



First report of *Euryhelmis* parasites (Trematoda, Heterophyidae) in Africa: conservation implications for endemic amphibians

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Received: 2 January 2018 / Accepted: 24 May 2018 / Published online: 2 June 2018
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Abstract

In this study, we report, through molecular identification, the first African records of a digenean trematode parasite of the genus *Euryhelmis*. We recovered metacercariae encysted in an anuran, the endemic Moroccan painted frog (*Discoglossus scovazzi*), and a vulnerable caudate, the North African fire salamander (*Salamandra algira*), from four localities in North Africa (Morocco). Our records go back to the past century and have been confirmed in successive fieldwork seasons thereafter. Metacercarial stages of these parasites require amphibians as the last intermediate host, but the exact identity of the primary hosts and predators of the infected animals in Africa remain unknown. Our searches with basic local alignment search tool (BLAST) from Genbank revealed that hosts were infected by parasites of *Euryhelmis costaricensis*, which showed almost the same genetic identity (with only one substitution) to previous reports from Costa Rica and Japan, suggesting a recent introduction in Morocco. We proceed to discuss the likely role of introduced mustelids as the potential definitive hosts of trematode adults. Under this assumption, we conclude that the infestation of *Discoglossus scovazzi* and *Salamandra algira* might pose a risk to these threatened species.

Keywords Metacercaria · *Euryhelmis* · *Salamandra algira* · *Discoglossus scovazzi* · Morocco · Amphibian conservation

Introduction

Species' increasing declines are a central topic in conservation biology and represent a major challenge for this century (Houlahan et al. 2000; Alford et al. 2001). According to predictive models, many species could become extinct over the next few decades (Stuart et al. 2004; Miraldo et al. 2016). Amphibian decline is often used as an example of the loss of global biodiversity (Collins and Storer 2003; Hopkins 2007). Since the nineteenth century, populations have declined globally and amphibian extinction threats remain higher than those

of either birds or mammals (Lips et al. 2006). The extinctions of amphibian populations throughout disparate regions are a consequence of combined factors acting simultaneously, such as the introduction of pathogens in natural systems, even in protected areas (Young et al. 2001; Bosch and Martinez-Solano 2006).

Although there are several explanations for the origin of pathogens affecting amphibian populations (Rachowicz et al. 2005), the anthropogenic introduction of exotic species has a crucial role in wildlife infectious diseases. Reports of emerging infectious diseases appearing in wild populations or expanding their geographical range along, with an increase of incidence and virulence, have become common in the last decades (Daszak et al. 1999, 2003; Collins and Storer 2003; Johnson et al. 2004; de Castro and Bolker 2005; Rachowicz et al. 2006; Sato et al. 2010). The causes for the relatively synchronous emergence of amphibian diseases globally include human-assisted introductions to previously unexposed amphibian populations or an alteration of pre-existing host-parasite relationships (Pounds et al. 2006). A classical example, the amphibian chytridiomycosis (*Batrachochytrium* sp.), has been recorded worldwide causing potentially fatal epidermal infections in amphibians (Weldon et al. 2004; McDonald et al. 2005; Skerratt et al. 2007; Duffus and Cunningham 2010). From a recent Asian origin (O'Hanlon et al. 2018),

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Batrachochytrium dendrobatidis has resulted in extensive disease, mass mortality and extinctions in a wide variety of amphibian species across three orders: Anura, Urodela and Gymnophiona, while *Batrachochytrium salamandrivorans* affects exclusively Urodela (Martel et al. 2014).

The family Heterophyidae Leiper, 1909 are intestinal flukes including important human pathogens. *Euryhelmsis* spp. (Trematoda, Heterophyidae) are a wide-bodied trematode parasite with a complex lifecycle, which require at least three hosts to complete it. Eggs released in water hatch into miracidia and usually infect freshwater molluscs. The released cercariae, emerging from the snails in an aquatic habitat, penetrate the exposed skin of second intermediate hosts and, consequently, encyst in the subcutaneous connective tissue developing into metacercariae. Wild amphibian or fish harbouring metacercariae are consumed by different predators, such as mustelids, to become definitive hosts of the adult intestinal parasites or flukes, facilitating the completion of the entire life cycle (Anderson and Pratt 1965; Simon 1972; Bray et al. 2008). Finally, even humans can be infected if they were to eat uncooked or improperly cooked fish or frog legs (Bowman et al. 2008).

Little is known about the phylogenetic relationships in *Euryhelmsis* spp. The family Heterophyidae Leiper, 1909 belong to the superfamily Opisthorchioidea Looss, 1899, which comprise three families with similar lifecycles. On the basis to morphological and molecular characters, the relationships among genera and species within families remain uncertain (Waikagul and Thakham 2014).

Genetic data suggests that at least one species of *Euryhelmsis* may have been introduced from Central America to Eastern Asia. *Euryhelmsis costaricensis* Brenes, Arroyo, Quirós et Jiménez, 1960 was firstly recorded from *Mustela frenata costaricensis* in Costa Rica (Brenes et al. 1960). Specimens of this parasite have also been identified in the intestine of Japanese martens (*Martes melampus*) and Japanese badgers (*Meles anakuma*) (Sato et al. 1999), and more recently, it was reported in cutaneous nodular lesions and cysts of Japanese hynobiid salamanders (*Hynobius lichenatus*). The frequency, severity and the distribution range of diseased salamanders have increased massively since the year 2000, constituting a threat for the species (Sato et al. 2010).

Herein, we collected samples of trematodes infecting the threatened North African fire salamander (*Salamandra algira*) and the Moroccan painted frog (*Discoglossus scovazzi*) from four localities (Table 1) from the Rift region in North Morocco, and we compared with another specimen deposited in British Museum from a cave in the region of Taza (Middle Atlas) (Fig. 1). We conduct a genetic identification of cysts to the species level based on the closest match through the local alignment search tool (BLAST) from Genbank, and we report on the likely vectors and the consequences of infection mode.

Table 1 Details of the four localities in Morocco (coordinates, altitude) with infected individuals, habitat description and species accounts at the sites

Infected species	Locality/year	Coordinates	Altitude (m)	Habitat description	Species account	Observations
Two <i>Salamandra algira splendens</i> post-metamorphics	Akshur—near Talembot 1999	N 35.24762 W 5.19623	329	Small channelled source that drains to main river	<i>Abytes maurus</i> , <i>Pelophylax saharicus</i> , <i>Discoglossus scovazzi</i> , <i>Bufo spinosus</i> , <i>Hyla meridionalis</i>	Close to Jebel Kelaia locality
Two newly metamorphosed <i>Salamandra algira tingitana</i> , one <i>Discoglossus scovazzi</i> (all sequenced)	Jebel Bu Kilaia 2010	N 35.15311 W 5.76483	258	See Fig. 4b	<i>Hyla meridionalis</i> , <i>Pelophylax saharicus</i> , <i>Bufo spinosus</i> , <i>Discoglossus scovazzi</i> Potential predators: <i>Pleurodeles waltl</i> , <i>Muremys leprosa</i> , <i>Emys orbicularis</i> , <i>Natrix maura</i>	One salamander with a high degree of infestation near a stream with <i>Planorbis</i> sp. water snails. The painted frog did not show external sign of infestation
One <i>Salamandra algira tingitana</i> post-metamorphic (sequenced)	Cudia Quertquera 2013	N 35.46011 W 5.41900	135	Irrigation ditch by river. See Fig. 4a	<i>Pelophylax saharicus</i> , <i>Sclerophrys mauritanica</i> , <i>Discoglossus scovazzi</i> and <i>Muremys leprosa</i>	Highly infested animal lethargic behaviour.
One <i>Salamandra algira splendens</i> post-metamorphic	Jebel Kelaia 2015	N 35.22366 W 5.24357	830	Stream in terrace croplands	<i>Discoglossus scovazzi</i> and <i>Pelophylax saharicus</i>	Same mountain of the terra typica of the salamander subspecies

Fig. 1 Voucher (CWP 56 3-3^a) of *Salamandra algira splendens* from Ikfou Ouan Cave, (Taza region), 1330 m above sea level (Morocco). Natural History Museum London (year 1973).



Material and methods

Fieldwork, sampling and identification

Newly metamorphosed specimens of *Salamandra algira* were found with subcutaneous cysts (Fig. 2) throughout several expeditions to North Morocco (between 1999 and 2015) by one of the authors (DD-B) (Fig. 3). We collected between two to five cysts per individual by excision of tail or toeclipping for molecular analysis from three localities (Jebel Bu Klila, Cudia Queriquera and Jebel Kelaia) (Figs. 3 and 4). In addition, one dry carcass of *Discoglossus* sp. found at Jebel Bu Klila was also collected for DNA analyses. Samples (host and cyst) were immediately preserved in 100% ethanol avoiding biological contamination from other sites.

Molecular analysis

DNA was extracted with a Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the instructions from the manufacturer. The nuclear 18S rDNA and the mitochondrial cytochrome *b* (*MT-CYB*) were targeted to identify the pathogen and hosts, respectively. The primers used were 18S rDNA C-for 5'-ATGG CTCATTAAATCAGCTAT-3', A-Rev 5'-TGCT

TTGAGCACTCAAAT TTG-3' (Routtu et al. 2014) and *MT-CYB*; L15172 5'-TGAGGACAAATATCATTCTG AGG-3' and H15557 5'-GGCGAATAGGAARTATCATT C-3' (Hillis et al. 1996). Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous base calls in Sequencher v.4.9. Sequences were aligned in Seaview v.4.2.11 (Gouy et al. 2010) under ClustalW2 (Larkin et al. 2007) default settings. Searches with BLAST were carried out in Genbank to identify pathogens and hosts to the species level. All PCRs were tested with positive and negative controls.

Results

Cysts were sequenced from Jebel Bu Klila, Cudia Queriquera and Jebel Kelaia and hosts from Jebel Bu Klila and Cudia Queriquera (the host sample from Jebel Kelaia failed to amplify clean sequences and were excluded from analyses). All controls were clear for contamination. PCR amplification of the hosts and subsequent Genbank blast searches of the fragment of *MT-CYB* (circa 300 base pair) from Cudia Queriquera and Jebel Bu Klila matched *Salamandra algira tingitana* (100% match), belonging to the larviparous clade of *S. a. tingitana* (Donaire and Bogaerts 2016). The anuran sequence matched *Discoglossus scovazzi* (99% match, 1 base pair difference), in agreement with this species' known distribution (Vences et al. 2014). *Euryhormis* was amplified from three cyst samples of the salamanders and five times per individual. The sequenced 700 base pair fraction of the 18S rDNA Genbank blast search matched with a 99% identity to *Euryhormis costaricensis* (Genbank accessions; AB521797-800) with a difference of one base pair. The only divergence between the 18S rDNA gene fragments amplified from the parasite of *D. scovazzi* and *S. algira* was an ambiguity of a C-T in the former and a C in the latter, in one polymorphic site (Genbank accessions, MH025805-MH025808). For all salamander trematodes analysed, we recovered the same haplotype.



Fig. 2 Infected *Salamandra algira* with visible cysts of *Euryhormis*. Locality: Cudia Queriquera (2013)

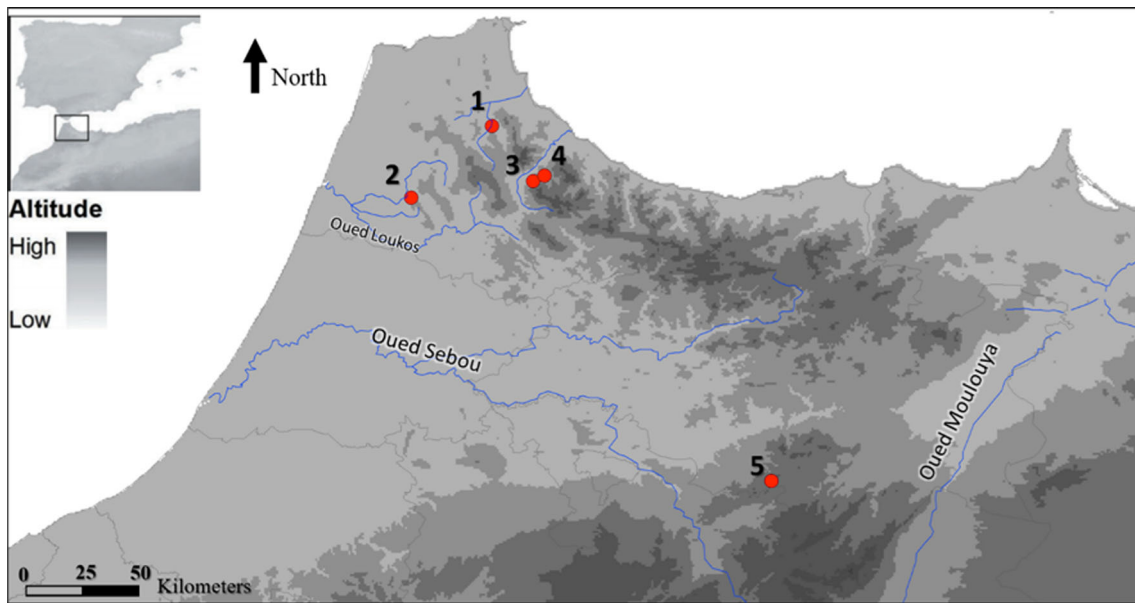


Fig. 3 Map of sampled localities in Morocco and years where metacercaria have been found. 1 Cudia Queriquera (2013), 2 Jebel Bu Klila (2010), 3 Jebel Kelaa (2015), 4 Akshur (1999), 5 Ikfou Ouan Cave (1973)

Discussion

We report the first African records of the *genus Euryhelmis* Poche, 1926 (Heterophyidae; Opisthorchiida). Until now, larval forms of *Euryhelmis* sp. have been described in European amphibians from tadpoles of *Pelophylax kl. esculentus* (Walton 1949), *Rana perezi* (Jiménez 2003) and *Rana temporaria* (Grabda-Kazubska 1980); *Amolops afahanus* in Thailand (Wongsawad et al. 2004); and *Lithobates catesbeianus* (Mata-Lopez et al. 2010) and several other anuran species in the USA (Anderson and Pratt 1965; Knutson et al. 2002; Bowman et al. 2008).

The results herein reveal the presence of the same cysts described in the Tohoku salamander (*Hynobius lichenatus*) in post-metamorphic individuals of *Salamandra algira* from Africa. All Genbank blast searches of the pathogen sequences matched *Euryhelmis costaricensis*. Pathogen sequences from salamanders recovered the same haplotype, while that of *Discoglossus scovazzi* just differed by one base pair substitution to the pathogen sequence of the salamander. However, caution is needed to ascertain the identity of the pathogen based to this conserved region, as other closely related *Euryhelmis* sp. sequences are highly similar. For example, nucleotide identities between the partial 5.8S-ITS2 and 28S rDNA regions between *Euryhelmis zelleri* and *E. costaricensis* matched 98% (Heneberg et al. 2015). The homologous fraction of the 18S rDNA is not available in Genbank at present for full genetic comparison.

Our analysis identified the presence of *Euryhelmis* cysts in intermediate hosts to nearly two decades ago. These findings suggest that this particular parasite in Morocco is not a sporadic or localised episode but rather



Fig. 4 Photographs of localities where the infected *Salamandra* and *Discoglossus* where found. **a** Cudia Queriquera (DD-B.). **b** Jebel Bu Klila (photo by Wouter Beukema)

well established throughout an extensive region. Furthermore, a voucher from the Middle Atlas of an infected *S. algira splendens*, apparently with the same cysts, seems to indicate its presence there for over 40 years (dated from 1973, London Natural History Museum; Fig. 1), suggesting that this genus has likely long gone unnoticed. There are no reports of amphibians infected by *E. costaricensis* or *E. zelleri* in southern Europe. Despite the inherent difficulty to collect infected amphibians related to the short time of cyst expression, only present in the skin of newly metamorphosed individuals, a molecular analysis of metacercariae of the genus *Euryhelminis* is desirable. A full comparative study of helminth fauna from the Iberian Peninsula will contribute to clarify the presence of these species.

Potential final hosts

The predators of the infected amphibians and the primary hosts of the trematodes in Morocco remain unknown. Yet, it is likely that the final hosts might be introduced riparian mustelids in North Africa. *Euryhelminis* adults have been reported from the intestine of riparian mustelids in Europe, America, Asia and New Zealand (Hoberg et al. 1990; McDonald and Lariviere 2001; Torres et al. 2008; Sato et al. 2010). In southwestern Europe, *Euryhelminis squamula* (Rudolphi, 1819), which is morphologically the closest species to *E. costaricensis*, has been isolated in polecat (*Mustela putorius* L., 1758), European mink (*Mustela lutreola* L., 1761), American mink (*Neovison vison* Schereber, 1777), marten (*Martes martes*), badger (*Meles meles*) and otters (*Lutra lutra*) (Torres et al. 2006, 2008).

The Western Palearctic polecat *Mustela putorius*, ranging from the Iberian Peninsula to the Urals (Wolsan 1993), has been recovered in Morocco's Rif region, but the origin of the population remains controversial (Thévenot and Aulagnier 2006). In the Iberian Peninsula, *Mustela putorius* plays an essential role in the life cycle of *Euryhelminis squamula*, more so than *Mustela lutreola*, *Mustela nivalis*, *Meles meles* or *Neovison vison* (Torres et al. 2006; Bakhoum et al. 2009). *Mustela putorius*, *Martes foina* and *Lutra lutra* are also host species in Bulgaria (Yanchev 1987).

The geographical distribution of *Mustela nivalis* is Holarctic, all along the northern hemisphere, throughout Asia, America and Europe (Sheffield and King 1994) and recently reported in North Africa. The phylogeography of these host species include two subclades, one in western Europe, from the Iberian Peninsula to Finland including the British Islands, and another in eastern Europe, which also includes Morocco and introduced populations in Mediterranean islands (Mallorca, Menorca, Corsica, Sardinia, Sicily and Crete) (Lebarbenchon et al. 2010). However, despite the occasional predation on amphibian by *Mustela nivalis* (Blas-Aritio 1970; Fragoso and Santos-Reis 2000), they do not constitute a major part of their diet.

Another amphibian predator, the otter (*Lutra lutra*) (Clavero et al. 2003), can also be infected by *Euryhelminis squamula* throughout the species' European range (Yanchev 1987). Reports have described otters predated on amphibians in Morocco, and the importance of amphibians in its diet has been highlighted in southernmost European localities (Clavero et al. 2005). All these reports are characterised by strong variations in the type of prey, depending on seasons and localities. Two studies refer on the diet of *Lutra lutra* in North Africa: the first deals with droppings collected mainly in Saharan rivers of the High Atlas and Middle Atlas (Broyer et al. 1988), and the second is from Beth Wadi, a typical river of the Middle Atlas (Morocco) (Libois et al. 2015). Fish made up more than 70% of the catches' relative abundance, while the remaining preys were mainly anuran. In Morocco, anurans from the genus *Pelophylax*, *Hyla*, *Discoglossus* and *Bufotes* are known to be part of the otters' diet (Clavero, pers. com.). Although the occupied habitat is not exactly the same as that of our study area, otter diet can include salamanders, conditioned over time by climatic and anthropogenic factors.

In Japan, the infection of salamanders has increased alarmingly after the introduction of the American raccoon (*Procyon lotor*) (Kishimoto 2005; Shimatani et al. 2010). Although there seems to be a correlation between the expansion of infected salamanders with *E. costaricensis* metacercariae (Sato et al. 2010) and the increase population of introduced mammals at the same areas and timing, further monitoring and identification of parasites in feral American minks and raccoons should help clarify this supposed relationship. In any case, it is therefore apparent that other species not strictly described as primary predators of *Euryhelminis* sp. should not be discarded a priori.

Parasite vectors and life cycle

Tadpoles and their role as vectors of metacercariae have been the focus of numerous studies (Hamann and González 2009). From a parasitological perspective, the study of tadpoles acting as vectors is significant in two ways: (a) to assess the link between aquatic and terrestrial ecosystems (Combes et al. 2002) and (b) to understand the effect of the pathogen throughout the host metamorphosis, especially when larvae are potential reservoirs (infected tadpoles can appear healthy while post-metamorphic animals express the infection). The fact that flukes just express after metamorphosis could be related to the nature of the cyst structures, only visible in keratinized tissue. According to previous reports, the infection in metamorphic individuals of hynobiid salamanders can be general, compromising their survival (Sato et al. 2010). Undoubtedly, a high number of metacercarial cysts per individual affects the intensity and prevalence of infection, as occurs with other parasites (Schotthoefer et al. 2003; Johnson and Lunde 2005), enhancing transmission, particularly in amphibian hosts. Furthermore, the sole ingestion of a

few infected amphibians is sufficient to cause advanced infection states in final hosts (Sato et al. 2010). On the other hand, the infections produced by the parasites cause no clinical signs (e.g. *Felis silvestris catus* or *Mustela putorius furo*), and thus, the definitive host becomes an asymptomatic carrier of the disease (Bowman et al. 2008).

Despite sampling close to the distribution range of the sublineages of viviparous salamanders, where pueriparity seems to be exclusive (Beukema et al. 2010; Donaire and Bogaerts 2016; Dinis and Velo-Antón 2017), the presence of *Euryhalmis* sp. was only confirmed in post-metamorphic individuals of the larviparous form of *S. algira tingitana*. The non-infection of viviparous salamanders, whose metamorphosed juveniles skip the aquatic phase, could be an adaptive advantage against infections. However, thorough sampling efforts have to be made in this direction to fully assess such a hypothesis. A better understanding of the natural history incidence and geographic extent of the parasite in North Africa has pivotal importance especially in threatened salamander species.

Conclusion

In summary, herein, we describe for the first time the presence of *Euryhalmis* trematodes in African amphibians, anuran and caudate species, in four localities of North Africa. However, the predators of the infected amphibians in Morocco remain unknown. If our hypothesis is confirmed and the final host is a recent introduced carnivore (mustelidae), the infestation of *Salamandra algira* could compromise the status of this threatened species and become a menace to other endemic species, at the same time that it may entail a risk to humans.

Acknowledgements We are grateful to Tahar Slimani and El Hassan El Mouden for their collaboration.

Permits Joint research project of the Natural History Museum of Braunschweig and the University of Marrakech (BMBF:01DH13015) and former Orde du mission issued to (DD-B.) by the University of Marrakesh Cadi Ayyad.

Funding This project was funded by the International Collaborative Research Grant by the National Institute of Ecology (South Korea) to MJJ and personal funds from DD-B.

References

- Alford RA, Dixon PM, Pechmann JH (2001) Ecology: global amphibian population declines. *Nature* 412:499–500
- Anderson GA, Pratt I (1965) Cercaria and first intermediate host of *Euryhalmis squamula*. *J Parasitol* 51:13–15
- Bakhoun AJS, Bâ CT, Fournier-Chambrillon C, Torres J, Fournier P, Miquel J (2009) Spermatozoon ultrastructure of *Euryhalmis squamula* Rudolphi, 1819 (Digenea, Opisthorchioidea, Heterophyidae), an intestinal parasite of *Mustela vison* (Carnivora, Mustelidae). *Rev Iber-Latinoam Parasitol* 1:37–45
- Beukema W, De Pous P, Donaire D, Escoriza D, Bogaerts S, Toxopeus AG, De Bie CAJM, Roca J, Carranza S (2010) Biogeography and contemporary climatic differentiation among Moroccan *Salamandra algira*. *Biol J Linn Soc* 101:626–641
- Blas-Artilo L (1970) Vida y Costumbres de los Mustélidos Españoles, 1st edn. Servicio de Pesca Continental, Caza y Parques Nacionales, Ministerio de Agricultura, Madrid
- Bosch J, Martínez-Solano I (2006) Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in Peñalara National Park, Spain. *Oryx* 40:84–89
- Bowman DD, Hendrix CM, Lindsay DS, Barr SC (2008) Feline clinical parasitology. John Wiley & Sons. Iowa University Press, Iowa
- Bray RA, Gibson DI, Jones A (2008) Keys to the Trematoda, Vol 3. CAB International and Natural History Museum, London
- Brenes RR, Arroyo G, Jiménez-Quirós O (1960) Helminths of the República de Costa Rica XVIII. Una nueva especie de *Euryhalmis* (Trematoda: Heterophyidae), parásito de *Mustela frenata costaricensis*. *Rev Biol Trop* 8:247–251. <https://doi.org/10.15517/rbt.v8i2.30303>
- Broyer J, Aulagnier S, Destre R (1988) La loutre, *Lutra lutra angustifrons* (Lataste, 1885) au Maroc. *Mammalia* 52:361–370
- Clavero M, Prenda J, Delibes M (2003) Trophic diversity of the otter (*Lutra lutra* L.) in temperate and Mediterranean freshwater habitats. *J Biogeogr* 30:761–769
- Clavero M, Prenda J, Delibes M (2005) Amphibian and reptile consumption by otters (*Lutra lutra*) in a coastal area in southern Iberian Peninsula. *Herpetol J* 15:125–131
- Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. *Divers Distrib* 9:89–98
- Combes C, Bartoli P, Theron A (2002) Trematode transmission strategies. In: Lewis EE, Campbell JF, Sukhdeo MWK (eds) The behavioural ecology of parasites. CAB International and Natural History Museum, Wallingford, pp 1–12
- Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R (1999) Emerging infectious diseases and amphibian population declines. *Emerg Infect Dis* 5:735–748
- Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. *Divers Distrib* 9:141–150
- De Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. *Ecol Lett* 8:117–126
- Dinis M, Velo-Antón G (2017) How little do we know about the reproductive mode in the north African salamander, *Salamandra algira*? Pueriparity in divergent mitochondrial lineages of *S. a. tingitana*. *Amphibia-Reptilia* 38:540–546
- Donaire D, Bogaerts S (2016) Sobre los límites de *Salamandra algira tingitana* Donaire-Barroso & Bogaerts, 2003 vivípara y consideraciones ecológicas. *Butll Soc Cat Herp* 23:64–70 <https://soccatherp.files.wordpress.com/2016/02/salamandra-algira-tingitana-viviparc3adsmo-larviparc3adsmo.pdf>. Accessed 10 April 2018
- Duffus AL, Cunningham AA (2010) Major disease threats to European amphibians. *Herpetol J* 20:117–127
- Fragoso S, Santos-Reis M (2000) Utilização dos recursos tróficos pela doninha no Parque Natural das Serras de Aire e Candeeiros. *Rev Biol* 18:23–32
- Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224
- Grabda-Kazubska B (1980) *Euryhalmis zelleri* sp. n. and *Euryhalmis squamula* Rudolphi, 1819 (Trematoda, Heterophyidae), metacercariae from *Rana temporaria* L., from the Babia Gora National Park, Poland. *Acta Parasitol Pol* 26:115–128 <https://www.cabdirect.org/cabdirect/abstract/19800877445> Accessed 10 April 2018

- Hamann MI, González CE (2009) Larval digenetic trematodes in tadpoles of six amphibian species from Northeastern Argentina. *J Parasitol* 95:623–628
- Heneberg P, Faltynkova A, Bizos J, Mala M, Ziak J, Literak I (2015) Intermediate hosts of the trematode *Collyriclum faba* (Plagiorchiida: Collyriclidae) identified by an integrated morphological and genetic approach. *Parasit Vectors* 8:646
- Hillis DM, Moritz C, Mable BK (1996) *Molecular systematics*. 2nd edn. Sinauer Associates, Inc. Sunderland
- Hoberg EP, Aubry KB, Britnell JD (1990) Helminth parasitism in martens (*Martes americana*) and ermines (*Mustela erminea*) from Washington, with comments on the distribution of *Trichinella spiralis*. *J Wild Dis* 26:447–452
- Hopkins WA (2007) Amphibians as models for studying environmental change. *ILAR J* 48:270–277
- Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL (2000) Quantitative evidence for global amphibian population declines. *Nature* 404:752–755
- Jiménez MS (2003) Contribución al conocimiento de la parasitofauna de *Rana perezi* Seoane, 1885 (Amphibia:Ranidae) de la provincia de Ávila. Tesis doctoral, Facultad Complutense de Madrid
- Johnson PT, Lunde KB (2005) Parasite infection and limb malformations: a growing problem in amphibian conservation. In: Lannoo M (ed) *Amphibian declines: the conservation status of United States species*. University of California Press, Berkeley, pp 124–138
- Johnson PT, Sutherland DR, Kinsella JM, Lunde KB (2004) Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. *Adv Parasitol* 57:191–253
- Kishimoto R (2005) Invasion of an alien species, American mink (*Mustela vison*), into the upper area of Chikuma river. *Bull Nagano Environ Res Inst* 1:65–68 (in Japanese)
- Knutson MG, Richardson WB, Weick S (2002) Farm ponds as critical habitats for native amphibians: Field Season 2001 Interim Report. USGS-Upper Midwest Environmental Sciences Center. La Crosse, Wisconsin https://umesc.usgs.gov/documents/reports/2001/interim2_report.pdf Accessed 10 April 2018
- Larkin MA, Backshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948
- Lebarbenchon C, Poitevin F, Arnal V, Montgelard C (2010) Phylogeography of the weasel (*Mustela nivalis*) in the western-Palaearctic region: combined effects of glacial events and human movements. *Heredity* 105:449–462
- Libois R, Fareh M, Brahim A, Rosoux R (2015) Régime alimentaire et stratégie trophique saisonnière de la Loutre d'Europe, *Lutra lutra*, dans le Moyen Atlas (Maroc). *Rev Ecol (Terre Vie)* 70:314–327 <http://hdl.handle.net/2042/56926> Accessed 10 April 2018
- Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *PNAS* 103:3165–3170
- Martel A, Blooi M, Adriaensen C, Van Rooij P, Beukema W, Fisher MC, Lips KR (2014) Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346:630–631
- Mata-Lopez R, Garcia-Prieto L, Leon-Regagnon V (2010) Helminths of the American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), from Pawnee Lake, Lancaster, Nebraska, USA with a checklist of its helminth parasites. *Zootaxa* 2544:1–53 <http://www.mapress.com/jzt/article/view/9201> Accessed 10 April 2018
- McDonald RA, Lariviere S (2001) Diseases and pathogens of *Mustela* spp, with special reference to the biological control of introduced stoat *Mustela erminea* populations in New Zealand. *J R Soc N Z* 31: 721–744
- McDonald KR, Mendez D, Müller R, Freeman AB, Speare R (2005) Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. *Pac Conserv Biol* 11:114–120
- Miraldo A, Li S, Borregaard MK, Flórez-Rodríguez A, Gopalakrishnan S, Rizvanovic M, Nogués-Bravo D (2016) An Anthropocene map of genetic diversity. *Science* 353:1532–1535
- O'Hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Bataille A, Kosch TA, Murray KA, Brankovics B, Fumagalli M, Martin MD, Wales N, Alvarado-Rybak M, Bates KA, Berger L, Böll S, Brookes L, Clare F, Courtois EA, Cunningham AA, Doherty-Bone TM, Ghosh P, Gower DJ, Hintz WE, Höglund J, Jenkinson TS, Lin CF, Laurila A, Loyau A, Martel A, Meurling S, Miaud C, Minting P, Pasmans F, Schmeller DS, Schmidt BR, Shelton JMG, Skerratt LF, Smith F, Soto-Azat C, Spagnoletti M, Tessa G, Toledo LF, Valenzuela-Sánchez A, Verster R, Vörös J, Webb R, Wierzbicki C, Wombwell E, Zamudio KR, Aanensen DM, James TY, Gilbert MTP, Weldon C, Bosch J, Balloux F, Garner TWJ, Fisher MC (2018) Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360:621–627. <https://doi.org/10.1126/science.aar1965>
- Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MP, Foster PN, Ron SR (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439:161–167
- Rachowicz LJ, Hero J, Alford RA, Taylor JW, Morgan JA, Vredenburg VT, Collins JP, Briggs CJ (2005) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conserv Biol* 19:1441–1448
- Rachowicz LJ, Knapp RA, Morgan JA, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ (2006) Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683
- Routtu J, Grunberg D, Izhar R, Dagan Y, Guttel Y, Ucko M, Ben-Ami F (2014) Selective and universal primers for trematode barcoding in freshwater snails. *Parasitol Res* 113:2535–2540
- Sato H, Inaba T, Ihama Y, Kamiya H (1999) Parasitological survey on wild carnivora in northwestern Tohoku, Japan. *J Vet Med Sci* 61: 1023–1026
- Sato H, Ihara S, Inaba O, Une Y (2010) Identification of *Euryhalmis costaricensis* metacercariae in the skin of Tohoku hynobiid salamanders (*Hynobius lichenatus*), northeastern Honshu, Japan. *J Wildl Dis* 46:832–842
- Schotthoefner AM, Cole RA, Beasley VR (2003) Relationship of tadpole stage to location of echinostome cercariae encystment and the consequences for tadpole survival. *J Parasitol* 89:475–482
- Sheffield SR, King CM (1994) *Mustela nivalis*. *Mamm Species* 454:1–10
- Shimatani Y, Fukue Y, Kishimoto R, Masuda R (2010) Genetic variation and population structure of the feral American mink (*Neovison vison*) in Nagano, Japan, revealed by microsatellite analysis. *Mammal Study* 35:1–7. <https://doi.org/10.3106/041.035.0101>
- Simon MJ (1972) *Euryhalmis cotti* N. Sp. (Trematoda: Heterophyidae) with observations on its life cycle. Dissertation and Theses, Paper 960. Portland State University. <https://doi.org/10.15760/etd.960>
- Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Kenyon N (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134
- Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786
- Thévenot M, Aulagnier S (2006) Mise à jour de la liste des mammifères sauvages du Maroc. Janvier 2006. *Go-South Bull* 3:6–9 http://go-south.org/wp-content/uploads/2014/08/go-south_bull_3_6-91.pdf Accessed 10 April 2018
- Torres J, Miquel J, Casanova JC, Ribas A, Feliu C, Morand S (2006) Endoparasite species richness of Iberian carnivores: influences of host density and range distribution. *Biodivers Conserv* 15:4619–4632

- Torres J, Miquel J, Fournier P, Fournier-Chambrillon C, Liberge M, Fons R, Feliu C (2008) Helminth communities of the autochthonous mustelids *Mustela lutreola* and *M. putorius* and the introduced *Mustela vison* in south-western France. *J Helminthol* 82:349–355
- Vences M, De Pous P, Nicolas V, Díaz-Rodríguez J, Donaire D, Hugemann K, Hauswaldt JS, Amat F, Barnestein JAM, Bogaerts S, Bouazza A, Carranza S, Galán P, Gonzalez de la Vega JP, Joger U, Lansari A, El Mouden H, Ohler A, Sanuy D, Slimani T, Tejedo M (2014) New insights on phylogeography and distribution of painted frogs (*Discoglossus*) in northern Africa and the Iberian peninsula. *Amphibia-Reptilia* 35:305–320. <https://doi.org/10.1163/15685381-00002954>
- Waikagul J, Thakham U (2014) Approaches to research on the systematics of fish-borne trematodes. Academic Press. 67–69
- Walton AC (1949) Parasites of the Ranidae (Amphibia). *Trans Am Microsc Soc JSTOR* 68:49–54. <https://doi.org/10.2307/3223205>
- Weldon C, Du Preez LH, Hyatt AD, Muller R, Speare R (2004) Origin of the amphibian chytrid fungus. *Emerg Infect Dis* 10:2100–2105
- Wolsan M (1993) *Mustela putorius* Linnaeus, 1758–Waldiltis, Europäischer Iltis, Iltis. In: Stubbe M, Krapp F (eds) *Handbuch der Säugetiere Europas*. Band 5/II, Carnivora 2 (Fissipedia): 699–769. Aula Verlag, Wiesbaden
- Wongsawad C, Rojtinnakorn J, Wongsawad P, Rojanapaibul A, Marayong T, Suwattanacoupt S, Sirikanjana P, Sey O, Jadhav BV (2004) Helminths of vertebrates from Mae Sa Stream, Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health* 35:140–146 <http://www.tn.mahidol.ac.th/seameo/2004-35-suppl-1/25-140.pdf> Accessed 10 April 2018
- Yanchev Y (1987) The morphology, taxonomy and distribution of *Euryhalmis squamula* Rudolphi 1819 (Trematoda, Heterophiidae) in some Mustelidae in Bulgaria. *Khelmintologiya* 23:50–58
- Young BE, Lips KR, Reaser JK, Ibanez R, Salas AW, Cedeño JR, Muñoz A (2001) Population declines and priorities for amphibian conservation in Latin America. *Conserv Biol* 15:1213–1223