Helminth fauna of the invasive American red-eared slider *Trachemys scripta* in eastern Spain: potential implications for the conservation of native terrapins

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**ABSTRACT**
In this study we report on the helminth fauna of the invasive American red-eared slider *Trachemys scripta* in five localities from eastern Spain where this species co-occurs with two native, endangered freshwater turtles, i.e. *Emys orbicularis* and *Mauremys leprosa*. In total, 46 individuals of *T. scripta* were analysed for parasites. Adult individuals of three helminth species were found: the monogenean *Neopolystoma orbiculare*, the digenean *Telorchis solivagus* and the nematode *Serpinema microcephalus*. *Telorchis solivagus* and *S. microcephalus* are trophically transmitted parasites of native turtles that probably infected *T. scripta* through shared infected prey. *Neopolystoma orbiculare* infects *T. scripta* in its native Nearctic range and probably survived the overseas shipping of hosts due to the combination of a direct life cycle, long lifespan in turtles and crowding conditions that allowed frequent (re)infections. These findings entail potentially significant conservation consequences that deserve further attention. First, there was a successful spill-back of *Telorchis solivagus* and *S. microcephalus* to large invasive populations of *T. scripta* in the study area, which could eventually increase infection levels in native turtles. Second, the possibility of spill-over of *N. orbiculare* to native turtles should not be underestimated as this phenomenon has recently been reported in France. Given the pathogenic potential of *N. orbiculare*, its transference to native turtles could be troublesome.

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**Introduction**
Over recent decades, most of the biogeographic barriers that had isolated continental biotas for millions of years have been erased due to increased human global trade. As a consequence, exchange of alien species among biogeographic regions has accelerated greatly (Hulme 2009). Nonetheless, only a small fraction (1%) of alien species become
invaders that are able to alter the delicate balance of an ecosystem and become a threat to native biota (Williamson and Fitter 1996; Mooney and Cleland 2001). However, invasive species are currently considered a major cause of animal extinctions (Clavero and Garcia-Berthou 2005).

The American red-eared slider, *Trachemys scripta* (Thunberg in Schoepff, 1792), is considered one of the 100 most harmful invasive alien species in the world (Lowe et al. 2000). This species has been marketed as a pet since the 1950s, and around 52 million individuals were exported from the USA between 1989 and 1997 (Telecky 2001). Since then, red-eared sliders have become naturalized and breed successfully in wetlands of western Europe and Asia (Cadi et al. 2004; Ramsay et al. 2007; GISD 2010; Alarcos-Izquierdo et al. 2010; references therein).

In the Iberian Peninsula, the red-eared slider has been reported to prey on carp *Cyprinus carpio* (Linnaeus, 1758) (Martínez-Silvestre and Soler 2009) and on tadpoles of two native amphibians, the Iberian water frog, *Pelophylax perezi* (Seoane, 1885) and the Iberian spade-foot toad, *Pelobates cultripes* (Cuvier, 1829), which do not recognize the red-eared slider as a potential predator (Polo-Cavia et al. 2010). In addition, the red-eared slider competes directly with two native freshwater turtles – the European pond turtle, *Emys orbicularis* (Linnaeus, 1758), and the Spanish pond turtle, *Mauremys leprosa* (Schweigger, 1812) – for food, and basking and nesting sites (Cadi and Joly 2003, 2004; Polo-Cavia et al. 2009). It is currently considered a significant negative factor for the conservation of both native species (Keller and Andreu 2002; Da Silva 2002; Cadi and Joly 2004; Polo-Cavia et al. 2011). *Emys orbicularis* is listed as ‘Near Threatened’ in the Red List of Threatened Species of the IUCN (Tortoise & Freshwater Turtle Specialist Group 1996) and ‘Vulnerable’ in the Red Book of Amphibians and Reptiles of Spain (Keller and Andreu 2002), whereas *M. leprosa* is included as ‘Vulnerable’ in the Red Book of Amphibians and Reptiles of Spain (Da Silva 2002). Both species are included in Annex II and IV of the Habitats Directive of the European Union (Species protected under the Habitats Directive 2015).

A neglected element in the interaction between invader and native species is parasitism. Invaders could bring exotic parasite species into their new habitats, infecting native species as long as the latter are competent hosts; this would represent a ‘spill-over’ effect (Kelly et al. 2009). Likewise, the invader could be a competent host for native parasites, thus being capable of disseminating infective stages to native hosts; this would produce a ‘spill-back’ effect (Kelly et al. 2009). However, if the invader were a non-competent host for native parasites, it could act as a ‘sink’, reducing infection levels in the native fauna and generating a ‘dilution effect’ (Heimpel et al. 2003; Keesing et al. 2006). Spill-over, spill-back and dilution effects could bring about significant conservation consequences depending on the pathogenic potential of both exotic and native parasites for invasive and native hosts in a community context (Prenter et al. 2004; Poulin et al. 2011; Telfer and Bown 2012). For instance, the invader could boost or alleviate detrimental effects of parasites that are highly pathogenic to the native host population through spill-back and dilution effects, respectively. Or, native parasites could be detrimental only for the invader, so having a net beneficial effect by hampering the invasive process.

The parasite fauna of *T. scripta* has been studied widely in its native range (Rosen and Marquardt 1978; Baker 1979; Esch et al. 1993; Moravec and Vargas-Vázquez 1998).
but surveys in its areas of invasion are relatively scarce. In Japan, red-eared sliders have been reported to harbour native parasites of the freshwater turtles *Mauremys japonica* (Temminck and Schlegel, 1835) and *Chinemys reevesii* (Gray, 1831), but also exotic parasites typically found in North America (Asakawa 2005; Oie et al. 2012). In southwestern Spain, Hidalgo-Vila et al. (2009) reported four parasite species typically infecting the native freshwater turtles *E. orbicularis* and *M. leprosa*. Interest in the conservation consequences associated with parasite exchange between *T. scripta* and native turtles has been shown recently (Hidalgo-Vila et al. 2011; Verneau et al. 2011).

In this study we examine the parasitic fauna of introduced *T. scripta* in eastern Spain, an area where a large population of this species co-exists with two species of native freshwater turtles (Martínez-Silvestre et al. 2011) and information about parasite fauna of any of these species is scarce (Aparicio et al. 2008). Our study is particularly aimed at providing a reliable diagnosis of potentially exotic parasite taxa. We also provide a preliminary assessment on the conservation consequences of parasitological findings for native freshwater turtles.

**Material and methods**

This study was conducted in 2011 within the framework of the ‘LIFE-Trachemys’ Programme for the control of exotic turtles. We examined the helminth fauna of 46 individuals of *T. scripta* captured in five localities of eastern Spain (Figure 1). In all localities, *T. scripta* coexisted with *E. orbicularis* and *M. leprosa*. Based on Pérez-Santigosa et al. (2008), individuals of *T. scripta* were classified as juveniles \[n = 20; \text{mean curved carapace length} \pm \text{SD (range)} = 9.7 \pm 1.9 \text{ cm (7.7–15.1)}\] and adults \[n = 26; 16.2 \pm 3.8 \text{ cm (11.9–26.5)}\]. The adult sample comprised nine females \[20.5 \pm 2.7 \text{ cm (17.2–26.5)}\] and 17 males \[14.0 \pm 2.0 \text{ cm (11.9–16.5)}\].

Turtles were humanely killed using a thiopental sodium injection (Tiobarbital; Braun Medical). The mouth, oesophagus, stomach, intestine, liver, kidney, lung and urinary bladder were examined for helminths. Each organ was dissected and visually examined for helminths, then washed with Ringer’s solution over a 200-µm mesh sieve, solid contents were collected and examined under a stereomicroscope (×20). Parasites were counted and preserved in 70% ethanol for later identification. Digeneans and monogeneans were stained with Mayer’s acid carmine, dehydrated, fixed in dimethyl phthalate and mounted in Canada balsam. Nematodes were cleared in Amman’s lactophenol solution, put on a glass slide for identification and then returned to the preservative. Species were identified and described according to Price (1939), López-Román (1974), Baker (1979), Font and Lotz (2008) and Morrison (2010). Voucher specimens are deposited at the Natural History Museum, London [Accession Nos. NHMUK 2015.3.6.12 (*Neopolystoma orbiculare*) and NHMUK 2015.3.6.3-4 (*Telorchis solivagus*)].

The 95% confidence interval (95% CI) for prevalence was calculated with Sterne’s exact method (Reiczigel 2003), and for mean values of intensity and abundance with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa et al. 2000). Calculations were performed using the free software Quantitative Parasitology v. 3 (Reiczigel and Rózsa 2005).
Figure 1. Sampling localities of the American red-eared slider, *Trachemys scripta* in eastern Spain. (A) Protected wetland ‘Marjal de Peñíscola’; (B) ‘Cabanes-Torreblanca’ Natural Park; (C) Protected Landscape ‘Desembocadura del Mijares’; (D) Protected wetland ‘Marjal de Gandía’; (E) Site of Community Importance ‘Marjal de La Safor’.
Results

Three helminth species were found (Table 1). The digenean *Telorchis solivagus* (Odhner, 1902) (Figure 2A) was collected from the digestive tract of 14 hosts from all localities. All individuals were gravid and contained a large number of eggs in the uterus. A single adult female of the nematode *Serpinema microcephalus* (Dujardin, 1845) (Figure 2B) was collected from the digestive tract of one host in the protected wetland ‘Marjal de Peñíscola’ (Figure 1). The monogenean *N. orbiculare* (Stunkard 1916) (Figure 2C, D) was collected from the urinary bladder and cloaca of five hosts from the protected wetland ‘Marjal de Gandía’ (Figure 1).

Given that *N. orbiculare* is a putative exotic species, a detailed description of specimens follows. Morphometric measurements are based on five individuals and given as mean ± SD with range in parentheses (in µm unless otherwise stated).

**Table 1.** Infection parameters of helminth species found in 46 red-eared sliders, *Trachemys scripta* collected in five localities from eastern Spain.

|                      | Prevalence (%) (95% CI) | Mean intensity ± SD (95% CI) [Range] | Mean abundance ± SD (95% CI) | Microhabitat       |
|----------------------|-------------------------|--------------------------------------|-----------------------------|--------------------|
| **PLATYHELMINTHES**  |                         |                                      |                             |                    |
| Monogenea (Polystomatidae) |                        |                                      |                             |                    |
| *Neopolystoma orbiculare* | 10.9 (4.3–23.0) | 1.4 ± 0.9 (1.0–1.8) [1–3]          | 0.2 ± 0.5 (0.0–0.4)         | Urinary bladder and cloaca |
| **Trematoda (Telorchidae)** |                  |                                      |                             |                    |
| *Telorchis solivagus* | 30.4 (18.0–45.0) | 10.3 ± 11.6 (5.6–17.5) [1–38]     | 3.1 ± 7.9 (1.5–6.4)        | Intestine          |
| Nematoda Chromadorea (Camallanidae) |               |                                      |                             |                    |
| *Serpinema microcephalus* | 2.2 (0.0–11.6) | 1.0                                    | 0.02 ± 0.15 (0.0–0.07)     | Small intestine    |

**Identification of Neopolystoma orbiculare**

Body oval-shaped and elongated [length: 2.93 ± 1.10 mm (2.16–4.32 mm); maximum width: 1.05 ± 0.31 mm (0.65–1.44 mm)]. Oral sucker diameter 433 ± 111 (330–570). Pharynx 192 ± 51 (120–220) long and 237 ± 62 (140–260) wide. Oesophagus not seen due to overlap with vitelline follicles. Intestine branches not convergent posteriorly. Genital pore ventral [diameter: 7.5 ± 2.6 (5–11)], posterior to intestinal bifurcation, with 16 genital spines [length: 3 ± 0.4 (0.3–3.8)]. Oval testis, in medial zone of the body 229 ± 108 (130–380) long and 206 ± 64 (120–270) wide. Vaginal pores ventral, posterior to ovary [separated by 1.07 ± 0.2 mm (1.01–1.33 mm)]. Vitelline follicles extending from posterior part of pharynx to haptor. One individual bearing one pear-shaped egg (Figure 2D), 183 long and 108 wide. Haptor slightly circular, 0.84 ± 0.27 mm (0.56–1.13 mm) long and 1.00 ± 0.40 mm (0.67–1.61 mm) wide. Six suckers distributed radially and equidistant from each other, 303 ± 96 (190–410) in diameter. Sixteen larval hooks 17 ± 2 (14–20) long and 9 ± 1 (7–10) wide situated as follows: six between the two anterior suckers, four between posterior suckers and one in each sucker.
Figure 2. Parasites found in the American red-eared slider, *Trachemys scripta* in eastern Spain. (A) *Telorchis solivagus*; (B) *Serpinema microcephalus* (anterior part detail); (C) *Neopolystoma orbiculare* (immature); (D) *Neopolystoma orbiculare* (adult). Scale bars: A, B, D, 1 mm; C, 0.5 mm.
Remarks

Species of *Neopolystoma* (Price, 1939) are distinguished from those of allied genera, i.e. *Polystomoides* (Ward, 1917) and *Polystomoidella* (Price, 1939), by the lack of hamuli (Morrison 2010). Other diagnostic traits are the presence of one testis, short uterus and ovary in the anterior part of the body. Also, ovarian follicles extend into the posterior part of the body and vaginas are present.

The genus *Neopolystoma* currently contains 21 species that are distributed worldwide except in the Afro-tropical region, and usually occur in the urinary bladder, cloaca and, occasionally, nasal mucosa and conjunctival sac of freshwater turtles (Price 1939). *Neopolystoma orbiculare* was described in *T. scripta* and *Chrysemys picta* (Schneider, 1783) in North America (Morrison 2010). Diagnostic traits of *N. orbiculare* found in the present study agree with the measurements and other features provided for the holotype established by Stunkard (1916) and with re-descriptions by Price (1939) and Lamothe-Argumedo (1972). The species can be separated from the four other sympatric species of *Neopolystoma* from the Nearctic region as follows (Stunkard 1916; Caballero 1938). *Neopolystoma domitilae* (Caballero, 1938) infects the urinary bladder and cloaca of *Trachemys ornata* (Gray, 1831) and has 19–21 genital spines and a larger body size (4.04–4.06 by 1.32–1.72 mm). *Neopolystoma elizabethae* (Platt, 2000) infects the conjunctival sac of the eye of *Chrysemys picta bellii* (Gray, 1831) and has eight genital spines. *Neopolystoma terrapenis* (Harwood, 1932) infects the urinary bladder and cloaca of *Terrapene carolina* (Linnaeus, 1758); Price (1939) considered that this species was very similar to *N. orbiculare*, but Harwood (1932) stated that *N. terrapenis* has a smaller body size than *N. orbiculare* (1.90–2.50 by 0.72–0.82 mm), the vitellaria do not crowd into the intercaecal area posterior to the testis, and the pharynx and cirrus sac are also smaller. *Neopolystoma chelodinae* (MacCallum, 1918) infects the urinary bladder and cloaca of *Chelodina longicollis* (Shaw, 1794); this species is very similar to *N. orbiculare* but differs from all other species of *Neopolystoma* in that it has a testis with irregular margin, and vitelline follicles with irregular shape and variable size (Price 1939; Pichelin 1995) and more variability in the number of genital spines (12–16; Morrison 2010).

In the Palaearctic region, three species of *Neopolystoma* have been described. *Neopolystoma exhamatum* (Ozaki, 1935) is found in the urinary bladder and cloaca of *Mauremys japonica* from Japan, but it was recently reported from *T. scripta* in the same region (Oi et al. 2012). This species resembles *N. orbiculare* regarding haptor shape, but its testis has different morphology and size (1.20–1.60 by 0.70–1.30); *N. exhamatum* also has more genital spines (16–18; Morrison 2010). *Neopolystoma palpebrae* Strelkov, 1950, which occurs on the lower eyelid of *Pelodiscus sinensis* (Wiegmann, 1834), is very similar to *N. orbiculare* but differs in the shape and size of genital spines (Morrison 2010). Finally, *Neopolystoma euzeti* Combes and Katari, 1976 infects the urinary bladder and cloaca of the Spanish pond turtle, *Mauremys leprosa* in North Africa (Combes and Katari 1976). However, compared with the specimens examined in the present study, *N. euzeti* has more hooks in the genitalia (33–36) with a larger size (length: 48–57 µm); body (4.54 ± 0.75 × 1.51 ± 0.08) and haptor (1.20 ± 0.17 × 1.67 ± 0.32) are also larger, and vitelline follicles are more widely extended.
Based on the above evidence, we conclude that our material is conspecific with *N. orbiculare*.

**Discussion**

There are few studies on the helminth fauna of *T. scripta* outside its natural range, and they all indicate that this species can acquire parasites in the area of invasion, mainly from native turtles. In Japan, Asawaka (2005) reported the digeneans *Telorchis clemmydis* Yamaguti, 1933 and *Telorchis geoclemmydis* Yamaguti, 1933, and the nematodes *S. microcephalus* and *Falcaustra* sp. In other localities from Japan, Oi et al. (2012) found *Telorchis clemmydis*, *S. microcephalus* and the monogeneans *Neopolystomoides exhamatum* and *Polystomoides japonicum* Ozaki, 1935. In southwestern Spain, Hidalgo-Vila et al. (2009) reported four nematode species including *S. microcephalus*, *Falcaustra donanaensis* Hidalgo-Vila et al., 2006, *Falcaustra* sp. and *Physaloptera* sp. Interestingly, all these species were shared with the syntopic native turtles *E. orbicularis* and *M. leprosa* (Hidalgo-Vila et al. 2009). Recently, Martínez-Silvestre et al. (2013) found *S. microcephalus* in *T. scripta* from northeastern Spain, and Yamauchi et al. (2012) recorded the leech *Ozobranchus jantseanus* Oka, 1912 in Japan. Overall, these observations suggest that the specific composition of native elements of the parasite community in invasive individuals of *T. scripta* depends on both geographic factors and the local pool of available parasite species provided by native turtles.

In the present study, *T. scripta* was also observed to be infected with two species presumably acquired from native turtles. The digenean *Telorchis solivagus* is a common parasite infecting freshwater turtles in Europe, Africa and Asia (Roca et al. 2005 and references therein). *Serpinema microcephalus* naturally infects freshwater turtles throughout the Palaearctic region (Baker 1979); in Spain, the most common definitive host is *M. leprosa* (Hidalgo-Vila et al. 2009). Recently, Martínez-Silvestre et al. (2013) found *S. microcephalus* in *T. scripta* from northeastern Spain, and Yamauchi et al. (2012) recorded the leech *Ozobranchus jantseanus* Oka, 1912 in Japan. Overall, these observations suggest that the specific composition of native elements of the parasite community in invasive individuals of *T. scripta* depends on both geographic factors and the local pool of available parasite species provided by native turtles.

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Oi et al. (2012) first reported on exotic parasites from *T. scripta* in invaded areas; in particular, two vascular digeneans, *Spororchis artericola* (Ward, 1921) and *Spororchis elegans* Stunkard, 1923, and the nematode *Falcaustra wardi* (Mackin, 1936) in Japan. In our study area, a previous survey reported a digenean preliminarily identified as *Telorchis attenuata* Goldberger, 1911 (Aparicio et al. 2008). If this identification were to be confirmed, this would represent the first report in Spain.
of an exotic species typical from *Trachemys scripta*. With regard to the monogenean *N. orbiculare*, Verneau et al. (2011) identified eggs of this species in individuals of *T. scripta* from a turtle farm in southern France using molecular methods. Recently, Meyer et al. (2015) also detected eggs of *N. orbiculare* in naturalized populations of *T. scripta* from the same area. In the present study, we report for the first time adults of *N. orbiculare* in wild individuals of *T. scripta* outside its natural range. The presence of this species in the region does not seem to be anecdotal as there are preliminary records of putative *N. orbiculare* in *T. scripta* from localities close to those sampled in the present study (F. Domènech unpub. data). The occurrence of *N. orbiculare* in non-native areas could have resulted from the interplay of three factors. First, *N. orbiculare* seems to be a common parasite of individuals of *T. scripta* sold as pets by some commercial suppliers in the native area. For instance, Henke et al. (1990) reported prevalences over 40% in commercial samples of *T. scripta* from Wisconsin (USA). Second, polystomatid monogeneans have a long lifespan (up to 3 years in extreme conditions, see Tinsley 1999), which could allow survival of the parasite during the overseas shipping of hosts. Third, *N. orbiculare* has a direct life cycle that includes a free-living oncomiracidium and *T. scripta* as the single host (Verneau 2004). Therefore, transport of overcrowded loads of *T. scripta* could have increased probabilities of host–parasite encounter (Verneau et al. 2011) allowing the completion of the life cycle of *N. orbiculare* first in captivity and later in the wild.

The finding of both native and exotic parasites in invasive populations of *T. scripta* from eastern Spain could entail potential conservation consequences that would deserve attention in the future. First, there is clear evidence of spill-back effects. Both *Telorchis solivagus* and *S. microcephalus* are generalist parasites of freshwater turtles that are able to reproduce in *T. scripta*, as shown in this study. In fact, the prevalence of *Telorchis solivagus* found in the present study (30.4%) is close to the upper range of prevalence of this species in *M. leprosa*, i.e. from 2.8 to 42.9% (López-Román 1974; Mishra and González 1978; Roca et al. 2005). In contrast, the prevalence of *S. microcephalus* in *E. orbicularis* and *M. leprosa* is generally higher (i.e. > 30%) than that found in the present study (Yildirimhan and Sahin 2004; Roca et al. 2005; Hidalgo-Vila et al. 2009). However, Kirin (2001) found *S. microcephalus* with a prevalence of just 1% in a sample of *E. orbicularis* from south Bulgaria (n = 69), suggesting that local factors strongly influence infection levels in native turtles. Overall, evidence suggests that *T. scripta* appears to be a competent host for at least some parasites of native turtles. However, the ecological impact of this phenomenon would depend on both the ability of *T. scripta* to produce infective stages, and the pathogenic potential of parasites. The red-eared slider has large populations in coastal wetlands from eastern Spain where both *E. orbicularis* and *M. leprosa* co-occur (LIFE-Trachemys 2011). Therefore, the possibility that *T. scripta* could help to significantly increase populations of native parasites cannot be ruled out. However, it is unclear whether this putative increase of parasite populations could have a pathogenic impact on native turtles, invasive turtles, or both. Interestingly, Hidalgo-Vila et al. (2011) suggested that *S. microcephalus* appears to be especially pathogenic to *T. scripta*, in contrast to the species of *Serpinema* that naturally infect this turtle in its native area. These authors hypothesized that the exotic population of *T. scripta* in southern Spain was immunosuppressed due to maladaptation to the
newly colonized area and, therefore, they were prone to the pathogenic effects of native parasites.

On the other hand, the possibility of spill-over effects should not be underestimated. Verneau et al. (2011) and Meyer et al. (2015) demonstrated by DNA barcoding analysis of parasite eggs that host-switching of *N. orbiculare* from *T. scripta* to *E. orbicularis* and *M. leprosa* were successful under both captive and natural conditions. In the present study, the prevalence of *N. orbiculare* was lower (10.9%) than that reported in their natural range (20–74%; Everhart 1957). However, this monogenean exhibits low specificity among freshwater turtles (Harwood 1932; Price 1939; Zerecero 1948; Barus and Moravec 1966; Lamothe-Argumedo 1972) and, therefore, the possibility exists that the parasite can easily be transmitted in areas of high turtle density. Henke et al. (1990) reported that putative eggs of *N. orbiculare* provoked inflammatory lesions in the urinary bladder of *T. scripta*. If this is true, then this species could be of potential pathogenic significance, at least for invasive turtles.

In summary, whether or not potential spill-over and spill-back events have a population impact on native and non-native turtles is difficult to predict and cannot easily be assumed. However, parasites are an integral part of ecosystems, and this study highlights the need to monitor the complex effects of parasite exchanges between native and invasive species, as emphasized in recent reviews on the subject (e.g. Torchin et al. 2002; Heimpel et al. 2003; Keesing et al. 2006; Kelly et al. 2009).

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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