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Research article

SEASONAL AND HABITAT PATTERNS OF DOMINANT *HAEMAPHYSALIS PUNCTATA* WITH *HAE. CONCINNA* (ACARI: IXODIDAE) RECORDED BEYOND KNOWN RANGE IN THE KARST EDGE, SLOVENIA

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Ticks of the genus *Haemaphysalis* are important vectors of various pathogens, yet their ecology and distribution remain poorly documented in certain regions. This study investigated the seasonal patterns, habitat preferences, and population densities of the dominant *Haemaphysalis punctata*, alongside the occurrence of *Hae. concinna* beyond its previously known range in the Karst Edge, Slovenia. Sampling was conducted at five sites encompassing pastures and forest habitats, targeting both adult and nymph stages. *Hae. punctata* was the dominant species (96.3% of sample size), with 47 males, 55 females, and 393 nymphs recorded, and it was present at all sites except the forest habitat in Osp. Most *Hae. punctata* ticks (70.7%) were collected from pastures, and the rest from the forest. Adults were detected throughout most of the year, but none were recorded between mid-June and mid-September. Nymphs occurred throughout much of the sampling period, with high abundances in spring and from early August to mid-October; they were absent in December-January and reappeared in February. Seasonal densities of *Hae. punctata* per 100 m² ranged from 0.21 at Osp to 10.36 individuals at Zazid (all pastures), up to over 30-fold higher than *Hae. concinna* at sites when both species occur concurrently. The latter was rare, with 4 males, no females, and 15 nymphs (3.7% of sample), and was recoreded at three sites: pastures in Osp and Hrastovlje, and forests in Zazid and Hrastovlje, over a period from mid-April to early July. *Hae. concinna* often co-occurred with *Hae. punctata* adults or nymphs. These findings confirm the dominance of *Hae. punctata* across studied habitats and document the first evidence of *Hae. concinna* in new localities along the Karst Edge, representing an extension of its known distribution in Slovenia. The study underscores the influence of habitat type on tick abundance and seasonality and provides critical baseline data for monitoring tick populations and potential vector-borne disease risks in the region.

Keywords: ecology, habitat, phenology, ticks, vector surveillance

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INTRODUCTION

Ticks (Arthropoda: Arachnida: Acari: Ixodida) are obligate hematophagous parasites belonging to three families: hard ticks (Ixodidae), soft ticks (Argasidae), and the non-European Nuttalliellidae [1]. They are recognized as major vectors of a wide range of pathogens affecting humans, livestock, and wildlife [2,3]. Their involvement in the transmission of bacterial, viral, and protozoan pathogens [4] makes them a central focus of ecological and epidemiological research, particularly in regions where tick-borne diseases are emerging or expanding as a consequence of climate change, land-use alteration, and wildlife population dynamics [5]. In terms of the number of pathogens transmitted, hard ticks surpass all other arthropod vectors except mosquitoes [1,6].

Tick activity is strongly influenced by temperature and humidity, with most species being active during the warmer months, while others remain inactive during the cold season [7]. All developmental stages of most hard ticks are susceptible to desiccation [8]; thus, temperature and humidity limit their distribution and activity [9]. Generally, ticks prefer shaded habitats that provide protection from desiccation, such as forests, shrubs, and dense grasslands, although habitat preferences vary among species. Most are concentrated in areas where potential hosts are abundant [10]. Ticks detect hosts by sensing body heat and exhaled CO_2 [10] and attach when a host brushes against the vegetation on which they are questing [7]. Feeding occurs through the hypostome inserted into the host's skin, and the bite is often unnoticed because tick saliva contains anesthetic compounds [11].

Of the more than 900 described tick species, approximately 70 occur in Europe [11]. European ticks belong to seven genera: *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus* (all Ixodidae), and *Argas* and *Ornithodoros* (both Argasidae). In Slovenia, 16 species of hard ticks (Ixodidae) have been recorded [2,12-21], representing all European genera.

Within the family Ixodidae, the genus *Haemaphysalis* comprises species with diverse ecological niches, host associations, and vector capacities. Two species of particular interest in Central Europe are *Haemaphysalis punctata* Canestrini and Fanzago 1878 and *Haemaphysalis concinna* Koch 1844, both exhibiting distinct habitat preferences, seasonal activity patterns, and host ranges [3,22]. Both species are known from Slovenia but have been reported as geographically localized [20,21]. Species of *Haemaphysalis* are often understudied regarding their vector roles, largely because medical and epidemiological attention has focused on the widespread *Ixodes ricinus*, which is monitored by the European Centre for Disease Prevention and Control [22]. Nevertheless, several *Haemaphysalis* species may parasitize humans and are of veterinary importance or maintain pathogens in wildlife hosts that serve as reservoirs for human infections [23].

Hae. punctata is widely distributed across the Palearctic region [11], including the Balkan Peninsula and Slovenia, where it is commonly recorded on birds, small mammals, and occasionally larger hosts [2]. It is a three-host tick with a one to three-year life cycle [24]. It predominantly inhabits open environments such as pastures, meadows, and shrublands, showing marked seasonal activity peaks influenced by temperature, humidity, and vegetation structure [5]. In Slovenia, it has been recorded from a variety of landscapes, although detailed information on its population density, sex ratio, host range, and habitat preferences remains limited. Understanding these ecological characteristics is important, as *Hae. punctata* is implicated in the transmission of pathogens affecting livestock (*Babesia major*, *B. motasi*, and *Theileria* spp. [6,9]) and potentially humans, including *Ehrlichia* sp., *Francisella tularensis* [9], and viral agents such as the tick-borne encephalitis (TBE) virus [9,23]. TBE is a viral disease of the central nervous system endemic to Slovenia. Slovenia has one of the highest incidence rates of TBE in the European Union, which poses a health risk mainly to individuals frequenting natural habitats during the tick season [25,26]. Moreover, tick paralysis caused by a neurotoxin secreted by *Hae. punctata* has been reported, occasionally leading to paralysis in animals and, rarely, in humans [9].

In contrast, *Hae. concinna* is considered a relict species with a more restricted distribution in temperate Eurasian forests, including Central and Eastern Europe [3,27]. It is a triannual, three-host species with minor host preference [28]. It primarily parasitizes large mammals, such as ungulates, while immature stages feed on small mammals and birds [29]. *Hae. concinna* is of medical and veterinary relevance due to its ability to transmit *Rickettsia sibirica* and *R. heilongjiangensis* (causative agents of Siberian tick typhus and Far-Eastern spotted fever), *Francisella tularensis* (causing tularaemia), TBE virus (causing tick-borne encephalitis), virus of Russian spring-summer encephalitis (RSSE) and *Babesia* spp. [9,23]. Despite confirmed records in neighboring countries (closest to our study site from Krk island in Croatia [30]), reports from Slovenia are scarce and mostly isolated. In Slovenia, this species was recorded in the Prekmurje region in the northeast in 2002 and subsequently confirmed in later studies [21]. Since then, its known distribution has not expanded, although its true range may be underestimated [3,23,31]. Expanding knowledge of its occurrence and distribution is essential for improving the understanding of regional tick biodiversity and assessing emerging disease risks.

The Karst Edge region of Slovenia provides an ecologically diverse setting for studying these species, characterized by a mosaic of pastures, shrublands, and forest patches. Such habitat heterogeneity can strongly influence tick distribution, abundance, and seasonal dynamics [5,32]. The potential co-occurrence of *Hae. punctata* and *Hae. concinna* in overlapping habitats could have implications for pathogen transmission dynamics and interspecific interactions.

The aim of this study was to investigate the seasonal dynamics, habitat preferences, and population densities of *Hae. punctata*, and to assess the potential presence of other *Haemaphysalis* species in the study area. The research was designed to characterize patterns of tick activity across different habitats, particularly pastures and forests, and to evaluate how climatic conditions and host availability may influence their abundance. By integrating multi-site field sampling, the study provides baseline ecological data essential for long-term monitoring of tick populations, assessing vector-borne disease risks, and supporting wildlife, livestock and public health management. Additionally, documenting the distribution and activity of *Haemaphysalis* ticks in previously unreported localities contributes to understanding their ecological adaptability and potential responses to ongoing environmental and climatic changes.

MATERIALS AND METHODS

Study area

Tick sampling was carried out at five sites along the Karst Edge in southwestern Slovenia: Kastelec–Socerb, Osp, Prešnica, Zazid, and Hrastovlje (Figure 1). Each site comprised two habitats, pasture and adjacent mixed deciduous–coniferous forest (Figure 1, Table 1), predominantly representing characteristic karst terrain at elevations between approximately 250 and 650 m a.s.l. Pastures consisted of open grasslands, often grazed by cattle, horses, or small ruminants such as sheep and goats (based on field observations). The vegetation was typically short to medium in height, with scattered shrubs providing partial shade. These dry karst grasslands were dominated by a mixture of grasses and herbaceous species [33,34], and regular grazing and animal movement created favourable conditions for questing ticks.

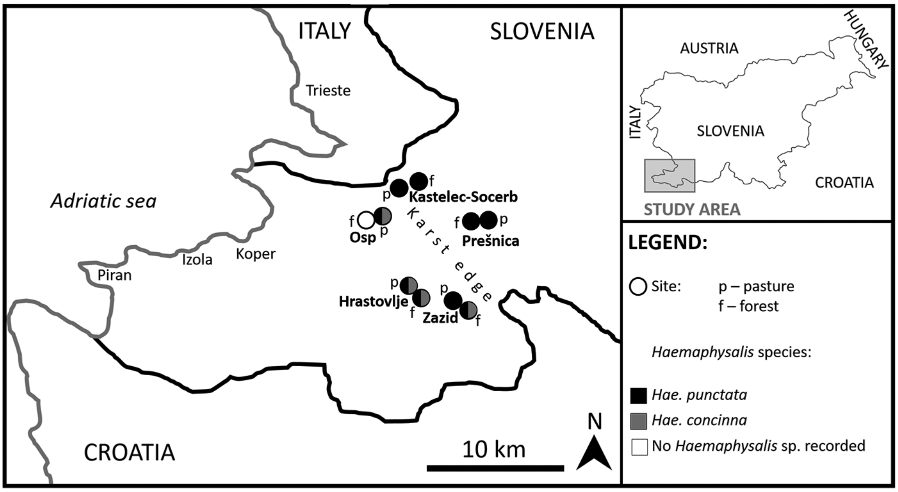

Figure 1. Map of sampling sites along the Karst Edge, SW Slovenia, showing habitat type (**p** = pasture, **f** = forest) and the presence of *Haemaphysalis* species. Each site × habitat type is represented by a coloured symbol indicating species presence; symbols are divided into two colours where both *Hae. punctata* and *Hae. concinna* co-occur.
For exact tick densities, see Table 1.

Table 1. Sampling sites, habitat characteristics, and abundance of *Haemaphysalis* ticks (nymphs and adults) recorded in the Karst Edge area (southwestern Slovenia). Densities of ticks (number per 100 m^2) are given in brackets. Symbol “×” represents mixed habitat type.

Site	Habitat	X	Y	Altitude [ma.s.l.]	Habitat description / Domestic animals	Habitat type [34]	*Hae. punctata*	*Hae. concinna*
Kastelec-Socerb	Pasture	45.58460	13.86125	550	Dry karst pasture, SW slope / cattle	Sub-Mediterranean–Illyrian pastures and dry stony grasslands.	13**⚥**, 6♂, 10♀ (2.07)	-
	Forest	45.58897	13.86432	630	Mixed deciduous-coniferous forest, SW slope.	Shrubby coniferous forests and areas undergoing succession with coniferous tree species; Forests.	7**⚥**, 1♂, 5♀ (0.93)	-
Osp	Pasture	45.57396	13.84781	257	Dry karst pasture, levelled / horses	Fenced pasture × Ruderal communities; Central European mesotrophic to eutrophic lowland grasslands × Ruderal communities; Sub-Mediterranean–Illyrian semi-dry extensive grasslands on flysch × Shrubby deciduous forests and areas undergoing succession with deciduous tree species.	2**⚥**, 0♂, 1♀ (0.21)	3**⚥**, 1♂, 0♀ (0.29)
	Forest	45.57444	13.84698	258	Mixed deciduous-coniferous forest, levelled.	Forests of the sub-Mediterranean region and western Eurasian steppes and semi-steppes, dominated by deciduous and partly deciduous thermophilous oak species × Forest stands of Robinia pseudoacacia.	-	-
Prešnica	Pasture	45.56439	13.93103	660	Dry karst pasture, SW slope / cattle	Sub-Mediterranean–Illyrian pastures and dry stony grasslands × Juniper scrub as a successional stage of dry grasslands.	81**⚥**, 15♂, 9♀ (7.50)	-
	Forest	45.56365	13.92964	658	Mixed, dominantly coniferous forest, SW slope	Forest islands.	32**⚥**, 3♂, 7♀ (3.00)	-
Zazid	Pasture	45.49181	13.93742	629	Dry karst pasture, SW slope / cattle	Sub-Mediterranean–Illyrian pastures and dry stony grasslands × Central European thermophilous basiphilous shrublands.	139**⚥**, 3♂, 3♀ (10.36)	-
	Forest	45.49162	13.93872	623	Mixed deciduous-coniferous forest, SW slope	Juniper scrub as a successional stage of dry grasslands × Shrubby coniferous forests and areas undergoing succession with coniferous tree species.	59**⚥**, 6♂, 5♀ (5.00)	8**⚥**, 2♂, 0♀ (0.71)
Hrastovlje	Pasture	45.52324	13.88970	323	Dry karst pasture, levelled / cattle, sheep, goats, horses	Sub-Mediterranean–Illyrian pastures and dry stony grasslands × Shrubby deciduous forests and areas undergoing succession with deciduous tree species.	44**⚥**, 12♂, 12♀ (4.86)	1**⚥**, 1♂, 0♀ (0.14)
	Forest	45.51955	13.89692	321	Mixed deciduous-coniferous forest, levelled	Thermophilous and coastal oak forests / Shrubby deciduous forests and areas undergoing succession with deciduous tree species.	16**⚥**, 1♂, 3♀ (1.43)	3**⚥**, 0♂, 0♀ (0.21)
SUM						Pasture	279**⚥**, 36♂, 35♀ (5.00)	4**⚥**, 2♂, 0♀ (0.09)
						Forest	114**⚥**, 11♂, 20♀ (2.07)	11**⚥**, 2♂, 0♀ (0.19)
						Pasture + Forest	393**⚥**, 47♂, 55♀ (3.54)	15**⚥**, 4♂, 0♀ (0.14)

The adjacent forests were primarily mixed deciduous–coniferous stands with a multilayered canopy and a well-developed understory of shrubs and herbaceous plants. Dominant tree species included *Quercus pubescens*, *Carpinus betulus*, *Pinus nigra*, and *Fagus sylvatica* [33]. Leaf litter, fallen branches, and low understory vegetation contributed to a humid microenvironment suitable for tick survival. A detailed characterisation of both habitats, categorized according to their structural features, is provided in Table 1.

The region has a sub-Mediterranean climate, characterized by warm, dry summers and mild, humid winters, influenced by both Mediterranean and continental air masses. Mean annual air temperatures range between 12 °C and 14 °C, and annual precipitation typically exceeds 1000 mm, with the majority falling in late autumn and winter [35]. Seasonal variability and frequent Bora (north-eastern) winds are typical climatic features of the Karst Edge area, shaping local vegetation and microhabitat conditions relevant for tick activity and survival [32].

Sampling dates

Sampling was carried out on 14 occasions between April 2024 and April 2025 to capture seasonal variations in tick activity. Ticks were collected monthly during the main activity season (April–December 2024) and continued at regular intervals through the winter and early spring (January–April 2025). Sampling dates were: 12 April, 11 May, 5 June, 20 June, 3 July, 8 August, 18 September, 16 October, 7 November, and 5 December 2024, and 15 January, 7 February, 7 March, and 11 April 2025.

Tick collection and identification

Ticks were collected using the standard dragging method (cf. [32]), which involves dragging a 1 × 1 m white cotton cloth over vegetation to capture questing specimens. At each sampling date and site, ticks were collected in both habitats (forest and pasture) by performing ten drags of 10 meters each, corresponding to a total sampled area of 100 m² per habitat. Over the entire study period, this resulted in a cumulative sampling area of 1400 m² for each habitat type at each site. The sampling protocol and effort were standardized across all locations and dates to ensure data comparability.

Collected ticks were placed alive in labelled vials (indicating date, habitat, and site), containing a small amount of fresh vegetation to maintain humidity prior to identification. Specimens were sorted under a stereomicroscope (Leica MZ 125), and *Haemaphysalis* ticks were morphologically identified to species and developmental stage following the identification keys of Estrada-Peña et al. [11] (see also [30] for *Hae. concinna* nymphs). Following identification, ticks were preserved in 70% ethanol for long-term storage. Although additional tick genera were encountered during sampling, this study focused exclusively on *Haemaphysalis* species, and other taxa were not systematically analysed. Hence broader patterns of tick community composition in the study area remain beyond the scope of this work.

Ethical approval

The conducted research is not related to animal use.

Data analysis

For each sampling site and habitat, the total numbers of *Haemaphysalis* nymphs, males, and females were recorded. The proportion of adults of each species was calculated separately for pasture and forest habitats to assess habitat-specific distribution patterns. Seasonal dynamics were described based on dates of occurrence, illustrating temporal changes in species presence throughout the 2024–2025 sampling period. Data on mean monthly temperatures (°C) and monthly rainfall (mm) were retrieved from ARSO (Slovenia environment agency) data archive [31] for the nearest meteorological station from the Karst area (Godnje), inspecting the possible connection between species abundance and both environmental parameters. Spatial distribution was analyzed according to site and habitat, and instances of co-occurrence between the recorded *Haemaphysalis* species were identified to determine where and in what ratios they appeared together. Tick density for each *Haemaphysalis* species was calculated as the number of individuals collected per 100 m² of sampled area, both for the total dataset and separately by habitat (pasture and forest). Densities were also computed separately for each habitat and site to assess spatial differences between species and habitats. For each sampling occasion, tick frequencies were used to evaluate temporal trends of each species. All data were processed and analyzed using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA).

The analyses performed in this study were primarily descriptive, focusing on patterns of seasonal dynamics, habitat preferences, and spatial distribution of *Haemaphysalis* species. No formal inferential statistical tests were applied, as the aim was to provide baseline ecological data and identify general trends rather than test specific hypotheses.

RESULTS

Besides the widespread *Hae. punctata* (N_{males} = 47, $N_{females}$ = 55, N_{nymphs} = 393, Figure 2), a small number of specimens of *Hae. concinna* (Figure 2) were also recorded (N_{males} = 4, $N_{females}$ = 0, N_{nymphs} = 15), accounting for approximately 3.7% of the total *Haemaphysalis* sample (Figure 3). Among the collected specimens, two additional species were also identified: *Ixodes ricinus* and *Hyalomma marginatum*.

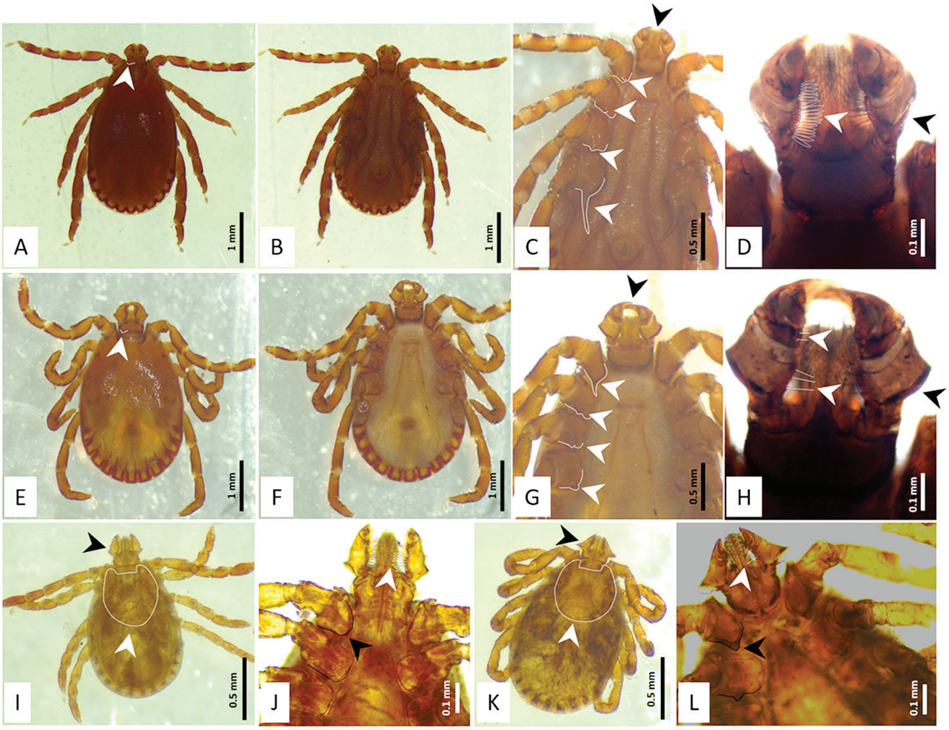

Figure 2. Male of *Haemaphysalis punctata* (**A–D:** Prešnica – forest, 18 September 2024) and male of *Haemaphysalis concinna* (**E–H:** Zazid – forest, 3 July 2024) from the Karst Edge (SW Slovenia). (**A, E**) Dorsal view (white arrows: note the prominent *cornua* in *Hae. concinna*); (**B, C, F, G**) Ventral view (white arrows: internal spurs of coxae I–IV; **black arrows:** note the overlap of article III of the palps in *Hae. concinna*); (**D, H**) Palps (**white arrows:** note the difference in number and width of setae on palp articles II–III, *Hae. punctata* > *Hae. concinna*; black arrows: note the difference in palp shape). In females (not shown), the relative lengths of internal spurs of coxae I–IV correspond to those observed in males. Nymph of *Hae. punctata* (**I, J:** Prešnica – pasture, 18 September 2024) and nymph of *Hae. concinna* (**K, L:** Zazid – forest, 3 July 2024). Note the shape of palps (black arrows: I, K), shape of scutum (white arrows: I, K), setae on palps (white arrows: J, L), and internal spurs of coxae I–II (black arrows: J, L).

Hae. punctata, the dominant *Haemaphysalis* species, was present at all five sites (Kastelec-Socerb, Osp, Prešnica, Zazid, Hrastovlje), and was absent only from the forest habitat at one site (Osp; Figure 1). The species was markedly more abundant in pastures, where almost three quarters (70.7%) of all individuals were collected. At Osp (pasture), livestock consisted only of horses, whereas at other sites also other livestock was present (in most cases cattle, but also horses, goats, and sheep in Hrastovlje; Table 1).

Although *Hae. punctata* adults were detected throughout most of the year, a mid-summer decline was observed between mid-June and mid-September, coinciding with high mean monthly temperatures (>18 °C) and low rainfall (minimum in July, approximately 31 mm). In contrast, nymphal abundance peaked in spring and from early August to mid-October (Figure 3). No *Hae. punctata* nymphs were found between December and January, but they reappeared in February (Figure 3).

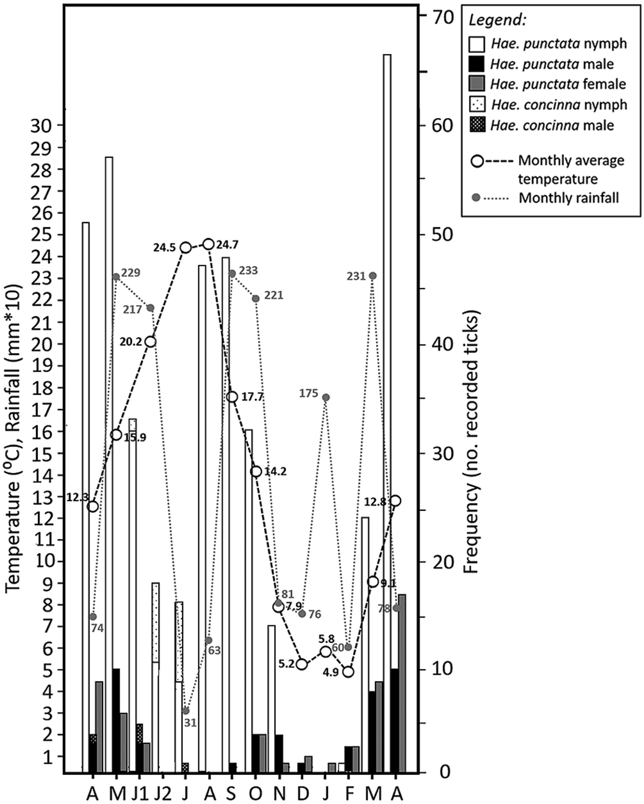

Figure 3. Numbers of collected *Haemaphysalis* ticks (*Hae. punctata* and *Hae. concinna*) across 14 sampling occasions (April 2024 – April 2025) and five sampling sites (each including two habitats, forest and pasture). Except for June 2024, where two samples were taken on 5 June and 20 June (denoted with J1 and J2 respectively), one sample was collected per each month (abbreviated with first letter of the month, ordered chronologically). Symbols: ○ – mean monthly temperature (°C, values added in black); ● – monthly rainfall (mm, values added in grey). Meteorological data were obtained from the Godnje meteorological station [31].

Hae. concinna was recorded at three sites: at Osp (pasture), Zazid (forest) and Hrastovlje (pasture and forest; Figure 1). In pasture at Osp livestock included horses while in Hrastovlje, there were horses together with sheep, goats and cattle. At pasture in Zazid, only cattle were present (Table 1). Its occurrence spanned nearly three months, from mid-April to early July (sampling dates: 12 April, 5 June, 20 June and 3 July 2024). Approximately two-thirds of all individuals (68.4%, n = 13) and half of the adults (50.0%, n = 2) were collected in forest habitats. *Hae. concinna* often co-occurred with *Hae. punctata* adults or nymphs (Table 2).

Table 2. Records of *Haemaphysalis concinna* and numbers of co-occurring *Hae. punctata* collected during the same sampling occasion at the same site and habitat. Notably, no *Hae. punctata* were recorded in Osp - forest, whereas the species was found in Zazid - pasture and Hrastovlje - forest (see Table 1).

		Hae. concinna			***Hae. punctata***		
Date	**Site**	⚥⚥	♂♂	♀♀	⚥⚥	♂♂	♀♀
12 April 2024	Osp – pasture	0	1	0	0	0	0
5 June 2024	Zazid – forest	0	1	0	13	1	1
5 June 2024	Hrastovlje – pasture	0	1	0	0	1	2
5 June 2024	Osp – pasture	1	0	0	0	0	0
20 June 2024	Osp – pasture	2	0	0	0	0	0
20 June 2024	Zazid – forest	1	0	0	5	0	0
20 June 2024	Hrastovlje – forest	3	0	0	0	0	0
20 June 2024	Hrastovlje – pasture	1	0	0	1	0	0
3 July 2024	Zazid – forest	7	1	0	1	0	0

Hae. punctata was overall more abundant than *Hae. concinna*, with seasonal densities of 3.54 and 0.14 individuals/100 m², respectively. Where present, the density of *Hae. punctata* varied from 0.21 (Osp – pasture) to 10.36 (Zazid – pasture), exceeding *Hae. concinna* also by over 30-fold at one co-occurring site (pasture at Hrastovlje). At sites where both species were scarce, such as Osp pasture, densities were comparable (0.21 vs. 0.29 individuals/100 m² for *Hae. punctata* and *Hae. concinna*, respectively; Table 1). *Hae. punctata* was considerably more abundant in pastures (5.00 individuals/100 m²) than in forests (2.07 individuals/100 m²), whereas it was the opposite but with a small difference for *Hae. concinna* (0.19 vs. 0.09 individuals/100 m² in forest and pasture, respectively; Table 1).

DISCUSSION

This study provides a comprehensive analysis of the ecology, seasonal dynamics, and morphology of *Hae. punctata*, together with the first record of *Hae. concinna* in the Karst Edge region of Slovenia. The results confirm the ecological dominance of *Hae. punctata*, which was present across all five surveyed sites and in multiple habitat types (see Table 1) and was, until now, the only previously known *Haemaphysalis* species from the sub-Mediteranean part of Slovenia (cf. [21]). In contrast, *Hae. concinna* was detected for the first time in this region, representing a considerable extension of its known distribution range [3,32] exceeding 200 kilometres southwest from the nearest known continental Slovenian localities in Prekmurje where the southern limit of its distribution had previously been established [21]. When considered in a broader geographic context, the apparent range expansion is smaller in northwest direction from the Mediterranean island of Krk [30] in Croatia (i.e., approximately

60–70 kilometres). Nevertheless, given its Palearctic distribution [37] and confirmed presence in neighbouring countries (Croatia: [30,38,39]; Austria, Hungary, Italy: [11]; Serbia: [40,41]; Bosnia and Herzegovina: [42]), its presence in the study area is not unexpected.

The identification of *Hae. concinna* was based on distinct morphological characteristics observed in adult male and nymph specimens, allowing reliable differentiation from *Hae. punctata* (Figure 2). Male *Hae. concinna* exhibited a prominent internal spur on the coxa of the first leg, which was longer than the internal spurs on other legs, and the second and third palp articles had only a few conical setae on the inner surfaces. Additionally, the cornua was prominent, and the third palp articles overlapped ventrally. In contrast, *Hae. punctata* males display shorter cornua, a long internal spur on coxa IV, short internal spurs on coxae I–III, and lack a ventral spur on the third palp article [11].

Additionally, nymphs of both species were identified mainly according to difference in the shape of scutum (more elongated in *Hae. punctata*) and the palps (as described in males [11,30]). Similar to males, *Hae. concinna* nymphs exhibited fewer and narrower setae on the palps. However, the lengths of the internal spurs on the coxae, a distinctive trait in adults, were highly variable and therefore not reliable for species identification. These features, however, allowed clear separation between the two species and highlight the importance of detailed morphological assessment even in the presence of rare or newly recorded species [23,43]. Because of the consistency of species-specific traits with published identification keys [11,30], no PCR-based confirmation (e.g., using 16S rRNA markers) was performed. Nevertheless, incorporating molecular approaches in future studies would provide additional support for species determination, particularly in cases of low abundance or morphologically similar taxa.

The low number of *Hae. concinna* specimens, together with the absence of females, suggests that the species may occur at low densities or may be in the early stages of establishment in the region [42,44]. Larvae might have been neglected during collection owing to their small size, or an inventory of their primary hosts, such as small mammals and birds [45,46], should be done to confirm their presence in the area. The detection of *Hae. concinna* in both pastures and forest habitats demonstrates its ecological flexibility, which may facilitate future range expansion under changing environmental conditions [3,47]. *Hae. concinna* may have reached the Karst Edge via infested migratory birds, as its larvae and nymphs are bird ectoparasites [29,45]. Transport of ixodid ticks by migratory birds is well documented [48,49] and has already been hypothesized as a possible pathway for *Hae. concinna*'s range expansion [42]. Nevertheless, *Hae. concinna* may have been present in the study area but, due to its close resemblance to the dominant congeneric species (*Hae. punctata*), its short activity period, and apparent rarity, it may simply have remained undetected until now. A limitation of this study is the relatively low number of *Hae. concinna* specimens, which restricts more detailed statistical and ecological interpretation. In addition, the study included a limited number of sampling sites, which may not fully capture the spatial variability of tick populations in the Karst Edge region.

Seasonal patterns observed for *Hae. punctata* adults indicate activity throughout most of the year, with a distinct mid-summer pause, likely associated with high temperature and low humidity, which are known to limit tick questing behaviour [5]. Nymphs exhibited peak activity in spring and again from late summer to early autumn, suggesting asynchronous life-stage activity, a strategy that may maximize survival and reproduction under variable environmental conditions [9]. The absence of nymphs in December and January, followed by their reappearance in February, is consistent with overwintering diapause. Interannual differences further suggest sensitivity to climatic variability [50].

In contrast, *Hae. concinna* displayed a restricted unimodal activity period (e.g. [21]) from mid-April to early July, consistent with observations from other parts of Central Europe, where *Hae. concinna* shows a short spring–early summer activity window [32,44], although Hillyard [51] reported activity of all developmental stages from March to June and again in October. In the Prekmurje region, adults were recorded from April to September and immature stages from April to October [21], whereas on the Croatian Kvarner island of Krk, Hassl [30] found nymphs in May parasitising five reptile species.

Although the limited sample size precludes detailed phenological conclusions, the observed timing aligns with the species' thermophilic and relict ecological characteristics, reflecting adaptation to specific hydrothermal conditions [52]. Recorded activity at mean monthly temperatures between approximately 12 °C to 24 °C (Figure 3) supports previous findings on its temperature-dependent activity patterns [21, 46].

Habitat associations observed in this study indicate a clear preference of *Hae. punctata* for pasture environments, where the majority of individuals were collected. This is consistent with previous reports of the species adaptation to sun-exposed habitats and association with livestock and ground-dwelling birds [2,47]. In contrast, *Hae. concinna* was recorded in both pasture and forest habitats, often in transitional or bushy microhabitats, suggesting ecological flexibility [3,23]. Such habitat use corresponds with previous reports describing the species as associated with light humid leafy and mixed hornbeam-oak forests, forest clearings, and edges [45,51]. In the present study it was always found in bushy sites, either within pastures or forests, likely at the interface of both habitats [53] (see Table 1). Nosek [45] similarly noted the association of *Hae. concinna* with bushy areas, and Barandika et al. [53] reported its preference for intermediate habitats between forests and herbaceous vegetation. As this species is associated with wet habitats in some regions [45], its occurrence on impermeable flysch substrates in pastures near a river at Osp, as well as on permeable limestone in bushy, partially shaded sites (Hrastovlje – pasture and Zazid – forest), or near a small riverbed (Hrastovlje – forest), is consistent with previous observations. Finally, besides autumn, spring represents the wettest period of the year in the study area (Figure 3).

Population density estimates revealed substantial differences between the two species, with *Hae. punctata* being considerably more abundant across most sites. Local density variation likely reflects differences in habitat suitability, host density, and microclimatic conditions [9,11]. Although *Hae. concinna* occurred at lower densities, its co-occurrence with *Hae. punctata* at some sites suggests potential ecological interactions, which may have implications for host use and pathogen transmission dynamics [50,54].

Both investigated tick species are competent vectors of pathogens of veterinary and medical importance, including *Francisella tularensis*, TBE virus, and *Babesia* species [2,23,43]. The presence of *Hae. concinna* in new areas may influence local pathogen transmission dynamics, increasing the potential risk to humans and animals. Climate change, land-use alterations, and shifts in host distribution could facilitate further expansion, necessitating ongoing monitoring and integrated vector management strategies [11,47].

Continued monitoring with increased sampling effort, integrating morphological and molecular methods is critical to detect emerging species, assess their establishment, and understand the ecological and epidemiological consequences of tick-borne diseases in Central Europe. Finally, broader patterns of tick community composition in the study area (i.e., more species included) should be addressed in future research.

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Author's contributions

JJ conceived and designed the study, participated in field sampling and species identification, analysed and interpreted the data, and drafted and revised the manuscript. The author read and approved the final manuscript.

Declaration of competing interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SEZONSKI I STANIŠNI OBRAZCI DOMINANTNE *HAEMAPHYSALIS PUNCTATA* I *HAE. CONCINNA* (ACARI: *IXODIDAE*) ZABELEŽENIH VAN POZNATOG PROSTIRANJA U ZONI KRASA, SLOVENIJA

Jure JUGOVIC

Krpelji roda *Haemaphysalis* su važni vektori raznih patogena, ali njihova ekologija i rasprostranjenost ostaju slabo dokumentovani u određenim regionima. Ova studija je istraživala sezonske obrasce, preferencije staništa i gustinu populacije dominantne vrste *Haemaphysalis punctata*, zajedno sa pojavom *Hae. concinna* izvan njenog ranije poznatog areala na kraškom rubu u Sloveniji. Uzorkovanje je sprovedeno na pet lokacija koje obuhvataju pašnjake i šumska staništa, ciljajući i odrasle i nimfe. *Hae. punctata* je bila dominantna vrsta (96,3% veličine uzorka), sa 47 mužjaka, 55 ženki i 393 nimfi, i bila je prisutna na svim lokacijama osim šumskog staništa u Osp. Većina krpelja *Hae. punctata* (70,7%) je sakupljena sa pašnjaka, a ostatak iz šume. Odrasle jedinke su registrovane tokom većeg dela godine, ali nijedna nije zabeležena u periodu od sredine juna do sredine septembra. Nimfe su bile prisutne tokom većeg dela perioda uzorkovanja, sa velikom brojnošću u proleće i od početka avgusta do sredine oktobra; nisu bile prisutne tokom decembra i januara, a ponovo su se pojavile u februaru. Sezonska gustina jedinki *Hae. punctata* na 100 m² kretala se od 0,21 u Ospu do 10,36 jedinki u Zazidu (svi pašnjaci), do preko 30 puta veće od *Hae. concinna* na lokalitetima gde se obe vrste javljaju istovremeno. Potonja je bila retka, sa 4 mužjaka, nijednom ženkom i 15 nimfi (3,7% uzorka), i zabeležena je na tri lokaliteta: pašnjaci u Ospu i Hrastovlju i šuma u Zazidu i Hrastovlju, u periodu od sredine aprila do početka jula. *Hae. concinna* se često javljala zajedno sa odraslim jedinkama ili nimfama *Hae. punctata*. Ovi nalazi potvrđuju dominaciju *Hae. punctata* u proučavanim staništima i dokumentuju prve dokaze o *Hae. concinna* na novim lokalitetima duž kraškog ruba, što predstavlja proširenje njene poznate rasprostranjenosti u Sloveniji. Studija naglašava uticaj tipa staništa na brojnost i sezonalnost krpelja i pruža ključne osnovne podatke za praćenje populacija krpelja i potencijalnih rizika od bolesti koje prenose vektori u regionu.