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New insights into the distribution, ecology, and systematic position of the rare water mite *Rutripalpus limicola* Sokolow, 1934 (Acari: Rutripalpidae)

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Original research

ABSTRACT

The water mite *Rutripalpus limicola* Sokolow, 1934, can be considered exceptionally rare. It shows a scattered distribution range and, until recently, was known from six sites across Europe only. This strictly spring-dwelling species has a highly localised distribution, presumably due to its specific ecological requirements. We present the first records of *R. limicola* in the Netherlands and review the current state of knowledge on its distribution and biology. In addition to the previously existing morphology-based investigations, we provide a phylogenetic placement based on 28S rRNA gene data and shed light on the controversial systematic position of *R. limicola*: In contrast to the previously hypothesised Lebertioidea-relatedness of the isolated, monotypic family Rutripalpidae, our results reveal a putative Hydryphantoidea association. Moreover, we discuss the host-parasite association of *R. limicola* larvae and the dipteran family Ptychopteridae. Combined with additional information derived from the new records from the Netherlands, we contribute to a better understanding of this elusive species' biology and phylogenetic position.

Keywords Rutripalpidae; distribution; parasite-host relationship; Ptychopteridae; phylogenetic placement; 28S; COI

Introduction

The extremely rare water mite *Rutripalpus limicola* Sokolow, 1934 (Figure 1) is confined to Europe, and knowledge of its distribution is restricted to six widely scattered locations. It occurs exclusively in spring habitats (Gerecke and Tuzovskij 2001), and in Europe, it is the only representative of the monotypic, holarctic family Rutripalpidae Sokolow, 1934 (Di Sabatino *et al.* 2010). The only other known member of this family is *Rutripalpus canadensis* Smith, 1991, with a single record from a spring on Cape Breton Island in North America (Smith 1991). Despite the relatively few records, *R. limicola* belongs to the fraction of spring-inhabiting species appearing in all three Limnofauna Europaea distribution belts (see Gerecke *et al.* 2018) and can be considered widely distributed but exceptionally rare. Such a mosaic-like distribution is frequently found among inhabitants of spring habitats: In fact, 30 out of 137 spring-dwelling water mite species are known from single sites only (Gerecke *et al.* 2018).


Rutripalpus limicola is a relatively small water mite species, with males measuring 670-750 µm and females 780-820 µm in length (Gerecke and Tuzovskij 2001). It has a sandy brown to reddish colour and a somewhat oval body shape (Figure 1). The surface of the idiosoma integument is widely soft, but several muscle attachments are sclerotised: in both sexes, four

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Figure 1 Dorsal view of an adult female of *Rutripalpus limicola*. Photo: Christophe Brochard.

pairs of dorsocentralia; in males, also six pairs of dorsolateralia (with weaker sclerotisation, probably smooth in freshly emerged specimens), and one pair of ventralia in lateral position. Legs lack swimming setae, and the species has a crawling mode of locomotion. The most striking feature that readily distinguishes *R. limicola* adults in both sexes and their deutonymphs from other species is the peculiar shape of the 5th palp segment (P-5). In dorsal view, it is foot-like enlarged and carries four nail-like claws. Sexual dimorphism is manifested in the shape of the palps, with males exhibiting a lateral indentation of P-5 (Figure 2) and the genital field.

The distinct male external genital organ in the shape of a rectangularly bent tube that can be protruded by increasing hemolymph pressure is a further striking feature of *R. limicola*. The ejaculatory complex of this species is considered rudimentary (Gerecke and Tuzovskij 2001), supporting the interpretation that the external genital organ facilitates spermatophore transmission. The female genital field consists of a long gonopore slit and three pairs of acetabula flanked by a pair of flaps. For detailed morphological descriptions of the larva,



Figure 2 *Rutripalpus limicola*: Sexual dimorphism in the shape of the palps in specimens from the Netherlands. Left: male. Right: female. Photos: Christophe Brochard.

deutonymph, adult male, and female, see Gerecke and Tuzovskij (2001).

The same authors proposed to consider larvae with characteristic morphological traits, found together with adults of *R. limicola*, as the larval stage of the latter. Furthermore, they suggested that the mite's apparent host is the phantom crane fly *Ptychoptera albimana* (Fabricius, 1787), of which the larvae can be found in the same habitat (Figure 3). Gerecke and Tuzovskij (2001) mentioned that the mite larvae they found on Ptychopterid larvae were not engorged. They suggested that the larvae, in line with some other mite families, were in a phoretic stage, waiting for the ecdysis of the adult host before initiating hemolymph sucking. Besides providing the larvae with nutrition for development, the adult ptychopterid will also serve as a vector and facilitates mite dispersal.

The family Rutripalpidae is currently placed within the superfamily Lebertioidea Thor, 1900 (Smit 2020). The unique morphological features of this isolated family and its systematic position has been intensively discussed by Gerecke and Tuzovskij (2001). Based on a reassessment of adult morphological character states, and larval and deutonymphal morphology, they suggested that Rutripalpidae branched off early from a clade that includes Teutoniidae and one or more genera presently placed within the family Anisitsiellidae. However, despite morphological similarities between Rutripalpidae, Teutoniidae and Anisitsiellidae, Rutripalpidae also show numerous differences, as similarly discussed by Smith (1991): (1) the Rutripalpidae-



Figure 3 Adult female of the phantom crane fly *P. albimana*, the apparent host of *R. limicola*. Photo: Jan Kersten.

specific shovel-like P-5; (2) male genital shaped in the form of a penis-like tube; (3) complete reduction of proximal appendages of the genital skeleton. The current conclusion about the Lebertioidea association of Rutripalpidae is based mainly on plesiomorphic larval character states and, therefore, needs thorough reassessment. To further investigate the systematic placement of Rutripalpidae, we analysed newly generated 28S (LSU rRNA gene) sequences that previously have shown to resolve higher taxonomic levels in water mites appropriately (Blattner *et al.* 2019), and performed phylogenetic placement of *R. limicola* relative to species representing major water mite taxa. Furthermore, we generated CO1-barcodes to enable genetic species identification of *R. limicola* for future work and spring ecosystem assessments.

This paper presents the first records of *R. limicola* from the Netherlands and assembles the current state of knowledge about its distribution. Its biology will be discussed, emphasising the presumed host-parasite association of *R. limicola* and phantom craneflies (Diptera: Ptychopteridae). Furthermore, it reassesses its assumed systematic position in light of phylogenetic placement relative to the most abundant spring-dwelling water mite taxa.

Material and methods

Description of the two Dutch sampling sites

Both sites where *R. limicola* was found are situated within the Drentsche Aa catchment area and National Park (designated as Natura 2000 area), located in the northern part of the Netherlands. The first site (Figure 4) is a ferruginous helocrenic spring on the unshaded face of the stream bank of the Gastersche Diep, a medium stream of the catchment area. The spring's water trickled down from the bankside into the main channel. Ochre formation by iron-oxidising bacteria was colouring the substrate orange. The soil underneath these deposits was black, saturated with groundwater and oxygen-depleted. The water overlaying the deposits was approximately 1 cm deep and slow to moderately flowing. The vegetation on the bankside consisted of a variety of coarse grasses and herbaceous plants, like Great manna grass (*Glyceria maxima*), Common bulrush (*Typha latifolia*) and Water mint (*Mentha aquatica*). At the time of sampling, the pH of the water measured 8.3, with a water temperature of 16.9 °C and an electrical conductivity of 616 µS/cm.



Figure 4 First Dutch sampling site where *Rutripalpus limicola* was collected. Ferruginous seepage discharge on the face of the stream bank of the Gastersche Diep. Photo: Rink Wiggers.

The second sampling site is an iron-rich helocrenic spring area situated on the face of the stream bank of the Looner Diep, about 4.5 km upstream from the first site (Figure 5). The discharged groundwater of the springs seeped down from the bankside into the main channel. The soil was saturated with groundwater. The site was partially shaded by Black alder (*Alnus glutinosa*), with an undergrowth of plants like Water horsetail (*Equisetum fluviatile*) and Lesser water-parsnip (*Berula erecta*). At the time of sampling, the pH of the water measured 7.0, with a water temperature of 7.9 °C and an electrical conductivity of 245 µS/cm.

Sampling, processing of samples and identification

Sampling of the site on the bankside of Gastersche Diep was conducted on 27 May 2018 and 5 May 2020, respectively. The site on the bankside of Looner Diep was sampled on 10 Apr. 2021. Samples were collected using an invertebrate hand net (mesh size 500 µm). In the laboratory, samples were divided into coarse and fine fractions by washing the material through a stack of sieves with decreasing mesh size (mesh width bottom sieve: 300 µm). Subsequently, the invertebrates remaining in the different fractions were sorted and preserved in Koenike-fluid (water mites) and ethanol (70%) (other invertebrates). Two specimens of *R. limicola* were preserved in ethanol (96%) for subsequent sequencing. Identifications of water mites and other freshwater invertebrates were executed using a stereo microscope.

Known distribution area

The few available records of *R. limicola* have all been published and could be assembled by consulting these resources (see Table 1). Occasionally, details of the records have been requested from the involved researchers or collectors.



Figure 5 Second Dutch sampling site where *Rutripalpus limicola* was collected. Ferruginous seepage discharge on the partially shaded face of the stream bank of the Looner Diep. Photo: Rink Wiggers.

Phylogenetic placement

DNA extractions, 28S and CO1 PCR amplification, as well as Sanger sequencing, were performed as outlined in Blattner *et al.* (2019) using the 28SHy_F/R (28S) and LCO_Hydr/HCO_Hydr (CO1) primer pairs. The male *R. limicola* specimen from the Gastersche Diep and a female specimen from the Looner Diep spring sites in the Netherlands were processed genetically (specimens R1 and R5, Figure 8). Furthermore, due to lacking sequence data on GenBank, we included a *Teutonia cometes* (Koch, 1837) specimen (V36) that was sampled in May 2018 near Tübingen in Germany (coordinates: 48.5578° N, 9.04989° E; Figure 8) to include a Teutoniidae representative for improved phylogenetic placement. For all other Hydrachnidia species, 28S sequence data were taken from Blattner *et al.* (2019) and downloaded from the respective sequence data archives.

Raw sequences were quality checked, trimmed, and aligned with MAFFT v7.490 (Kato and Standley 2013) in Geneious Prime v2022.2 (<https://www.geneious.com>). We used Gblocks v0.91b (Castresana 2000; Talavera and Castresana 2007) with default parameters and allowed gap positions to exclude misassembled segments in the final 28S alignment. Phylogenetic maximum likelihood (ML) inference was performed with IQ-TREE v1.6.12 (Minh *et al.* 2020) on the HPC infrastructure of the University of Basel (sciCORE). The appropriate nucleotide substitution model TVM+F+I+G4 was assessed with ModelFinder (Kalyaanamoorthy *et al.* 2017) and chosen according to the Bayesian information criterion (BIC) implemented in IQ-TREE. Branch support was examined with 10,000 bootstrap replicates, and we used a *Stygothrombium chappuisi* Walter, 1947 sequence (GenBank Accession no.: KM100937.1) from Dabert *et al.* (2016) as an outgroup to root the phylogenetic tree. 28S sequences were deposited on NCBI GenBank under the accession numbers: OP784269 (R1), OP784270 (R5) and OP784271 (V36), and CO1-barcoding data were uploaded to BOLDsystems under the IDs: LBCWS246-22 (R1), LBCWS247-22 (R5) and LBCWS248-22 (V36).

Association of a putative larva

To investigate the association of a *Rutripalpus*-like larval specimen (R2) that was attached to a *Ptychoptera albimana* larva from the Benninger Ried (Germany) sampling site sampled in 2017 (Table 1; for the exact site, see Gerecke 2006) with the adult specimens from the Netherlands, CO1 sequences were generated as outlined above. However, due to unsuccessful PCR amplification using LCO_Hydr/HCO_Hydr primer pairs, new *Rutripalpus* genus-specific primers were designed based on the adult CO1 sequences: Rutri_CO1_F (5'-AAC CAY AAA GAY ATT GGC ACC-3') and Rutri_CO1_R (5'-CGA ARA ATC AAA AAA GGT GTT G-3'). The newly generated larval CO1 sequence is available under NCBI GenBank accession: OP793138. Sequence similarity between the larva and the adult individuals was calculated in Geneious Prime.

Results

Distribution – current state of knowledge

Rutripalpus limicola is confined to Europe and, until recently, was only known from six widely scattered localities. The first description was based on two females from a helocrene near Gobzhitsy, a Russian village near St. Petersburg (Sokolow 1934). It was not until 1988 that the species was found again in a helocrene at the lake shore of Mindelsee, Baden-Württemberg, Southern Germany. This time, also a male was found and could be newly described (Schwoerbel 1991). Goldschmidt (1994) reported a female, male and a deutonymph from the same site, collected in 1992.

Gerecke and Tuzovskij (2001) mentioned another observation of a single male from a helocrene in Montenegro back in 1990. They also found a ptychopterid larva with numerous mite larvae attached to its body at the same site, and they tentatively attributed the larvae to *R. limicola*. Gerecke (2006) reported another record of mite larvae with morphological features in agreement with the Montenegrin specimens attached to an adult *Ptychoptera albimana* found in 2002 in Bavaria. During a later visit to the Bavarian site, larvae and pupae of *P. albimana* with identical mite larvae attached were observed. Unfortunately, despite several additional attempts when revisiting the collecting site, the presence of adults or deutonymphs of *R. limicola* could not be confirmed.

Further records stem from two rheohelocrenic springs lying close together in the Hoher Trauchberg area, a pre-alpine mountain range in the Ostallgäu, Bavaria (Gerecke *et al.* 2011). One spring (D528-a) is located below a pasture fence near a forest edge (47.644253° N, 10.861729° E) at 848 m a.s.l. One female was collected in March 2008, and another female one year later (June 2009) at the same location. The second spring site (D528-f) is situated on a meadow called “Roßvichweide” (= “horse pasture”; 47.642337° N, 10.845480° E) at 799 m a.s.l., where another female was collected in March 2008.

The most recent documented record of *R. limicola* is from 2010 in northwestern Poland (Bańkowska *et al.* 2015). A single female was found in a helocrenic spring in the valley of the river Krapiel.

Recently we recorded the species for the first time at two closely located sites in the Netherlands. At the first site (Gastersche Diep at Gasteren, Province of Drenthe, 53.041667° N, 6.643000° E, leg. R. Wiggers), a single adult female was collected in May 2018 from a helocrenic spring. When revisiting the site in May 2020, an adult male was also collected. In April 2021, two more females were collected further upstream from this site (Looner Diep at Balloo, Province of Drenthe, 53.014833° N, 6.627567° E, leg. R. Wiggers) (Figure 5). The new Dutch records bring the total number of currently known record sites of *R. limicola* to seven. A summary of the record sites is presented in Table 1 and shown in Figure 6.



Figure 6 Distribution of *Rutripalpus limicola*. New record from the Netherlands (red) and previously known locations (blue). For detailed information, see Table 1. The map was created in QGIS v3.22.10 with base map tiles by Stamen Design (<http://maps.stamen.com>) under CC BY 3.0.

Invertebrate assemblage of the Dutch sites

The encountered aquatic invertebrate community at the site on the stream bank of the Gastersche Diep consisted of species able to withstand low oxygen levels and species demanding a fair amount of oxygen.

The tubificid *Potamothrix heuscheri* Bretscher, 1900, and larvae of the tanypod *Psectrotanypus varius* Fabricius, 1787, were encountered in substantial numbers in the oxygen-depleted mud. Both species tolerate anoxic conditions (Milbrink 1999; Moller Pillot and Buskens 1990). Also, early-stage larvae of *Ptychoptera albimana*, the assumed host of *R. limicola*, were encountered in the mud. Their long siphon acts like a snorkel, enabling them to survive in oxygen-deprived biotopes (Figure 7).

Examples of species of oxygen-rich running water are larvae of the chironomids *Paratendipes albimanus* Meigen, 1818 and *Prodiamesa olivacea* Meigen, 1818, both encountered in fair numbers at the site. Oxygen diffusion from the air into the trickles enables these species to occur at such sites.

Only two other water mite species were observed. Besides *R. limicola*, a single specimen of *Thyopsis cancellata* (Protz, 1896) was encountered, a species reported from helocrenic springs (Gerecke and Schwoerbel 1992) but can also be found in other biotopes influenced by



Figure 7 A typical larva of a phantom crane fly (Ptychopteridae) with its long, caudal respiratory siphon. The larva depicted is from the site at Looner Diep and belongs to *Ptychoptera scutellaris*. Photo Christophe Brochard.

groundwater. Furthermore, a single specimen of *Torrenticola amplexa* (Koenike, 1908) was found in the sample, but this species has its stronghold in the main course of the stream. A substantial number of microcrustaceans, mainly ostracods and copepods, were also found at the site.

The invertebrate community at the site on the stream bank of the Looner Diep is more characteristic of spring biotopes in the area. Other water mites encountered at the site are *Arrenurus sculptus* Halbert, 1903, *Lebertia minutipalpis* K. Viets, 1920, and *Sperchon*

Table 1 Summary of the available records of the different life stages of *R. limicola* and observed hosts with larvae. LS = life stage: ad = adult, dn = deutonymph, la = larva, pu = pupa; G = gender, N = number encountered.

Country	Location	Date	LS	G	N	Host (N)	Reference
Russia	Near Gobzhitsy, Luga	1933-06-18	ad	♀	1		Sokolow (1934)
		1933-06-23	ad	♀	1		Sokolow (1934)
Germany	Mindelsee, Baden-Württemberg	1988-06-01	dn	-	1		Gerecke and Tuzovskij (2001)
		1988-06-16	ad	♀	2		Schwoerbel (1991)
		1988-06-16	ad	♂	1		Schwoerbel (1991)
		1992-05-19	ad	♀	1		Goldschmidt (1994)
		1992-05-19	ad	♂	1		Goldschmidt (1994)
	Benninger Ried, Bavaria	1992-09-23	dn	-	1		Goldschmidt (1994)
		2002-09-27	la	-	20	<i>P. albimana</i> ad (1)	Gerecke (2006)
		2017-05-29	la	-	n	<i>P. albimana</i> la (5)	Gerecke (unpublished)
	Hoher Trauchberg, Bavaria	2017-05-29	la	-	7	<i>P. albimana</i> pu (2)	Gerecke (unpublished)
		2008-03-30	ad	♀	1		Gerecke et al. (2011)
2008-03-31		ad	♀	1		Gerecke et al. (2011)	
2009-06-05		ad	♀	1		Gerecke et al. (2011)	
Montenegro	Murino, Mokra Planina, near Velika	1990-06-06	ad	♂	1		Gerecke and Tuzovskij (2001)
		1990-06-06	la	-	n	<i>Ptychoptera</i> la (1)	Gerecke and Tuzovskij (2001)
Poland	Valley of the river Krąpiel	2015-09-02	ad	♀	1		Bańkowska et al. (2015)
Netherlands	Gastersche Diep, Drenthe	2018-05-27	ad	♀	1		This publication
		2020-05-05	ad	♂	1		This publication
	Looner Diep, Drenthe	2021-04-10	ad	♀	2		This publication

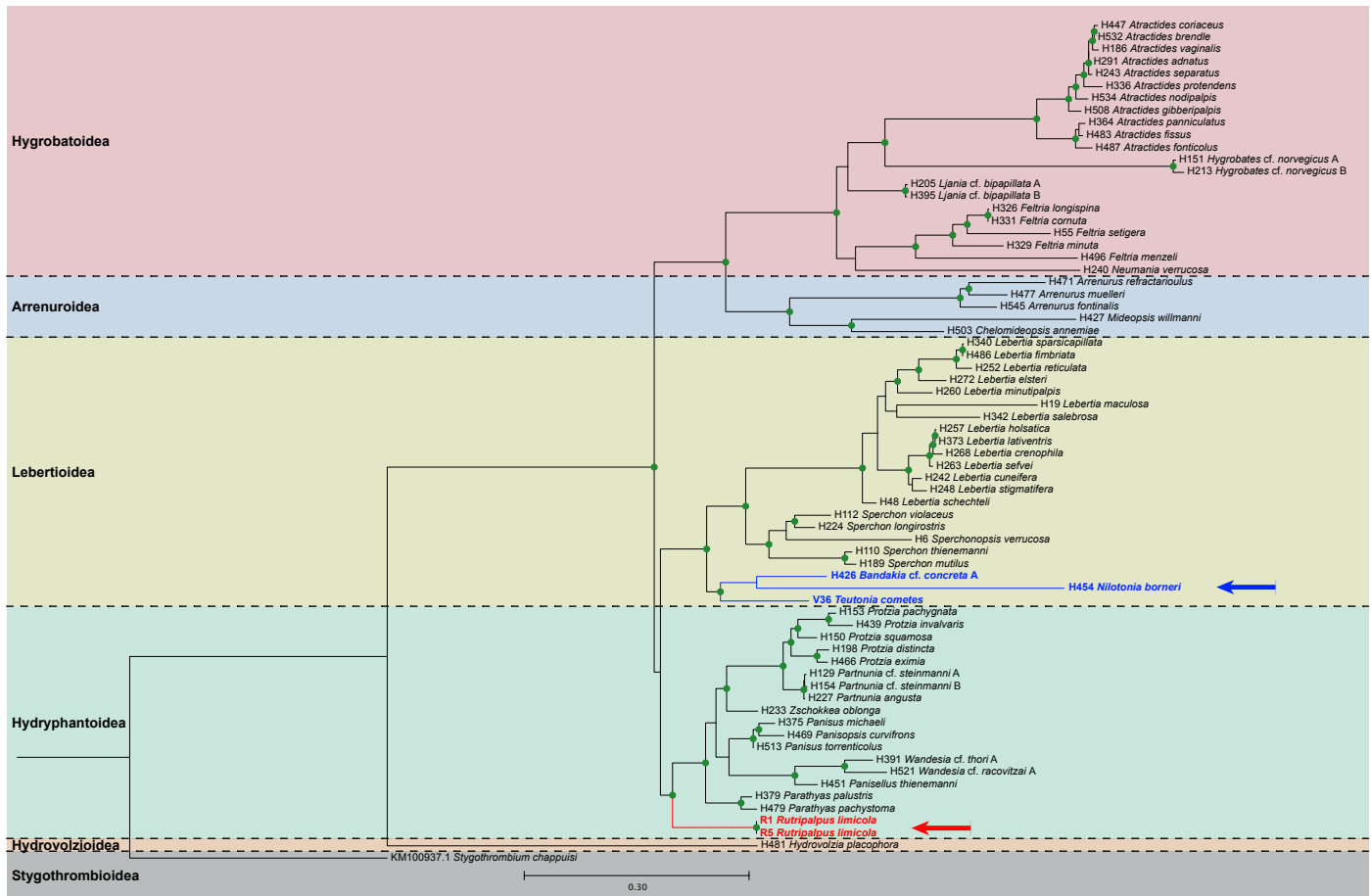


Figure 8 Phylogenetic placement of *Rutripalpus limicola* based on 28S maximum likelihood inference with major spring-related Hydrachnidia taxa. *Rutripalpus limicola* individuals are highlighted in red and indicated with the red arrow. The Lebertioidea clade previously assumed as *R. limicola* association is shown in blue and with a blue arrow. Sample IDs of the other specimens, except *Teutonia cometes* (this study) and the *Stygothrombium chappuisi* root specimen (GenBank Accession: KM100937.1), correspond to Blattner *et al.* (2019); thus, respective metadata can be found there. Nodes formed by well-supported branches (> 80% bootstrap support) are indicated with a green dot. Differently coloured backgrounds distinguish superfamilies.

squamosus Kramer, 1879. A selection of other invertebrates determined are the stoneflies *Nemoura dubitans* Morton, 1894 and *N. cinerea* (Retzius, 1783), larvae of the caddis flies *Beraea pullata* (Curtis, 1834) and *Plectrocnemia conspersa* (Curtis, 1834), and the chironomid larvae *Macropelopia adauca* Kieffer, 1916, *Brillia bifida* (Kieffer, 1909), *Conchapelopia melanops* (Meigen, 1818), *Corynoneura lobata* Edwards, 1924, and *Micropsectra cf. notescens* (Walker, 1856). The few ptychopterid larvae encountered belonged to *Ptychoptera scutellaris* Meigen, 1818 (Figure 7).

Systematic position and larval-adult association

The phylogenetic placement of *R. limicola* based on 28S maximum likelihood inference with major spring-related Hydrachnidia taxa is shown in Figure 8. The phylogenetic placement of *R. limicola* shows strong evidence for its Hydryphantoidea association (red arrow). The assumed Lebertioidea placement and relatedness of *R. limicola* with Teutoniidae (*Teutonia cometes*) and Anisitsiellidae (*Nilotonia* spp. and *Bandakia* spp.) is not evident (blue arrow). Furthermore, the Anisitsiellidae – Teutoniidae clade shows a relatively basal position within the Lebertioidea. Similarly, *R. limicola* can be found at a basal position relative to the presumably more modern Hydryphantoidea species.

The CO1 sequence from the larval specimen (R2) from the Benninger Ried sampling location shows relatively low sequence similarity (84.6%) relative to the adult *R. limicola* individuals from the Netherlands. Sequence identification on BOLDsystems showed the closest match with sequences from our adult *R. limicola* specimens.

Discussion

Habitat

Sokolow (1934) found the species at two sites and described the habitat as boggy seepages with ferruginous water. There, *R. limicola* occurred in depressions containing a substrate of black FPOM (Fine Particulate Organic Matter) on sand, overgrown with mosses and liverworts. The other site is described as a helocrene, located on the flood plain of the Oredezj River, with ferruginous water and black FPOM.

The site near the edge of lake Mindelsee is a muddy groundwater discharge in a mixed deciduous forest. It is situated only slightly above the level of, and in direct contact with the lake. In summer, nearly no open water is left. It is a typical helocrene with a very patchy structure and substantial variation in temperature and conductivity (Goldschmidt 1994). Schwoerbel (1991) found *R. limicola* at this exact location by sieving large amounts of FPOM.

Both the collecting site in Montenegro (Gerecke and Tuzovskij 2001) and the sites in Bavarian Ostallgäu (Gerecke *et al.* 2011) are unshaded, weakly seeping rheohelocrenes in pastureland. The other Bavarian site is situated within the extended groundwater outflow complex “Benninger Moos”, a Natura 2000 site famous for the last known populations worldwide of the sea thrift subspecies *Armeria maritima purpurea* (Plumbaginaceae) (Sonnberger 2020). Here, ptychopterid pupae and an adult parasitised by *R. limicola* larvae were found in a helocrene with *Sphagnum* and *Armeria*, shadowed by willows, on the border of a large spring stream draining the reed (Gerecke 2006).

Bańkowska *et al.* (2015) described the Polish site as a helocrene, associated with some small limnocrenes, situated amid willows with some sedges and mosses. The layer of water was thin (1-2 cm) and flowed over a muddy bottom with leaves. The pH measured was between 5.4-6.2, with an electrical conductivity ranging from 169 to 193 $\mu\text{S}/\text{cm}$ (personal communication A. Zawal).

The Dutch sites are both iron-rich helocrenes on the face of a stream bank. One site was an unshaded bankside with coarse grasses and herbaceous plants, whereas the other site was partially shaded (for more details, see material and methods).

In summary, it can be derived that *R. limicola* is a crenobiotic species with a preference for habitats characterised by weak but stable flow and extensive muddy, FPOM affluent areas, occurring both at forested and unshaded sites.

The presence of ptychopterids is likely to be a precondition but larvae of several species are commonly found in such habitats. Considering this, it is still unclear why *R. limicola* is so rare and not found more often at sites with seemingly similar conditions. Presumably, it is a species with highly specific requirements, which are not yet fully understood.

Phenology

The records of larvae, deutonymphs and adults of *R. limicola* are too few to provide a complete picture of the phenology of this species (see Table 1). Also, differentiation in the phenology of the populations occurring at the sites is not unlikely since the sites are situated in different regions.

It is apparent that the larvae are present in late spring and early summer. However, larvae of several spring-dwelling mite species are known to be present for more extended periods of time or during two distinct periods over a year (Martin and Stur 2006). If this is the case for larvae of *R. limicola* is unclear. A factor which might facilitate an extended larval presence

is that their apparent host, *P. albimana*, also has an extended flight period from late March to October with a two-peaked distribution (April and September) (Wilberg-Larsen *et al.* 2021).

The only two deutonymphs (Mindelsee, Germany) were found in early June and at the end of September. Adult males were only found in May and June, whereas females were observed over nearly all seasons (March, May, June, and September). More data are needed for interpreting the phenology of the species.

Purpose of the modified palps

Some speculations have been made on the purpose of the peculiar shape of P-5, with its nail-like claws. Sokolow (1934) suggested they could be used for digging in the mud. Gerecke and Tuzovskij (2001) tended to believe they enable the mite to feed on strongly sclerotised prey such as microcrustaceans. They also mentioned that the adapted palps could be involved in the mite's sexual biology, indicated by sexual dimorphism of the terminal palp segment (Figure 2). Gerecke (2006) reckoned that it is likely the deutonymphs and adults feed on the eggs of their host, using the adapted palps for cracking the eggshells. This presumed function is in line with water mite species of other families (Hydryphantidae, Hydrodromidae, Hydrachnidae) known to feed on insect eggs, which exhibit modified, chelate palps. However, behavioural studies are required to elucidate the true purpose of the modified palps.

Host-parasite association

Gerecke and Tuzovskij (2001) stated that their observation of *R. limicola* larvae on ptychopterid larvae is the first published record of a water mite species parasitising that dipteran family. Since then, another observation of an adult female of *Ptychoptera minuta* Tonnoir, 1919 with water mite larvae belonging to the genus *Hydryphantes* (unpublished data Bureau Biota, Figure 9) shows that representatives of the family are used as a host by mite species of other families as well.

From an evolutionary point of view, it is fascinating that the Natural History Museum in Stuttgart recently exhibited a ptychopterid male in a piece of Cretaceous Burmese amber (± 100 -110 million years old), parasitised by several mite larvae superficially resembling the larvae tentatively attributed to *Rutripalpus* and attached predominantly to the insect's abdomen (Figure 10). The piece was on loan from a private collector, who kindly gave permission to study it in detail. However, the discriminating features required for a definite attribution of the mite larvae to *Rutripalpus* were not distinguishable with microscopic observation methods. A further attempt to better visualise the decisive features using radiography (X-ray scans) did not prove successful.

The attribution of the larvae sampled in Montenegro to *R. limicola* was initially based on co-occurrence with adults, supported by morphological evidence that the observed larval character states deviated substantially from those of all other water mite taxa found in the area (Gerecke and Tuzovskij 2001). Furthermore, the occurrence of larvae of *Ptychoptera albimana* at one of our Dutch *R. limicola* sites, although without mite larvae attached, strengthens the assumption of a potential Rutripalpidae – Ptychopteridae association. However, the assumption that larvae of *R. limicola* use ptychopterid midges as hosts is not yet fully proven and further confirming data are required. In this context, it is interesting that the ptychopterid pupa and adult with mite larvae from the Bavarian *R. limicola* site also belong to *P. albimana* (personal communication P. Zwick). The species typically breeds in muddy puddles and mud at seepages with a preference for ochre (Wilberg-Larsen *et al.* 2021). It is possible that other species of ptychopterids are also utilised as hosts, e.g., larvae and adults of *Ptychoptera scutellaris* at the Dutch site on the stream bank of Looner Diep. It is a species typically found in spring and seepage habitats with some flow. Unfortunately, species attribution of the Montenegrin ptychopterid larva could not be retrieved.

Adult ptychopterids have a relatively short life, probably less than a week. Furthermore, they are rather sedentary, generally staying near their larval habitat (Wilberg-Larsen *et al.* 2021).



Figure 9 Preserved female *Ptychoptera minuta* with mite larvae of a species of the genus *Hydryphantes*. When alive, the mite larvae were red. Twijzelmieden, the Netherlands, 29 Jul. 2017. Photo Rink Wiggers.



Figure 10 Male ptychopterid from Cretaceous Burmese amber (ca. 100-110 Mya) with mite larvae morphologically resembling recent larvae currently attributed to *Rutripalpus*. Private collection P. Müller. Photo Christophe Brochard.

This implies that dispersal of mite larvae by ptychopterids will be very limited. Furthermore, springs can be considered insular environments (Cantonati *et al.* 2006) and existing populations of *R. limicola* are likely to be very isolated.

Attempts to sequence both adult and larval gene fragments were executed to further strengthen the attribution of the larvae found on ptychopterids to *R. limicola*. From the Dutch specimens, sequences of the CO1 and the 28S rDNA gene were generated, whereas only the sequence of the CO1 gene could be attained from the Benninger Ried larvae. However, the CO1 sequences from the Dutch adults and the German larvae differ by 15.4% in base composition. Since in water mites, the threshold in sequence dissimilarity to associate two samples to the same species is approximately 5-6% (Blattner *et al.* 2019), it can be concluded that the larvae likely belong to different species than the Dutch adults.

All in all, the evidence of this proposed host-parasite interaction is strong. But for irrefutable proof, either laboratory rearing or new attempts of linking the larvae to adult *R. limicola* would be necessary. If our assumption holds true, the discovery of this host-parasite relationship will be essential to a better understanding of the mite's biology. Given the fact that several water mite species belonging to Hydryphantoidea have a wider range of hosts, it cannot be ruled out that also other hosts rather than ptychopterids only are used by larvae of *R. limicola*. To study this, placement of emergence traps at some of the record sites might be a suitable method for collecting infected hosts.

Species complex?

From the CO1 sequence comparison, it cannot be inferred whether the Dutch adults and German larvae belong to different families or two separate species within Rutripalpidae. The lack of phylogenetic resolution in CO1 sequences (e.g., Blattner *et al.* 2019) makes it impossible to ascertain whether the larvae we investigated truly belong to *Rutripalpus*. To solve this, attempts to attain a 28S rDNA sequence from freshly preserved larvae should be undertaken.

The fact that the establishment of the CO1 sequence of the larvae was successful using the genus-specific primer, however, gives more weight to the possibility that adults and larvae belong to different species within the genus *Rutripalpus*. Since they found some morphological differences between German adult males and the male from Montenegro, Gerecke and Tuzovskij (2001) already reflected on the possibility of more than one *Rutripalpus* species in Europe. Considering the widely spread geographical distribution of *R. limicola*, populations could be genetically distinct, and further differentiation of Rutripalpidae is likely. This assumption appears appropriate, especially in strictly spring-bound species with narrow ecological niches (see Blattner *et al.* 2022). However, more research on morphological variance and the genetic differentiation of *R. limicola* populations is needed to provide justifiable statements on species delimitation within the genus *Rutripalpus*.

Systematic placement

In contradiction to the assumed relatedness of Rutripalpidae with Teutoniidae and Anisitsiellidae by Gerecke and Tuzovskij (2001), our molecular results show evidence for Hydryphantoidea association of *R. limicola*, supporting its morphological distinctiveness. However, despite the well-supported Lebertioidea and Hydryphantoidea main branches and the branch separating Hydryphantoidea/Lebertioidea from Arrenuroidea/Hygrobatoidea, the branch/node separating Hydryphantoidea – Lebertioidea shows only little bootstrap support (< 80%) and, therefore, is questionable. The basal position of *R. limicola* within Hydryphantoidea and the basal position of the Anisitsiellidae and Teutoniidae clade within Lebertioidea indicates phylogenetic proximity of these families but clear separation into the two main taxa, Lebertioidea and Hydryphantoidea. This pattern is also supported by the larval morphology of *R. limicola*, which shows the presumed plesiomorphic Lebertioidea character states and potentially indicates a “connecting link” between Hydryphantoidea and Lebertioidea. Overall, we assume that this unsupported branching event may be resolved by including more species and taxa, primarily

covering as much species diversity within Lebertioidea and Hydryphantoidea as possible, or through the discovery of a putative missing link between the two superfamilies.

Gerecke *et al.* (2018) proposed and discussed two potential spring adaptation scenarios in water mites. Rather basal water mite clades, i.e., Hydropholzioidea or Hydryphantoidea (see Dabert *et al.* 2016, Blattner *et al.* 2019 and Figure 8), are considered “paleocrenobionts” that originated from terrestrial trombidiform mite ancestors showing a preference for lentic spring types with extended areas of standing water such as helocrenes and rheohelocrenes. In contrast, the so-called “neocrenobionts” presumably derived from previously aquatic stream-dwelling species and prefer lotic spring environments such as strongly flowing rheocrenes. Due to our newly proposed systematic position of *Rutripalpus* at the basis of Hydryphantoidea (Figure 8) and its strong preference for weakly flowing helocrenic springs, *R. limicola* should be seen as a paleocrenobiont rather than a neocrenobiont.

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