Free-living, non-native pond sliders in Carinthia and their protozoan parasites: first recorded local occurrence of two remarkable *Eimeria* species.

Freilebende, nicht heimische Sumpfschildkröten in Kärnten und ihre einzelligen Parasiten: Erster Nachweis des lokalen Vorkommens zweier beachtenswerter *Eimeria*-Arten.

From Andreas R. HASSL & Andreas KLEEWEIN

Abstract

In Carinthia, allochthonous populations of free-living, nearctic pond sliders are occasionally suspected of constituting reservoirs for parasites that threaten domestic populations of the endangered European pond turtle. Within the scope of a monitoring study aimed at verifying this suspicion, 70 feces samples of free-living *Trachemys scripta* from the surroundings of Lake Wörthersee were screened for their load of apicomplexan oocysts by means of autofluorescence-microscopy. In 15 samples oocysts of *Eimeria mitraria* and *Eimeria gallaeciaensis* were definitely identified and classified, however oocysts of the widespread Eimerian parasite of European pond turtles, *Eimeria delagei*, could not be discovered. This study provides the first evidence for an occurrence of these *Eimeria* species in Carinthian, Austrian and Central European habitats. Moreover, the study confirms *T. scripta* as the second known host species of *E. gallaeciaensis*. An up-to-date data table of distinguishing characteristics of the most commonly recorded Central European *Eimeria* parasites of free-living pond turtles is provided. It seems that at least one Carinthian population of non-native pond sliders constitutes a recently emerged epidemiological reservoir of moderately host-specific Eimerian parasites. Yet this reservoir appears to have an insignificant impact on the domestic populations of *Emys orbicularis*. Hence, from a protozoological-parasitological perspective, allochthonous populations of nearctic pond sliders rather enhance domestic biodiversity than constitute a threat to European pond turtle populations.

Schlüsselwörter

Eimeria mitraria, Eimeria gallaeciaensis, apicomlexan parasite, *Trachemys scripta*, allochthonous population, Carinthia, Austria

Keywords

Eimeria mitraria, Eimeria gallaeciaensis, apicomlexer Parasit, *Trachemys scripta*, allochthone Population, Kärnten, Österreich

Zusammenfassung

In Kärnten werden allochthonen Populationen freilebender, nearktischer Sumpfschildkröten gelegentlich nachgesagt, dass sie als Reservoire für solche Parasiten fungieren, die die heimischen Populationen der gefährdeten Europäischen Sumpfschildkröte bedrohen. Im Rahmen einer Überwachungsstudie zur Überprüfung dieser Behauptung wurden 70 Kotproben von freilebenden *Trachemys scripta* aus der Umgebung des Wörthersees mittels Autofluoreszenz-Mikroskopie auf ihre Last an Oozysten von Apikomplexa untersucht. In 15 Proben wurden Oozysten von *Eimeria mitraria* und *Eimeria gallaeciaensis* unbestreitbar identifiziert und klassifiziert, Oozysten des weit verbreiteten *Eimeria*-Parasiten Europäischer Sumpfschildkröten, *Eimeria delagei*, konnten jedoch nicht nachgewiesen werden. Diese Studie liefert den ersten Nachweis für ein Vorkommen der genannten *Eimeria*-Arten in kärntner, österreichischen und mitteleuropäischen Lebensräumen. Darüber hinaus bestätigt die Studie *T. scripta* als zweite bekannte Wirtsart von *E. gallaeciaensis*. Eine Tabelle aktueller Daten von

Unterscheidungsmerkmalen der am häufigsten beobachteten mitteleuropäischen *Eimeria*-Parasiten freilebender Sumpfschildkröten wird bereitgestellt. Es scheint, dass mindestens eine Kärntner Population von nicht heimischen Sumpfschildkröten ein erst jüngst entstandenes epidemiologisches Reservoir von mäßig wirtsspezifischen *Eimeria*-Parasiten bildet. Vermutlich hat dieses Reservoir jedoch nur einen unbedeutenden Einfluss auf die heimischen Populationen von *Emys orbicularis*. Aus protozoologisch-parasitologischer Sicht fördern allochthone Populationen nearktischer Sumpfschildkröten daher eher die heimische Biodiversität, als dass sie eine Bedrohung für Bestände der Europäischen Sumpfschildkröte darstellen.

Introduction

Nowadays it is in vogue to assert that one of the main causes of the loss of biodiversity is the spread of introduced, invasive species, because these species are claimed to reduce local biodiversity (e. g. Rakauskas et al. 2016). However, the globally dominant form of life is parasitism, and all known vertebrate species are hosts to significantly more than one parasite species (Windsor 1998). A considerable number of these parasites are monoxenic ones, so more parasite species than host species exist on a large scale. Invasive host species either introduce their specific parasites into the local biocenosis or, in the case of abandoned pet animals with their significantly modified parasite microbiocenosis (Hassl 2004), free-living populations of alien hosts constitute additional epidemiological reservoirs of alien parasites or euroxenic domestic ones. In this context the spreading of invasive species may indeed threaten local populations of endangered taxa, in spite of conservation efforts. Such a threat is assumed in the case of the few populations of the highly endangered European pond turtle, *Emys orbicularis* (Linnaeus, 1758) in Austria (Gollmann 2007). Some experts argue that the establishment of allochthonous populations of nearctic pond turtles of the genera *Trachemys*, *Pseudemys*, and *Graptemys* is responsible for this threat (e. g. Kleewein & Wöss 2010).

During a protozoological and helminthological monitoring of free-living, once pet *Trachemys scripta* (Thunberg in Schoepff, 1792) of three subspecies *Trachemys scripta elegans*, *Trachemys scripta scripta* and *Trachemys scripta troostii* from the surroundings of Lake Wörthersee, congruent patterns of parasite acquisition by these sliders could not be ascertained (Hassl & Kleewein 2017). Within the scope of the laboratory procedure oocysts of two emydophilic *Eimeria* species were detected whose occurrences were not previously known for Central European habitats or for pond sliders.

Oocysts of the protozoan genus *Eimeria* are generally shed into the environment within the feces of the host. *Eimeria* is a species-rich genus of apicomplexan parasites. *Eimeria* protozoans are found in the intestines of vertebrates, especially in mammals and birds, but around 150 species infect reptiles, and more than twenty of these are known to infest Emydidae. Especially high incidences of bovine *Eimeria* oocyst excretion in livestock have been reported in Carinthia (Koutny et al. 2011), yet cross-contamination of slider-derived samples with oocysts shed by unanticipated hosts has never been uncovered.

Genus and species identification is still based on phenotypic characteristics of the oocysts. Each sporulated oocyst typically contains

four sporocysts with two sporozoites respectively (Duszynski et al. 2008). The oocysts of these coccidia are coated by an autofluorescent wall, making it easy to detect them in feces samples. *Eimeria* parasites are monoxenous by definition – their life cycle is completed within a single host individuum – and they are considered to be host-specific to a considerable extent, thus moderately stenoxenous. The size, shape and outer wall thickness of the oocysts, and the moderate stenoxenia of the genus are regular characteristics for species identification within the genus. Gene sequences suitable for diagnostic purposes are established for economically significant taxa only.

Material and methods

From May to September 2011, a total of 14 *Trachemys scripta* of the three subspecies *T. s. elegans*, *T. s. scripta* and *T. s. troostii* were captured or received at several of the known occurrence sites of these turtles in Carinthia (Kleewein 2007), in the area of the western Wörthersee basin (46° 36′ / 14° 02′; 460–550 m asl). Blood samples were obtained from injuries to animals brought in for care (fishing hooks, dog bites, etc.) and processed into a total of 48 blood smears. During a herpetological field trip in 2011 to Kunpeszer in Hungary (47° 03′ / 19° 16′; 100 m asl), a colleague working there on a genetic project of a German university could be asked to provide the first author with a blood and a fecal sample of a wild *Emys orbicularis* there. These samples, stored and processed in the same way, served as comparison samples.

These blood smears were dried on site, transported in this condition to the Medical University of Vienna (MUW), fixed in methanol and further processed within a few weeks. The blood smears were stained in Giemsa's azure-eosin-methylene blue solution stain with a pH of 7.4 for 10 min, differentiated in phosphate buffered saline (PBS), washed with methanol, and air-dried. Blood parasites were determined using morphological criteria according to the plates in Telford (2009).

70 samples, all collected at Bäckerteich near Velden (46° 36' 32'' / 14° 02' 10''; 460 m asl) from the feces of nine *Trachemys scripta* ssp. individuals, deposited either as a startle reaction or by individuals who could be observed during deposition, were collected immediately after deposition and fixed in 75 % ethanol. These samples were also transported to MUW, where they were stored in alcohol at 8 °C in the dark, and further processed in late 2016. A piece of approximately 1 ccm volume of each fecal sample was finely divided in 10 ml PBS, filtered through gauze, centrifuged at 300 g for 10 min, and the sediment suspended in 0.5 ml PBS. Occasionally detectable uric acid conglomerates were dissolved in low concentration sodium hydroxide solution and removed. Taxon affiliation of the producers of the eggs in the feces was determined by specimen at 400x magnification using a proprietary key. Protozoa that are usually only diagnosed in the active state, such as hexamites and trichomonads, could not be identified with the applied technique. Presumably the oocysts of all protozoa from the order Eimeriidae possess an autofluorescent envelope (e. g., Chen et al. 2013). They radiate so conspicuously in excitation light of 365 nm, and when a blocking filter of 445 nm is used, that even isolated oocysts can be reliably identified

in a microscopic preparation. The species affiliation was determined by comparing morphological and biometric properties in visible light.

Oocysts of apicomplexan parasites were allocated to a species according to their characteristics as depicted in table 1, and microphotographed.

Results

Oocysts of *Eimeria mitraria* (Laveran et Mesnil, 1902) could be definitely identified in eight feces samples (11 %), oocysts of *Eimeria gallaeciaensis* Segade et al., 2006 in seven samples (10 %). All feces samples containing *Eimeria* oocysts were acquired from sliders of the subspecies *T. scripta elegans*. All these sliders were free-living within the Velden municipal area in Carinthia. Oocysts of *Eimeria delagei* (Labbé, 1893) were detected in the single feces sample from the Hungarian *E. orbicularis*.

Tab. 1: Distinguishing characteristics of oocysts of *E. mitraria*, *E. gallaeciaensis* and *E. delagei*. Common features were omitted. Data assembled from Duszynski & Morrow (2014), Hassl & Kleewein (2017), Segade et al. (2006), Široký et al. (2006) and own acquisition.

Tab. 1: Unterscheidungsmerkmale der Oozysten von *E. mitraria*, *E. gallaeciaensis* und *E. delagei*. Gemeinsame Merkmale wurden weggelassen. Daten zusammengestellt aus Duszynski & Morrow (2014), Hassl & Kleewein (2017), Segade et al. (2006), Široký et al. (2006) und eigener Erfassung.

	E. mitraria	*E. gallaeciaensis*	*E. delagei*
Shape	Mitre-shaped with 3 protusions at the flat end and one on the far end	Subspherical to lightly ovoid-ellipsoid	Pear-shaped
Dimension in µm	13-14 x 9-11	17-22 x 15-18	20-22 x 16-17
Shape index	1.35	1.2	1.2
Residuum	Absent	Present	Present
Wall thickness in µm	0.5	0.7	ca. 1.0
Oocyst-sheding host taxon	Emydidae	*Emys orbicularis*, *Trachemys scripta* (this study)	*Emys orbicularis*
Host range of the *Eimeria* species	Euryxenic	Stenoxenic (according to this study)	Monoxenic
Geographical extension of the *Eimeria* species in Europe	Galicia, Carinthia (this study)	Galicia, Carinthia (this study)	Presumably ubiquitous, bound to naturally occurring host populations

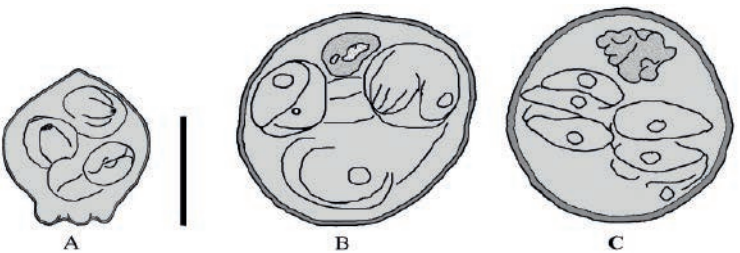

Fig. 1: Shaded line graphic created from microphotographs of oocysts of *Eimeria mitraria* (A), *Eimeria gallaeciaensis* (B), and *Eimeria delagei* (C), preserved in ethanol. The bar represents 10 µm. Photo: A. Hassl

Abb. 1: Nuancierte Linien-grafiken, angefertigt von Mikrofotografien von in Ethanol konservierten Oozysten von *Eimeria mitraria* (A), *Eimeria gallaeciaensis* (B) und *Eimeria delagei* (C). Der Balken repräsentiert 10 µm. Foto: A. Hassl

Discussion

Since the year 2000 twenty locations have been identified in Carinthia, where specimens of the domestic European pond turtle, *E. orbicularis*, were observed (Kleewein 2015). Some of these turtles have to compete with released, non-native pond turtles living in the same habitat (Kleewein 2007). Such a competitive co-existence occurs because of human intervention in the local fauna, and it can lead to the emergence of locally unknown infectious agents which may threaten the domestic fauna (Daszak et al. 2001). In this study, however, such a threat scenario could not be verified. *E. mitraria* seems to be a ubiquitous parasite with a holarctic distribution, and is clearly a parasite species with minor host-specificity, infecting a wide range of freshwater turtles. The existence of this *Eimeria* species has been demonstrated in *E. orbicularis*-populations in northwestern Spain (Segade et al. 2006). In Austria, the occurrence of this *Eimeria* species is herewith verified for the first time: presumably it has not been detected because of the small number of free-living pond turtles in Austria and the lack of any parasitological investigations regarding these turtles. Four *Eimeria* species (*E. delagei*, *E. emydis*, *E. gallaeciaensis* and *E. mitraria*) are currently known to infest *E. orbicularis* (McAllister & Upton 1989, Segade et al. 2006, Široký et al. 2006, this study). As far as *E. gallaeciaensis* is concerned, *E. orbicularis* was the only identified host until today (Duszynski & Morrow 2014). This species is not only proven to occur in Austria for the first time ever, but the study also names an additional host unknown until now, *T. scripta*. On the other hand, the distinguished and common Eimerian species of red-eared sliders, *Eimeria trachemydis* (McAllister & Upton 1988), could not be identified in any sample from Carinthia. Apparently, this nearctic parasite could not establish itself in Carinthia, but its ecological niche was filled up by the acquisition of a presumably ubiquitous generalist originating from the new habitat (Hassl 2004).

This survey of the apicomlexean endoparasites of free-living, non-native pond sliders shows that these animals are suitable hosts for so far locally unknown *Eimeria* species. The slider populations thus constitute recently arisen reservoirs of moderately host-specific Eimerian parasites. However, in Carinthia these reservoirs obviously have an insignificant impact on the populations of domestic pond turtles. The protozoan parasites identified in this study appear to enrich the local fauna and to increase Central European biodiversity. This may be an unorthodox parasitophilic point of view – but there is no evidence that parasites of Emydid neobiota, already living in Central Europe for a long time, will now suddenly threaten local species by means of a virulence enhancement.

REFERENCES

Daszak P., Cunningham A. A. & Hyatt A. D. (2001): Anthropogenic environmental change and the emergence of infectious diseases in wildlife. – Acta Tropica, 78: 103–116.

Duszynski D. W. & Morrow J. J. (2014): The Biology and Identification of the Coccidia (Apicomplexa) of Turtles of the World. – Academic Press, London, San Diego, Waltham, Oxford, 222 S.

Duszynski D. W., Upton S. J. & Couch L. (2008): Taxonomic Summary of Genera within the Eimeriidae. – University of New Mexico supported by NSF-PEET DEB 9521687: http://biology.unm.edu/biology/coccidia/genera.html [access 16.08.2022].

Gollmann G. (2007): Rote Liste der in Österreich gefährdeten Lurche (Amphibia) und Kriechtiere (Reptilia): 37–60. In: Zulka K. P. (Ed.) (2007): Rote Listen gefährdeter Tiere Österreichs. Checklisten, Gefährdungsanalysen, Handlungsbedarf. Teil 2: Kriechtiere, Lurche, Fische, Nachtfalter, Weichtiere. – Grüne Reihe des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 14/2, Böhlau, Wien, 515 S.

Hassl A. (2004): Microbiological Conservation Medicine and Exotic Pets. Supplement no. 4. – The Central European Journal of Medicine, 116: 53–57.

Hassl A. & Kleewein A. (2017): Die Parasitenfauna allochthoner, nearktischer Sumpfschildkröten in Kärnten und deren Konsequenz im Kontext von Artenschutzmaßnahmen. – Carinthia II, 207./127.: 517–526.

Kleewein A. (2007): Verbreitung der Rotwangen-Schmuckschildkröte (*Trachemys scripta elegans*) in Kärnten. – Carinthia II, 197./117.: 53–58.

Kleewein A. (2015): Die Europäische Sumpfschildkröte (*Emys orbicularis*) in Kärnten – Untersuchung zur Frage: allochthones oder autochthones Faunenelement? – Carinthia II, 205./125.: 559–572.

Kleewein A. & Wöss G. (2010): Niedliche Tierchen als ökologischer Zündstoff: Über faunenfremde Schildkröten in Österreich: 105–111. – In: Rabitsch W. & Essl F. (Hrsg.) (2010): Aliens. Neobiota und Klimawandel-Eine verhängnisvolle Affäre? – Ausstellungskatalog des Landesmuseums Niederösterreich, Nr. 485, Linz, 160 S.

Koutny H., Joachim A., Tichy A. & Baumgartner W. (2011): Bovine Eimeria species in Austria. – Parasitology research, 110(5): 1893–1901.

McAllister C. T. & Upton S. J. (1988): *Eimeria trachemydis* n. sp. (Apicomplexa: Eimeriidae) and other Eimerians from the red-eared slider, *Trachemys scripta elegans* (Reptilia: Testudines), in northcentral Texas. – Journal of Parasitology, 74(6): 1014–1017.

McAllister C. T. & Upton S. J. (1989): The Coccidia (Apicomplexa: Eimeriidae) of Testudines; with description of three new species. – Canadian Journal of Zoology, 67: 2459–2467.

Rakauskas V., Masiulytė R. & Pikūnienė A. (2016): Predator-prey interactions between a recent invader, the Chinese sleeper (*Perccottus glenii*) and the European pond turtle (*Emys orbicularis*): A case study from Lithuania. – Acta Herpetologica, 11: 101–109.

Segade P., Crespo C., Ayres C., Cordero A., Arias M. C., García-Estévez J. M. & Iglesias Blanco R. (2006): Eimeria species from the European pond turtle, *Emys orbicularis* (Reptilia: Testudines), in Galicia (NW Spain), with description of two new species. – Journal of Parasitology, 92: 69–72.

Široký P., Kamler M. & Modrý D. (2006): A new *Eimeria* (Apicomplexa: Eimeriidae), possessing mitra-shaped oocysts, from the Neotropical chelid turtle *Batrachemys heliostemma* (Testudines: Chelidae), and its comparison with *Eimeria mitraria* (Laveran & Mesnil 1902). – Memórias do Instituto Oswaldo Cruz, 101: 555–558.

Telford S. R. (2009): Hemoparasites of the Reptilia: color atlas and text. – CRC Press Taylor and Francis Group, Boca Raton, 376 S.

Windsor D. A. (1998): Most of the species on Earth are parasites. – International Journal for Parasitology, 28: 1939–1941.

Address of the authors

ao. Univ.-Prof. Dr. Andreas R. Hassl, Micro-Biology Consult Dr. Andreas Hassl, Ameisgasse 63/4/12, 1140 Vienna
E-Mail: office@hassl.at

Mag. Dr. Andreas Kleewein, Ingenieurbüro für Biologie Andreas Kleewein, Erlenweg 8, 9220 Velden am Wörther See
E-Mail: andreas.kleewein@gmx.net