

New distributional data on *Haemogregarina stepanowi* (Apicomplexa) and *Placobdella costata* (Hirudinea) parasitising the Sicilian pond turtle *Emys trinacris* (Testudines)

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Abstract - The host-parasite system “*Emys trinacris* - *Placobdella costata* - *Haemogregarina stepanowi*” is known for Sicily, but scarce information is available to date about the distribution of the two parasites *P. costata* and *H. stepanowi* on the island. Therefore, an extensive sampling effort through visual census and collection and analysis of blood smears of the endemic Sicilian pond turtle *E. trinacris* was carried out in 46 water bodies scattered throughout mainland Sicily.

Our findings revealed that the distribution of both parasites is limited to the Nebrodi area, where the infection of *H. stepanowi* has shown a high incidence on the local turtle populations. Our data suggest no correlation between the current distribution of the two parasite species and environmental features. The current distribution of *H. stepanowi* and *P. costata* seems not to be relictual, but rather the outcome of a recent colonisation process. Considering the possible negative impact of both *H. stepanowi* and *P. costata* on their turtle host, their long-term effect on *E. trinacris* should be investigated.

Key words: blood parasites, conservation, Emydidae, Glossiphoniidae, Haemogregarinidae.

Riassunto - Nuovi dati sulla distribuzione di *Haemogregarina stepanowi* (Apicomplexa) e *Placobdella costata* (Hirudinea) parassiti della testuggine palustre siciliana *Emys trinacris* (Testudines).

In Sicilia è noto il sistema ospite-parassita “*Emys trinacris* - *Placobdella costata* - *Haemogregarina stepanowi*”, ma attualmente sono disponibili scarse informazioni sulla distribuzione in Sicilia dei parassiti *P. costata* e *H. stepanowi*. Pertanto, è stato condotto un ampio sforzo di campionamento attraverso il censimento visivo e la raccolta

e l'analisi di strisci di sangue della testuggine palustre siciliana *E. trinacris* in 46 corpi idrici distribuiti in tutta la Sicilia.

I nostri risultati hanno rivelato che la distribuzione di entrambi i parassiti è limitata alla zona dei Nebrodi, dove l'infezione da *H. stepanowi* ha mostrato un'elevata incidenza sulle popolazioni di testuggini locali. I dati raccolti non hanno suggerito alcuna correlazione fra l'attuale distribuzione delle due specie di parassiti e le caratteristiche ambientali. L'attuale distribuzione di *H. stepanowi* e di *P. costata* non sembra essere relictuale, ma piuttosto il risultato di un recente processo di colonizzazione. Considerando il possibile impatto negativo sia di *H. stepanowi* che di *P. costata* sul loro ospite, l'effetto a lungo termine su *E. trinacris* dovrebbe essere studiato.

Parole chiave: conservazione, Emydidae, Glossiphoniidae, Haemogregarinidae, parassiti del sangue.

INTRODUCTION

Sicily hosts the endemic pond turtle *Emys trinacris* Fritz, Fattizzo, Guicking, Tripepi, Pennisi, Lenk, Joger & Wink 2005, which is the only autochthonous freshwater turtle occurring on the island. This turtle species has a scattered distribution (Vamberger *et al.*, 2015; Marrone *et al.*, 2016a, 2016b; Vecchioni *et al.*, 2020b), and has suffered severe habitat destruction and fragmentation (Fritz *et al.*, 2005), which together with the species' limited dispersal ability (Iannella *et al.*, 2018) put it at risk. Furthermore, *E. trinacris* suffers the pressure of invasive alien species (Marrone & Naselli-Flores, 2015; Naselli-Flores & Marrone, 2019; Liuzzo *et al.*, 2020), occasional hybridisation with the congeneric *E. orbicularis* (Linnaeus, 1758) (Vamberger *et al.*, 2015; Scardino *et al.*, 2020), possible parasite spill-over (Arizza *et al.*, 2016), and potential threats due to angling (Vecchioni *et al.*, 2020a).

The only Palaearctic representative of the glossiphoniid leech genus *Placobdella* Blanchard 1893 is *P. costata* (Müller 1846) which has a strict, but not exclusive, trophic relationship with aquatic turtles of the genus *Emys* Duméril 1806 (Bielecki *et al.*, 2012; Romero *et al.*, 2014; Kvist *et al.*, 2022). These two taxa constitute a host-parasite system in which a further species is involved i.e. *Haemogregarina stepanowi* (Danilewsky 1885) a widespread apicomplexan haemoparasite of aquatic turtles (Dvořáková *et al.*, 2014; Arizza *et al.*, 2016). This last species has a complex life cycle that requires an intermediate host (e.g. *E. trinacris*) and a definitive host (i.e.

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P. costata), in which the haemogregarine's gametogenesis occurs in both, with the subsequent formation of the zygote (Siddall & Desser, 1990; Mihalca *et al.*, 2008; Dvořáková *et al.*, 2014).

Marrone *et al.* (2016a) and Vecchioni *et al.* (2021) investigated the system “*E. trinacris* + *P. costata*” in an attempt to assess the possible existence of a cophylogenetic pattern between them. However, no evidence supporting this hypothesis was retrieved.

We here describe the distribution of the “*E. trinacris* - *P. costata* - *H. stepanowi*” system in Sicily, in light of the possible impact of the two parasites on the populations of the endemic Sicilian pond turtle.

MATERIALS AND METHODS

Samples were collected in 46 sites located on mainland Sicily from 2014 to 2017 (no record of *E. trinacris* is available for the small circum-Sicilian islands) as shown in Fig. 1 and Tab. 1, including both low-altitude water bodies and the highest localities where *E. trinacris* is known to occur (Marrone *et al.*, 2016b; Ottonello *et al.*, 2021), including permanent lakes and ponds, brackish lakes and watercourses. Pond turtles were caught by hand or with baited hoop traps as described in Marrone *et al.* (2016a).

After the identification of the species *in situ*, a blood sample was obtained from the dorsal coccygeal vessel of each turtle. Each pond turtle was also carefully inspected for the presence of leeches; when these were present, they were collected and fixed *in situ* in 90% ethanol and identified according to Minelli (1977). Afterwards, the animals were immediately released in nature. Blood smears were prepared *in situ* according to Arizza *et al.* (2016). Leech samples and blood smears are stored at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) of the University of Palermo under the curatorial responsibility of one of the authors (LV).

The distribution of the sampled population of *E. trinacris* in Sicily, the occurrence of its parasites, and the presence of parasitized individuals were mapped in ESRI ArcGIS 10.2. The distribution of the captured turtles and parasitized individuals has been analysed with respect to bioclimatic data (Bazan *et al.*, 2015), lithological features (Basilone, 2018) and habitats recorded in the “Carta della Natura” (ISPRA, 2012). Because the Shapiro-Wilk test (Shapiro & Wilk, 1965) showed that data were not normally distributed, non-parametric Spearman correlation test was used in order to explore the possible link between turtle distribution/presence and environmental features.

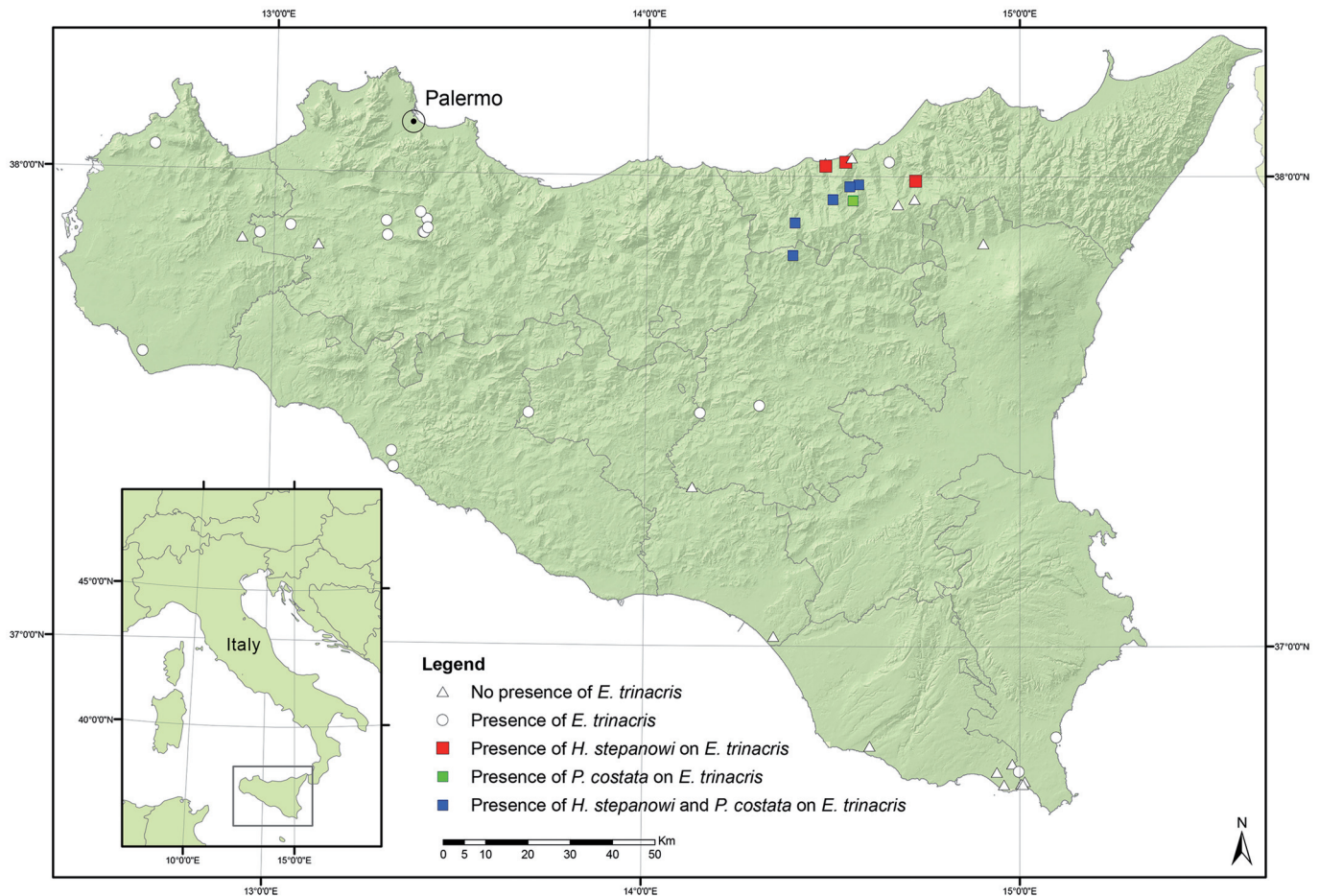


Fig. 1 - Sites in Sicily where the occurrence of *E. trinacris*, *P. costata* and *H. stepanowi* has been observed. / Siti in Sicilia in cui è stata osservata la presenza di *E. trinacris*, *P. costata* e *H. stepanowi*.

Tab. 1 - List of the sampled sites, captured individuals of *E. trinacris*, collected *P. costata* individuals and synopsis of the studied blood samples of *E. trinacris* in which *H. stepanowi* occurred. Geographical coordinates are expressed as decimal degrees (MapDatum: WGS84). The type of water body is shown in brackets: A) Artificial; N) Natural. / Elenco dei siti campionati, individui catturati di *E. trinacris*, individui raccolti di *P. costata* e sinossi dei campioni di sangue studiati di *E. trinacris* in cui *H. stepanowi* era presente. Le coordinate geografiche sono espresse in gradi decimali (MapDatum: WGS84). Tra parentesi è indicato il tipo di corpo idrico: A) Artificiale; N) naturale.

* Pond turtles kept in captivity. / Testuggini palustri tenute in cattività.

Province	Municipality	Site name	Latitude N	Longitude E	Elevation (m a.s.l.)	<i>E. trinacris</i> (n. ind)	Naturalness Evaluation Index ("NEI")	<i>P. costata</i> (p/a)	<i>H. stepanowi</i> (p/a)	N. parasitized individuals (by <i>H. stepanowi</i>)
Agrigento	Casteltermini	Fiume Platani (N)	37.493897	13.687256	138	7	0.38	-	-	-
Agrigento	Montallegro	Lago Gorgo (A)	37.408766	13.324239	65	26	0.72	-	-	-
Agrigento	Siculiana	RNO "Torre Salsa" (N)	37.374883	13.329954	18	17	0.49	-	-	-
Caltanissetta	Gela	Biviere di Gela (N)	37.020923	14.345073	6	-	0.77	-	-	-
Caltanissetta	Riesi	C.da Pietralunga (N)	37.337828	14.126060	220	-	0.40	-	-	-
Catania	Randazzo	Lago Gurrída (A)	37.857812	14.900734	855	-	0.49	-	-	-
Enna	Enna	Fiume Imera (N)	37.494888	14.145615	261	11	0.51	-	-	-
Enna	Enna	Pergusa (N)	37.511532	14.304555	671	14	0.89	-	-	-
Enna	Nicosia	Laghetto Sambuchetti-Campanito (N)	37.832023	14.390823	1252	22	0.41	1	1	18
Enna	Nicosia	Laghetto Sambuchetti-Campanito 2 (N)	37.831846	14.390189	1261	1	0.39	1	-	-
Messina	Alcara Li Fusi	Alcara Li Fusi (A)	37.991153	14.719617	644	3	0.50	-	1	2
Messina	Alcara Li Fusi	Lago di Maulazzo (A)	37.94059	14.672646	1470	-	0.98	-	-	-
Messina	Caronia	Lago Gianferraro (N)	37.951383	14.49746	1010	2	0.96	1	1	2
Messina	Caronia	Marina di Caronia (N)	38.027674	14.476765	65	-	0.36	-	-	-
Messina	Caronia	Marina di Caronia 2 (A)	38.022166	14.477595	159	1	0.42	-	1	1
Messina	Caronia	San Fratello (A)	37.98346	14.566497	500	2	0.73	1	1	1
Messina	Caronia	San Fratello 2 (A)	37.979295	14.542903	403	8	0.57	1	1	7
Messina	Caronia	Stagno di Pantana (N)	37.949314	14.551364	922	44	0.83	1	n.a.	n.a.
Messina	Caronia	Torre del Lauro (A)	38.03035	14.531056	246	2	0.94	-	1	1
Messina	Caronia	Torre del Lauro 2 (A)	38.039912	14.547666	179	-	0.54	-	-	-
Messina	Cesarò	Lago Biviere (N)	37.95211	14.715968	1279	-	0.70	-	-	-
Messina	Mistretta	Lago Urio Quattrocchi (N)	37.901245	14.396302	1039	3	0.84	1	1	3
Messina	Sant'Agata di Militello	Lago di Spartà (A)	38.030587	14.647807	670	20	0.43	-	-	-
Palermo	Godrano	Gorgo Lungo (N)	37.901286	13.408673	894	11	0.70	-	-	-
Palermo	Godrano	Laghetto Coda di Riccio (A)	37.873226	13.398654	862	17	0.86	-	-	-
Palermo	Godrano	Laghetto Coda di Riccio 2 (A)	37.872622	13.401166	868	5	0.81	-	-	-
Palermo	Godrano	Laghetto presso Valle Maria (A)	37.882222	13.411111	651	4	0.71	-	-	-
Palermo	Monreale	Chiusa Arcera (A)	37.916111	13.391111	584	2	0.94	-	-	-
Palermo	Monreale	Laghetto agricolo di Tagliavia (A)	37.896613	13.299692	589	14	0.37	-	-	-
Palermo	Monreale	Lago presso C/da Costa del Renoso (A)	37.883611	13.043056	216	7	0.41	-	-	-
Palermo	Monreale	Ponte Calatrasi (N)	37.844453	13.119358	203	-	0.36	-	-	-
Palermo	Monreale	Stagno agricolo di Gallitello (A)	37.866954	12.961537	132	23	0.41	-	-	-
Palermo	Palermo	Laghetto agricolo del Frattina (A)	37.865877	13.30409	478	11	0.52	-	-	-

Province	Municipality	Site name	Latitude N	Longitude E	Elevation (m a.s.l.)	<i>E. trinacris</i> (n. ind)	Naturalness Evaluation Index ("NEI")	<i>P. costata</i> (p/a)	<i>H. stepanowi</i> (p/a)	N. parasitized individuals (by <i>H. stepanowi</i>)
Ragusa	Ispica	Canale Arezzi (A)	36.707172	14.957663	3	-	0.33	-	-	-
Ragusa	Ispica	Canale presso C/da Carruba (A)	36.751374	14.980002	27	-	0.33	-	-	-
Ragusa	Ispica	Pantano Gariffi (A)	36.733481	14.938490	2	-	0.33	-	-	-
Ragusa	Ragusa	Fiume Irminio (N)	36.788669	14.602031	18	-	0.42	-	-	-
Siracusa	Noto	Canale Longarini (A)	36.735227	14.996854	8	6	0.33	-	-	-
Siracusa	Noto	Pantani di Vendicari (N)	36.807613	15.095825	2	13	0.69	-	-	-
Siracusa	Pachino	Ispica (n.a.)	-*	-*	n.a.	7	-	-	-	-
Siracusa	Pachino	Pantano Longarini 1 (N)	36.713826	15.01244	0	-	0.75	-	-	-
Siracusa	Pachino	Pantano Longarini 2 (N)	36.708364	15.00215	0	-	0.87	-	-	-
Siracusa	Pachino	Pantano Longarini 3 (N)	36.707618	15.00772	1	-	0.68	-	-	-
Trapani	Calatafimi-Segesta	Laghetto presso C/da Anguillara (A)	37.856747	12.914465	204	-	0.36	-	-	-
Trapani	Custonaci	Lentina (A)	38.051115	12.674649	90	3	0.37	-	-	-
Trapani	Mazara del Vallo	RNI "Lago Preola e Gorgi Tondi" (N)	37.609118	12.653975	3	30	0.55	-	-	-

To evaluate the influence of landscape features on species distribution, the "Naturalness Evaluation Index" ("NEI", Baiamonte *et al.*, 2015) of the area surrounding the sites (defined with a 500 m buffer) was calculated. The index was used to measure the conservation status of habitats where 0 means total artificiality and 1 maximum naturalness. Values of NEI were correlated with the occurrence of turtles in each site and the occurrence of parasitized individuals.

RESULTS

Overall, 336 individuals of *E. trinacris* were captured in 30 different sites out of the 46 sampled water bodies (Tab. 1). In 15 cases, sites consisted of natural habitats including lakes (4), ponds (6), and watercourses (5). In 14 cases the species was present in anthropogenic habitats including artificial lakes (2) and farm ponds (12). One sample includes pond turtles kept in captivity. Conversely, no *E. trinacris* were recorded in the other 16 surveyed sites. Sampling sites were located within different landscape contexts such as agricultural systems, pastures, wood plantations, and natural forests from sea level up to 1470 m a.s.l. The NEI mean value of sites with the absence and presence *E. trinacris* was respectively 0.54 and 0.61. The Spearman correlation test between occurrence of turtles and NEI of surrounding landscape was not significant ($r = 0.211$).

Among the sites where pond turtles were found, only seven showed the occurrence of *P. costata*. These are both natural (5 sites) and artificial ponds (2 sites), all located within areas of high naturalness: the Nebrodi Natural Park and the Sambuchetti-Campanito Natural Reserve. The forenamed areas are characterized by geological substrata of Numidian Flysch formation and by three different al-

titudinal vegetation belts (*Quercus suber* L., *Q. cerris* L. and *Fagus sylvatica* L. forests). The NEI value of the sites where *P. costata* has been detected ranges from 0.39 to 0.96, with a mean value of 0.67.

Based on the study of *Emys* blood smear samples, 35 individuals from eight sites out of the 336 pond turtles examined were positive for haemogregarines (Tab. 1). Within the pond turtle populations where the presence of *H. stepanowi* was recorded, all of which located in the Nebrodi area, 81% of the individuals of *E. trinacris* proved to be infected with haemogregarines. *H. stepanowi* was detected in individuals living in quite well-preserved environmental contexts in terms of vegetation diversity (mean of NEI equal to 0.67) where the main anthropogenic disturbance is represented by grazing. However, the Spearman correlation test coefficient between occurrence of parasitized individuals and NEI value of surrounding landscape was 0.21.

Conversely, no evidence for the presence of the api-complexan and leech parasites was observed in the 293 *E. trinacris* collected in the remaining 21 sites where the Sicilian pond turtle was found to occur (Fig. 1).

DISCUSSION

Based on the results, the system "*Emys trinacris* - *Placobdella costata* - *Haemogregarina stepanowi*" proved to occur in the Nebrodi area only.

Oddly, in three different sites (i.e. "Alcara li Fusi", "Marina di Caronia 2" and "Torre del Lauro": see Tab. 1) we recorded the occurrence of *H. stepanowi* but not the occurrence of *P. costata*; this is possibly related to the limited number of Sicilian pond turtles collected in these sites. Conversely, in two locations (i.e. "Sambuchetti-

Campanito 2" and "Stagno di Pantana", see Tab. 1), despite the occurrence of *P. costata*, no haemogregarine were detected; however, a single blood smear was examined, from the single pond turtle individual collected there. No blood smears were prepared for the turtles collected in "Stagno di Pantana".

Interestingly, no correlation was observed between the water bodies origin (i.e. natural or artificial, see Tab. 1), altitude or landscape features and the occurrence of *P. costata* and/or *H. stepanowi*. In fact, the occurrence of leeches and apicomplexans was observed in different water bodies in a bioclimatic range from thermomediterranean dry to supramediterranean subhumid (between 159 and 1252 m a.s.l.), and occurring in various landscapes (Bazan *et al.*, 2015), from cultivated to forested, highlighting the euryecy of these species. The collected evidence thus suggests that no ecological factors possibly determined the observed pattern, as already reported for other leech species (e.g., Marrone *et al.*, 2021). This interpretation is supported by a not significant Spearman correlation between presence of *E. trinacris* and *H. stepanowi* and NEI values.

Moreover, it is unlikely that the absence of *Haemogregarina* spp. in other Sicilian areas where *E. trinacris* occurs is linked to constraints in species dispersal, especially in the case of *Placobdella* leeches, which are able to take advantage of several biological dispersal vectors (Davies *et al.*, 1982; Vamberger & Trontelj, 2007). Conversely, a recent natural or human-mediated colonisation of *P. costata* in Sicily, with a point-introduction in the Nebrodi area along with translocated *E. orbicularis* specimens from mainland Italy (e.g., Vamberger *et al.*, 2015), can be possibly advocated to explain the observed pattern.

In sites where haemogregarines were found, the incidence of the apicomplexan infection was high, interesting 81% of the captured pond turtles (i.e., 35 infected turtles out of 43 captured individuals, see Tab. 1), thus showing how *H. stepanowi* has great efficiency in exploiting its definitive host (i.e., *P. costata*) to infect *E. trinacris* individuals.

To date, scant information is available on the health status of the *Emys* spp. populations infected by haemogregarines (Ozvegy *et al.*, 2015; Úngari *et al.*, 2018). Monitoring programs would therefore be advisable in order to check for any possible long-term negative effects on the populations of the endemic Sicilian pond turtle, and to prevent them.

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