981) mentioned 2 members of the *P. esculentus* complex as occurring in HNP, *Pelophylax ridibundus* (Pallas, 1771) and *Pelophylax esculentus* (Linnaeus, 1758), the latter of which originates from the hybridization of *P. ridibundus* and *Pelophylax lessonae* (Camerano, 1882) (see Graf and Polls-Pelaz, 1989). Mészáros and Bartos (1978) and Hilbers (2008) recently reported *P. lessonae* from HNP as well. No voucher specimens exist for the *Pelophylax* spp. listed as hosts by Edelényi (1972) and Murai et al. (1983).

In this report we follow the DAMA protocol and present findings documenting the helminth fauna of water frogs in the HNP in eastern Hungary while discussing initial efforts in assessing that fauna with respect to previous studies (Edelényi, 1972; Murai et al., 1983).

MATERIALS AND METHODS

Study site

We collected water frogs from habitats associated with flowing water (47°26′43′′N; 21°10′10′′E and 47°26′38′′N; 21°08′26′′E), the fish pond system (47°37′13′′N; 21°04′35′′E), and marshland sites (47°36′03′′N; 20°52′53′′E) in the HNP.

Data collection

In many countries, including Hungary, all amphibian species are protected—some of them highly protected—and the collection of these individuals is strictly connected to research permits issued by the local authorities. These permits allow destructive sampling of a restricted number of individuals, which in most cases is not sufficient for assessing and monitoring parasite species. Water frogs were manually collected between May 2012 and August of 2013 using dip nets and by collecting fresh road kills. We collected 52 *Pelophylax* spp. individuals from 2 localities associated with flowing water (canals) and 49 individuals from Hortobágy fish ponds and the Egyek-Pusztakócs marsh system. Due to the high rate of hybridization among water frogs, we used molecular taxonomic techniques following the methods of Hauswaldt et al. (2012) to distinguish between *P. ridibundus*, *P. lessonae*, and the hybrid *P. esculentus*. This method is based on the PCR product size differences from the amplification of the serum albumin intron-1 gene fragment.

Preparation and fixation methods

We examined hosts for helminth parasites within 48 hr of capture. The frogs were overanaesthetized in ether-filled glass containers and the body cavity opened via longitudinal ventral incision. The heart, lungs, liver, gall bladder, and urinary bladder were separated and examined under a stereomicroscope. The alimentary tract was opened by cutting from the anterior esophagus to the rectum and the contents placed into Petri dishes containing Ringer's solution and examined under a stereomicroscope. All helminths collected for DNA data were placed in 96% EtOH for storage. Voucher specimens of adult digeneans were fixed in hot 4% formalin and stored in 70% EtOH. Nematodes were fixed in glacial acetic acid, washed in 70% EtOH, and stored in 70% EtOH + 5% glycerine. Acanthocephalans were relaxed in distilled water for 24 hr followed by fixation in alcohol formalin-acetic acid (AFA) for 24 hr and then stored in 70% EtOH. Digeneans and acanthocephalans were stained with aceto-carmine, dehydrated, cleared in cedar oil, and mounted in Damar gum. Nematodes were cleared in glycerol and examined as temporary mounts. Voucher specimens of parasites were deposited in the Parasitological collection and hosts were deposited to the Herpetological collection of the Hungarian Natural History Museum. Additional voucher specimens of *Rhabdias esculentarum* were deposited in the Helminthological collection of the Department of Parasitology, I. I. Schmalhausen Institute of Zoology, Kyiv, Ukraine.

Statistical analysis

We used generalized linear models (GLM) with a binomial error distribution term to determine the overall prevalence differences between sexes, host species, and collection sites. We used GLM with a Poisson error distribution term to determine the overall mean intensity of parasite species per hosts and for comparison of the previously mentioned variables. Based on the characteristics of collection sites, we pooled the data derived from the fish ponds and the Egyek-Pusztakócs marsh system, yielding 2 categories: habitats associated with still water (fish ponds and Egyek-Pusztakócs marsh system) and canals. Statistical analysis was carried out with QP. 3.0 (Rózsa et al., 2000) and in the R statistical environment (R Core Team, 2013). Overall prevalence and overall mean intensity data are followed by 95% confidence intervals; measurement data is presented as mean followed by minimum and maximun values in parentheses.

RESULTS

Table 1 summarizes our helminth findings for the 101 individuals (48 males and 53 females) of *P. ridibundus* and *P. esculentus* we collected. The overall prevalence of endoparasitic helminths was 76.2% (95% CI = 66.89–83.78) in the examined water frog population. The overall mean intensity was 2.44 (95% CI = 2.17–2.71) helminth species per host. No host contained more than 6 species of parasites: 20 (19.8%) of 101 *Pelophylax* spp. harbored 1 species, 24 (23.8%) harbored 2 species, 19 (18.8%) harbored 3 species, 9 (9%) harbored 4 species, 3 (3%) harbored 5 species, and 2 (2%) harbored 6 species. We found that *P. ridibundus* individuals inhabiting canals had a significantly greater overall mean intensity of helminth species per host than did the same species in still water ($\chi^2 = -4.782$, df = 1, $P = 0.002$). No other significant differences were detected. Based upon these data we divided our faunistical findings

Table 1. Species of parasites collected in this study from the Hortobágy National Park, Hungary.*

Species	Family	Host record	Location	Collection number
Haematoloechus variegatus	Haematoloechidae Freitas & Lent, 1939	*P. ridibundus*, *P. esculentus*	lungs	NHMH-18465
Opisthioglyphe ranae	Telorchiidae Looss, 1899	*P. ridibundus*, *P. esculentus*	SI, LI	NHMH-18463
Diplodiscus subclavatus	Diplodiscidae Cohn, 1904	*P. ridibundus*, *P. esculentus*	rectum	NHMH-18464
Pleurogenes claviger	Pleurogenidae Looss, 1899	*P. ridibundus*	SI	NHMH-18461
Pleurogenoides medians	Pleurogenidae Looss, 1899	*P. ridibundus*, *P. esculentus*	SI, LI	NHMH-18462
Oswaldocruzia filiformis	Trichostrongylidae Leiper, 1912	*P. ridibundus*	SI	NHMH-18466
Rhabdias esculentarum	Rhabdiasidae Railliet, 1915	*P. ridibundus*, *P. esculentus*	lungs	NN 6.1, NN 6.2, NN 6.3, NN 6.4
Acanthocephalus ranae	Echinorhynchidae Cobbold, 1879	*P. ridibundus*, *P. esculentus*	SI	NHMH-18467

* SI = small intestine; LI = large intestine; NHMH = Parasitological collection, Hungarian Natural History Museum, Budapest, Hungary; NN = Helminthological collection, Department of Parasitology, I. I. Schmalhausen Institute of Zoology, Kyiv, Ukraine. All specimens were adults.

into 3 categories: Persistent core species; Missing species; and Species new to the area.

Persistent core species

Digenea: *Haematoloechus variegatus* (Rudolphi, 1819) Looss, 1899; *Opisthioglyphe ranae* (Frölich, 1791) Looss, 1899; Nematoda: *Oswaldocruzia filiformis* (Goeze, 1782); and Acanthocephala: *Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911. These species were reported in HNP previously and are commonly reported species in European amphibians. Consequently, these species seem to form a strong and persistent core of parasite species in the anurans of HNP.

Missing species

These helminths were reported in previous studies, were confirmed by examination of deposited voucher specimens, and were absent from our inventory. Digenea: *Codonocephalus urnigerus* (Rudolphi, 1819) metacercaria; *Clinostomum complanatum* (Rudolphi, 1814) metacercaria; *Cephalogonimus retusus* (Dujardin, 1845) Odhner, 1910; *Gorgodera cygnoides* (Zeder, 1800) Looss 1899; *Haplometra cylindracea* (Zeder, 1800) Looss, 1899; *Haematoloechus asper* (Looss, 1899); *Pleurogenes loossi* (Looss, 1890) Africa, 1930; and Nematoda: *Cosmocerca ornata* (Dujardin, 1845).

Species new to the area

Diplodiscus subclavatus (Pallas, 1760) Diesing, 1836

Description ($n = 3$): Body pyramidal, 3.8 (2.7–5.7) mm long and 1.83 (1.1–2.6) mm wide. Oral sucker terminal 0.43 (0.3–0.6) mm wide with well developed oral diverticula. Oesophageal bulb 0.45 (0.35–0.6) mm long and 0.56 (0.4–0.9) mm in diameter. Acetabulum terminal and large with central accessory sucker. Acetabulum 1.93 (1.2–2.5) mm wide and one third of the total body length.

Site of infection: Rectum.

Type host and type locality: Rana spp., Germany (Goeze, 1782).

Other reported hosts and geography: European fire-bellied toad, *Bombina bombina* (Vojtková, 1961, former Czechoslovakia; Vojtková et al., 1963, former Czechoslovakia; Kozák, 1966, former Czechoslovakia; Vojtková and Krivanec, 1970, former Czechoslovakia); yellow-bellied toad, *Bombina variegata* (Kozák, 1966, former Czechoslovakia; Prokopic and Krivanec, 1975, former Czechoslovakia); common toad, *Bufo bufo* (Vojtková, 1961, former Czechoslovakia; Sey, 1964, 1968, Hungary; Kozák, 1973, former Czechoslovakia; Sey, 1991, Hungary; Cox, 1971, United Kingdom; Shimalov and Shimalov, 2001, Belarus); European green toad, *Bufo viridis* (Sey, 1964, 1968, 1991, Hungary; Vashetko and Siddikov, 1999, Uzbekistan; Murvanidze et al., 2008, Georgia); European tree-frog, *Hyla arborea* (Vojtková, 1961, former Czechoslovakia); lemon-yellow tree frog, *Hyla savignyi* (Yildirimhan et al., 2012, Turkey); edible frog, *Pelophylax esculentus* (Edelényi, 1942, 1960, Hungary; Sey, 1964, 1968, Hungary; Popovic and Mikes, 1989, Serbia; Sey, 1991, Hungary; Bjelic-Cabrilo et al., 2009, Serbia; Chikhlaev et al., 2009, Russia; Popiolek et al., 2011, Poland); pool frog, *Pelophylax lessonae* (Vojtková, 1961, former Czechoslovakia; Vojtková et al., 1963, former Czechoslovakia; Kuc and Sulgostowska, 1988b, Poland; Bakhoum et al., 2011, Belarus; Popiolek et al., 2011, Poland); marsh frog, *Pelophylax ridibundus* (Edelényi, 1960, Hungary; Buchvarov, 1962, Bulgaria; Sey, 1964, Hungary; Popovic and Mikes, 1989, Serbia; Sey, 1991, Hungary; Yildirimhan et al., 1996, Turkey; Düsen and Öz, 2006, Turkey; Murvanidze et al., 2008,

Georgia; Popiolek et al., 2011, Poland); *Pelophylax* spp. (Sey, 1983; Sey and Eöry, 1992, Hungary); moor frog, *Rana arvalis* (Kozák, 1973, former Czechoslovakia; Sey, 1991, Hungary); agile frog, *Rana dalmatina* (Kozák, 1973, former Czechoslovakia); Uludağ frog, *Rana macrocnemis* (Murvanidze et al., 2008, Georgia); common frog, *Rana temporaria* (Bovien, 1916, Denmark; Vojtková et al., 1963, former Czechoslovakia; Kozák, 1966, former Czechoslovakia; Prokopic and Krivanec, 1975, former Czechoslovakia; Kuc and Sulgostowska, 1988b, Poland; Sey, 1991, Hungary); alpine newt, *Ichthyosaura alpestris* (Barus et al., 1963, former Czechoslovakia); common newt, *Lissotriton vulgaris* (Prokopic and Krivanec, 1975, former Czechoslovakia; Cedhagen, 1988, Sweden; Bertman, 1994, Poland); northern crested newt, *Triturus cristatus* (Barus et al., 1963, former Czechoslovakia; Vojtková, 1963, former Czechoslovakia; Frandsen, 1974, Denmark; Bertman, 1994, Poland); sand lizard, *Lacerta agilis* (Lewin, 1992, Poland); European grass snake, *Natrix natrix* (Sey, 1991, Hungary; Bertman, 1993, Poland; Buchvarov et al., 2000, Bulgaria; Shimalov and Shimalov, 2000, Belarus; Murvanidze et al., 2008, Georgia); dice snake, *Natrix tessellata* (Buchvarov et al., 2000, Bulgaria); nose-horned viper, *Vipera berus* (Shimalov and Shimalov, 2000, Belarus).

Specimens deposited and collection numbers: One, deposited in the Parasitological Collection of the Hungarian Natural History Museum (NHMH-18464).

Remarks

Previous reports from HNP did not mention the presence of this digenean species even though *D. subclavatus* is a common and widely distributed endoparasite of amphibians throughout Europe. Members of *Diplodiscus* in Eurasia and closely related North American (*Megalodiscus*) and Neotropical (*Catadiscus*) representatives of the Paramphistomidae are among the most-commonly reported anuran digeneans.

Pleurogenes claviger (Rudolphi, 1819) Looss, 1896

Site of infection: Small intestine.

Type host and type locality: Bufo viridis (Laurenti, 1768), Europe (Rudolphi, 1819).

Other reported hosts and geography: Bufo bufo (André, 1912, Switzerland; Cox, 1971, United Kingdom; Frandsen, 1974, Denmark; Cedhagen, 1988, Sweden); *Bufo viridis* (Shimalov and Shimalov, 2001, Belarus); *Natrix natrix* (Mihalca et al., 2007, Romania); *Pelophylax esculentus* (André, 1913, Switzerland; Edelényi, 1942, Hungary; Sey, 1964, 1968, Hungary; Cox, 1971, United Kingdom; Frandsen, 1974, Denmark; Kuc and Sulgostowska, 1988b, Poland; Chikhlaev et al., 2009, Russia; Bjelic-Cabrilo et al., 2009, Serbia); *Pelophylax ridibundus* (Sey, 1964, Hungary; Combes and Gerbeaux, 1970, Spain; Kuc and Sulgostowska, 1988a, Poland; Sey and Eöry, 1992, Hungary; Oguz et al., 1994, Turkey; Yildirimhan et al., 1996, Turkey); *Pelophylax* spp. (Sey and Eöry, 1992, Hungary); *Rana arvalis* (Sey, 1964, Hungary); *Rana macrocnemis* (Yildirimhan et al., 1997; Yildirimhan, Bursey et al., 2006, Turkey); *Rana temporaria* (André, 1913, Switzerland; Bovien, 1916, Denmark; Cox, 1971, United Kingdom; Frandsen, 1974, Denmark; Cedhagen, 1988, Sweden; Tkach et al., 2000, Ukraine); *Lacerta agilis* (Sharpilo et al., 2001, Ukraine).

Specimens deposited and collection numbers: One, deposited in the Parasitological Collection of the Hungarian Natural History Museum (NHMH-18461).

Remarks

Previous reports from HNP did not mention the presence of this digenean species. A few reports from Hungary exist but their occurence is not frequent. We found only 1 specimen in the small intestine of *P. ridibundus* collected from canals and could not generate measurements because of the poor condition of the only available voucher specimen.

Pleurogenoides medians (Olsson, 1876) Travassos, 1921

Description ($n = 5$): Body elongate, 81.2 (65–94) μm long and 50 (35–65) μm wide. Oral sucker is subterminal and round, 15 (10–20) μm in diameter. The ventral sucker is round, 12.6 (12–13) μm in diameter. The testes are symmetrical, posterior to the cecum, 10 (9–11) μm in diameter. The ovary is elongate, lateral to the cecum in the left side, 11.5 (9–14) μm long and 7 (6–8) μm wide.

Site of infection: Small and large intestines.

Type host and type locality: B. viridis, Denmark (Olsson, 1876).

Other reported hosts and geography: Bombina bombina (Vojtková, 1961, former Czechoslovakia; Vojtková et al., 1963, former Czechoslovakia; Vojtková and Vojtek, 1975, former Czechoslovakia); *Bombina variegata* (Vojtková and Vojtek, 1975, former Czechoslovakia); *Bufo bufo* (Cox, 1971, United Kingdom; Kozák, 1973, former Czechoslovakia;

Vojtková and Vojtek, 1975, former Czechoslovakia; Fernandez et al., 1986, Spain; Shimalov and Shimalov, 2001, Belarus); *Bufo viridis* (Kolendo, 1959, Poland; Vojtková and Vojtek, 1975, former Czechoslovakia); natterjack toad, *Epidalea calamita* (Vojtková and Vojtek, 1975, former Czechoslovakia); *Hyla arborea* (Kozák, 1973, former Czechoslovakia; Vojtková and Vojtek, 1975, former Czechoslovakia; Düsen and Öz, 2004, Turkey); *Pelophylax esculentus* (Edelényi, 1942, 1960, Hungary; Sey, 1964, Hungary; Rodrigues et al., 1973 Portugal; Popovic and Mikes, 1989, Serbia; Chikhlaev et al., 2009, Russia); *Pelophylax lessonae* (André, 1913, Switzerland; Bovien, 1916, Denmark; Kopriva, 1957, former Czechoslovakia; Vojtková, 1961, former Czechoslovakia; Vojtková et al., 1963, former Czechoslovakia; Kozák, 1966, 1968, former Czechoslovakia; Sey, 1968, Hungary; Kozák, 1973, former Czechoslovakia; Vojtková and Vojtek, 1975, former Czechoslovakia; Buchvarov, 1977, Bulgaria; Kuc and Sulgostowska, 1988b, Poland; Tkach et al., 2003, Ukraine; Bjelic-Cabrilo et al., 2009, Serbia); *Pelophylax ridibundus* (Edelényi, 1960, Hungary; Buchvarov, 1962, 1977, 1983, Bulgaria; Combes and Gerbeaux, 1970, Spain; Cox, 1971, United Kingdom; Kozák, 1973, former Czechoslovakia; Kuc and Sulgostowska, 1988a, Poland; Popovic and Mikes, 1989, Serbia; Oguz et al., 1994, Turkey; Yildirimhan et al., 1996, Turkey; Hassan and Saeed, 2001, Iraq; Düsen and Öz, 2006, Turkey; Saglam and Arikan, 2006, Turkey; Murvanidze et al., 2008, Georgia); *Pelophylax* spp. (Sey and Eöry, 1992, Hungary); *Rana arvalis* (Kozák, 1973, former Czechoslovakia; Vojtková and Vojtek, 1975, former Czechoslovakia); *Rana dalmatina* (Kozák, 1973, former Czechoslovakia; Prokopic and Krivanec, 1975, former Czechoslovakia; Buchvarov, 1977, Bulgaria; Düsen et al., 2009, Turkey); *Rana macrocnemis* (Yildirimhan, Bursey et al., 2006, Turkey; Düsen, 2007, Turkey; Murvanidze et al., 2008, Georgia); *Rana temporaria* (Bovien, 1916, Denmark; Kopriva, 1957, former Czechoslovakia; Vojtková and Krivanec, 1970, former Czechoslovakia; Prokopic and Krivanec, 1975, former Czechoslovakia; Vojtková and Vojtek, 1975, former Czechoslovakia; Cedhagen, 1988, Sweden); *Lissotriton vulgaris* (Vojtková, 1963, former Czechoslovakia); *Triturus cristatus* (Vojtková, 1963, former Czechoslovakia); *Lacerta agilis* (Lewin, 1992, Poland; Sharpilo et al., 2001, Ukraine); *Natrix tessellata* (Buchvarov et al., 2000, Bulgaria).

Specimens deposited and collection numbers: One, deposited in the Parasitological Collection of the Hungarian Natural History Museum (NHMH-18462).

Remarks

This is a new record for HNP. It has been reported previously in water frogs from Hungary (Edelényi, 1942, 1960; Sey, 1964, 1968; Sey and Eöry, 1992).

Rhabdias esculentarum (Cipriani, Mattiucci, Paoletti, Santoro and Nascetti, 2012)

Description ($n = 4$): Body small, 4.99 (4.34–6.41) mm long, 283 (268–304) μm wide (maximum width at vulval region). Body cuticle partly inflated, especially at the anterior and posterior ends. Buccal capsule wide, 9 (8–10) μm depth, 12.25 (8–14) μm outer diameter, 10 (9–13) μm inner diameter. Oesophageal length 389.5 (362–433) μm (7.9 % of total body length), width 27.5 (26–30) μm at anterior end, 61.75 (57–65) μm at oesophageal bulb. Nerve-ring near middle of oesophagus. Vulva postequatorial, distance from anterior end to vulva 2,759.5 (2,209–3,581) μm. Tail simple and pointed, length 205.5 (183–240) μm (4.16% total body length).

Site of infection: Lungs.

Type host and type locality: Pelophylax lessonae, Lake Vico, Latium, central Italy (Cipriani et al., 2012).

Other reported hosts and geography: Pelophylax esculentus (Cipriani et al., 2012, Italy).

Specimens deposited and collection numbers: Four, deposited in the Helminthological collection of the Department of Parasitology, I. I. Schmalhausen Institute of Zoology (NN 6.1; NN 6.2; NN 6.3; NN 6.4).

Remarks

The shape of the anterior end, shape of the buccal capsule and oesophagus, and the presence of ventral inflation of the body wall posterior to the anus are the characters allowing the specimen to be assigned to *R. esculentarum*. Using these characters, these worms differ from *Rhabdias bufonis* (Schrank, 1788) Stiles and Hassall, 1905; from *Pelophylax* spp.; from *Rhabdias rubrovenosa* (Schneider, 1866) Semenov, 1929; and from *Rhabdias sphaerocephala* (Goodey, 1924), all of which are all known from European bufonids. *Pelophylax ridibundus* is a new host record for *R. esculentarum* and Hungary is a new geographic distribution record.

DISCUSSION

As suggested by Brooks et al. (2014), assessment in the DAMA protocol is a 2-part process. The first of these is comparison of data with baselines (i.e., what

is already known about the parasites, hosts, and geographic location of an inventory) to address the question of how current findings relate to previous findings. The latter requires the understanding and acceptance that new inventories of places and hosts assessed previously are just as important, and possibly even more so, than are inventories of new hosts and new places. This study provides an excellent example of this assertion.

The current amphibian helminth fauna in the HNP appears to differ significantly from that reported previously by Edelényi (1972) and Murai et al. (1983). First, the original study of amphibian helminths in the HNP listed *Pleurogenes loossi* as present. We did not find this species but did find *Pleurogenoides medians* and *Pleurogenes claviger*.

Second, Murai et al. (1983) listed 3 species of digeneans inhabiting the lungs of water frogs: 2 species of *Haematoloechus* (*H. variegatus* and *H. asper*) and *Haplometra cylindracea*. In contrast, we found only *H. variegatus* in this study. All 3 species are historically common: *H. variegatus* has been found in Bulgaria (Buchvarov, 1977), Georgia (Murvanidze et al., 2008), Hungary (Edelényi, 1942, 1960; Sey, 1964, 1968; Murai et al., 1983; Sey and Eöry, 1992), Ukraine (Tkach et al., 2000), Poland (Kuc and Sulgostowska, 1988a, b; Popiolek et al., 2011), Portugal (Rodrigues et al., 1973), Russia (Chikhlaev et al., 2009), Serbia (Popovic and Mikes, 1989; Bjelic-Cabrilo et al., 2009), Turkey (Yildirimhan et al., 1996; Saglam and Arikan, 2006; Yildirimhan, Goldberg et al., 2006), and the United Kingdom (Cox, 1971). *Haematoloechus asper* has been reported from Georgia (Murvanidze et al., 2008), Hungary (Sey, 1964, 1968; Murai et al., 1983), Ukraine (Tkach et al., 2000), and Russia (Chikhlaev et al., 2009); and *H. cylindracea* has been reported from Belarus (Ryzhikov et al., 1980), Bulgaria (Buchvarov, 1977), former Czechoslovakia (Vojtková and Vojtek, 1975), Hungary (Murai et al., 1983), Iran (Mashaii, 2005), Latvia (Ryzhikov et al., 1980), Lithuania (Ryzhikov et al., 1980), Russia (Ryzhikov et al., 1980), Sweden (Cedhagen, 1988), Turkey (Yildirimhan, Bursey et al., 2006; Düsen, 2007, 2012), Ukraine (Ryzhikov et al., 1980; Tkach et al., 2000), and the United Kingdom (Cox, 1971).

Murai et al. (1983) also reported *Cephalogonimus retusus* and *Cosmocerca ornata* in the small intestine and *Gorgodera cygnoides* in the urinary bladder of water frogs in the HNP. Neither species was found in our study. All 3 species are common parasites of amphibians: *C. retusus* has been reported from Armenia (Ryzhikov et al., 1980), Azerbaijan (Ryzhikov et al., 1980), Bulgaria (Buchvarov, 1977; Buchvarov et al., 2000), Georgia (Ryzhikov et al., 1980; Murvanidze et al., 2008), Hungary (Edelényi, 1942, 1960; Sey, 1968; Murai et al., 1983), Portugal (Rodrigues et al., 1973), Russia (Ryzhikov et al., 1980), Serbia (Popovic and Mikes, 1989), and the United Kingdom (Cox, 1971); and *C. ornata* has been reported from Belarus (Shimalov and Shimalov, 2001; Shimalov et al., 2000, 2001), Bulgaria (Buchvarov, 1977), Georgia (Murvanidze et al., 2008), Hungary (Murai et al., 1983), Iran (Mashaii, 2005), Italy (Galli et al., 2000, 2001), Poland (Kuc and Sulgostowska, 1988a, b; Grabda-Kazubska and Lewin, 1989; Popiolek et al., 2011), Russia (Chikhlaev et al., 2009), Sweden (Cedhagen, 1988), Turkey (Düsen and Öz, 2004, 2006; Yildirimhan, Bursey et al., 2006; Düsen, 2007), United Kingdom (Walton, 1933; Cox, 1971), and Uzbekistan (Vashetko and Siddikov, 1999). *Gorgodera cygnoides* has been reported from Azerbaijan (Ryzhikov et al., 1980), Belarus (Ryzhikov et al., 1980; Shimalov and Shimalov, 2001), Bulgaria (Buchvarov, 1977, 1983; Kirin, 2002), former Czechoslovakia (Vojtková and Vojtek, 1975), Georgia (Ryzhikov et al., 1980; Murvanidze et al., 2008), Greece (Hristovski et al., 2006), Hungary (Edelényi, 1942, 1960; Sey, 1964, 1968; Murai et al., 1983), Kyrgyzstan (Ryzhikov et al., 1980), Macedonia (Hristovski et al., 2006), Poland (Kuc and Sulgostowska, 1988a, b; Popiolek et al., 2011), Portugal (Rodrigues et al., 1973), Russia (Ryzhikov et al., 1980), Serbia (Popovic and Mikes, 1989), Tadzhikistan (Ryzhikov et al., 1980), Turkey (Yildirimhan et al., 1996; Düsen and Öz, 2006; Yildirimhan, Goldberg et al., 2006; Yildirimhan et al., 2009; Düsen and Oguz, 2010), Ukraine (Ryzhikov et al., 1980), United Kingdom (Cox, 1971), and Uzbekistan (Ryzhikov et al., 1980). The absence of *C. retusus*, *C. ornata*, *G. cygnoides*, *H. asper*, *H. cylindracea*, and *P. loossi* were confirmed by sampling in 2013.

Murai et al. (1983) did not report members of 2 common amphibian helminth taxa that we detected, including nematodes belonging to the genus *Rhabdias*, which includes species living in the lungs of both amphibians and some lizards (Cipriani et al., 2012; Kuzmin, 2013). Most studies of *Rhabdias* in European amphibians have reported *R. bufonis*, and Edelényi (1960) reported this worm from *P. esculentus*, *P. ridibundus*, and *Bufo viridis* near the HNP. However, Murai et al. (1983) did not list any species of *Rhabdias*. Recent research suggests that specimens identified as *R. bufonis* represent a species complex

(Tkach et al., 2014). Cipriani et al. (2012) recently described *R. esculentarum* from both *P. lessonae* and *P. esculentus* in Italy, and our specimens in water frogs from HNP correspond to that species. We have not collected parasites from *Bufo bufo* (Linnaeus, 1758) or *B. viridis*, so we do not know if both *R. bufonis* and *R. esculentarum* do, or did, co-occur in the HNP. If the report of *R. bufonis* in *Pelophylax* spp. by Edelényi (1960) actually referred to *R. esculentarum*, then *R. esculentarum* must be assigned to the group of persistent core species of helminths in the water frogs of HNP.

Interestingly, *D. subclavatus* occurs in the rectum of frogs throughout Europe. We are therefore surprised that neither Edelényi (1972) nor Murai et al. (1983) listed *D. subclavatus*. Given its widespread geographic and host range, we would expect this species to be 1 of the persistent core species as well.

If we provisionally assign *R. esculentarum* to the category of persistent core species, the changes in the helminth fauna of water frogs in HNP consists of the loss of 5 species of digenean, 1 species of nematode, and the addition of *D. subclavatus*. These species seem to have little in common with respect to their basic ecology: as a group they live in the rectum, small intestine, urinary bladder, and lungs. Their first intermediate hosts include both gastropods and bivalves, and their second intermediate hosts range from the surface of plants and the exoskeleton of crustaceans to odonate larvae and tadpoles.

One thing the missing species may have in common is their anuran host. Of the 3 members of the *P. esculentus* complex occurring in HNP, Edelényi (1972) and Murai et al. (1983) listed only *P. ridibundus* and *P. esculentus* as hosts, and all the missing helminth species were reported from these 2 species. Unfortunately, no vouchers of collected hosts were deposited, and it is possible that some of the hosts identified previously as *P. ridibundus* were actually *P. esculentus* or *P. lessonae*. In this study, we collected only *P. ridibundus* and *P. esculentus*, so it is tempting to suggest that the missing parasites still occur in the HNP but that they are restricted to *P. lessonae*, which we have not yet collected. Such an explanation leads to the question: Has this species experienced a decline, or even extirpation, in HNP?

The answer to the above question may be due to localized anthropogenic phenomena, incuding the effects of urbanization, agriculture, or both, which may have reduced or changed the composition of the invertebrate fauna necessary to maintain the transmission dynamics of the missing species of helminths and which may have allowed *D. subclavatus*, if it is truly new to HNP, to become established. Given the protected status of the study site, we do not believe that there have been such significant anthropogenic changes inside HNP, implying that regional effects, including climate change, may play an important role in this regard. At this higher level of assessment we would expect to see a geographic pattern distinguishing the species that have been lost from those that persist. However, at this level no such obvious patterns exist that might explain the differences in results between our study and the previous inventories.

Previous reports did not list information about the prevalence and intensity of infection, and all water frogs were identified as either *Rana ridibunda* (=*P. ridibundus*) or *Rana esculenta* (=*P. esculentus*). It is possible that the missing parasites were quite rare in previous studies, and that they still exist as rare species today, which is supported by our finding of only a single specimen of *P. claviger* during 2 yr of sampling. Alternatively, it is also possible that *P. lessonae* and the missing parasites were previously more common and that they have become rare in the HNP. We suggest that additional efforts be made to find and assess the status of *P. lessonae*, and their parasites, in the HNP.

A second class of baseline comparisons is evolutionary, via asking "Parascript" (Manter, 1966) questions about the phylogenetic context of the ecology and distribution of the parasites found in an inventory. Such data are rare, and not often applied to inventory studies in parasitology, despite a generation of calls for such action (e.g., Brooks and McLennan, 1993; Brooks, 1998; Brooks and Hoberg, 2000, 2006, 2007, 2013; Hoberg, 1997, 2010; Hoberg and Brooks, 2008, 2010, 2013; Hoberg et al., 2012, 2013; Brooks et al., 2014). Because the documentation phase of the DAMA protocol precludes the possibility of obtaining such direct documentation of statistically robust population estimates, our results must be considered highly preliminary, at best, given the small sample size necessitated by the constraints of collecting permits. Nevertheless, such population level data are a critical part of the assessment phase in the DAMA protocol and they can be obtained in the future using noninvasive methods that can provide both qualitative (presence–absence) and quantitative (relative abundance) data (e.g., Endo et al., 2009). As such, our data serve as a starting point and marker for the monitoring element of the HNP within the context of

the DAMA protocol. Thus, while it may seem somewhat clumsy to undertake an inventory and monitoring project in separate phases, we believe that this approach maximizes the amount of information obtained while minimizing the amount of destructive sampling needed over the long term. In short, we believe that the DAMA protocol initiated herein provides an excellent means of obtaining useful information from parasite inventories (both within the HNP and elsewhere), even though limited destructive sampling occurs. The addition of molecular genetic data derived from parasites collected in this study will allow additional critical information to be gathered using nondestructive techniques in the future.

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