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NEW EREYNETID MITES (ACARI: TYDEOIDEA) FROM KARSTIC AREAS: TRUE ASSOCIATION OR SAMPLING BIAS?

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A new genus and two new species of ereynetid mites, one edaphobitic, the other troglobitic, are described from three European karst areas. Free-living species of the Riccardoella*-complex exhibit ramified barbules in guard setae associated with tarsal solenidia, whereas parasitic species lack these characters. Ramified barbules in guard setae are thus considered specific adaptations to soil habitat. Free-living species of the* Riccardoella*-complex are seemingly restricted to karstic and other calcareous-rich areas while parasitic species live exclusively on slugs and snails. The relationship between calcium and* Riccardoella*-complex mites is discussed. A key is provided for the genera of Ereynetinae.*

The genus *Riccardoella* Berlese, 1923 includes five species, most of them being parasitic on gastropod mollusks (Fain & Van Goethem 1986; Fain & Klompen 1990; Fain 2004). The only species of the most evolved subgenus, *Riccardoella*, lives exclusively on Helicidae, the most evolved family among the land pulmonate gastropods. Species belonging to the subgenus *Proriccardoella* are more primitive and colonize more primitive gastropods such as species of the Milacidae and Limacidae (Fain & Van Goethem 1986). Recently, Fain & Barker (2004) described, from New Zealand, a new genus closely related to the genus *Riccardoella* and including a single species, also parasitic on gastropods.

The only free-living species of the genus *Riccardoella*, *R. canadensis*, was collected in Canada from a mull type soil with a mixed hardwood stand in which sugar maple (*Acer saccharum* Marsh) predominated (Marshall & McE. Kevan 1964: 60). The species was recently recorded from litter sample in Ukraine (Zabludovskaya 1995: 90-92). In addition, a specimen misidentified as *R. oudemansi*, was cited from the Amateurs' Cave in the Moravian karst (Zacharda 1978). Recently, an undetermined *Riccardoella* species was listed from grassy habitats in the Slovak karst (Kalúz 1998).

During a biospeleological survey of invertebrates from Belgian caves (Ducarme *et al*. 2003), numerous mites of a new genus close to *Riccardoella* were collected. They are described hereafter, together with specimens collected from the two other European karsts. Adaptive characters of free-living species and their distribution will be discussed.

MATERIALS AND METHODS

The material studied includes the holotype and paratypes of *R. canadensis*, plus specimens of two new species collected from three European karst areas.

For the mite description, we used photographs taken with a Leica TC200 digital camera mounted on a Leica DM LB microscope equipped with phase contrast. From the 402 source pictures (2.3 Mb), 124 photographs (624 Kb) were combined with the AUTO-MONTAGE program (version 3.03.0103 by Synoptics Ltd) as explained in André & Ducarme (2003). Terminology and chaetotaxal naming convention follow André (1980). Measurements in micrometers are given as mean ± standard deviation.

DESCRIPTIONS AND KEY

Hanriccardoella n. gen.

Like *Riccardoella* and *Austreynetes*, the new genus has 3-segmented palps. However, it is different from *Riccardoella* and *Austreynetes* in lacking lyrifissure *im* and having only two setae on the palptarsus. Leg IV has more setae than all *Riccardoella* species, with two setae on the basifemur and two on the coxa. The new genus is also different from *Austreynetes* characterized by a reduced tibial chaetotaxy, nude trochanters and the absence of opisthosomal trichobothridia.

Prodorsum (Fig. 1A-C): dehiscence line procurved (Fig. 3D) although setae (*vi*) are located slightly in front of (*ve*) (Fig. 1B-C), no lens and no eyespot. Opisthosoma: Poroidotaxy: 3 (*im* missing). Setae (*l*4) trichobothridial as in the genus *Riccardoella* (Fig. 1F). Genital chaetotaxy (Fig. 1G): Ad(0,3-6-4) TN(2 or 3-4), DN(1-2), PN and Lv(0-0); aedeagus-like structure in male (Fig. 1H); epimeral formulae: Ad & TN(3-1-3-3), DN(3-1-3-2), PN(3-1-2-0), Lv(3-1-2). Legs: chaetotaxy: I(12-6-4-6-1) II(9-3-4-4-1) III(8-3-3-3-0) IV(8-3-3-(2-2)-0) in the adult, tritonymph: idem minus the ventral seta on tarsi II, II and IV, DN: I(10-5-4-5-1) II(8-2-4-4-1) III(7-2-3-3-0) IV(7-2-3-3-0),PN: (10-5-4-5-0) II(6-2-4-4-0) III(5-2-3-3-0) IV(5-0-0-0-0), larva as the protonymph first three legs; solenidiotaxy: 3 (ereynetal organ included, Fig. 2D); femur IV undivided in the larva, proto- and deutonymph but divided in the next stases. Eupathidia on tarsus I: (*ft*), (*tc*), (*it*) and (*p*). Palp: (2-0-0) (Fig. 2K-L).

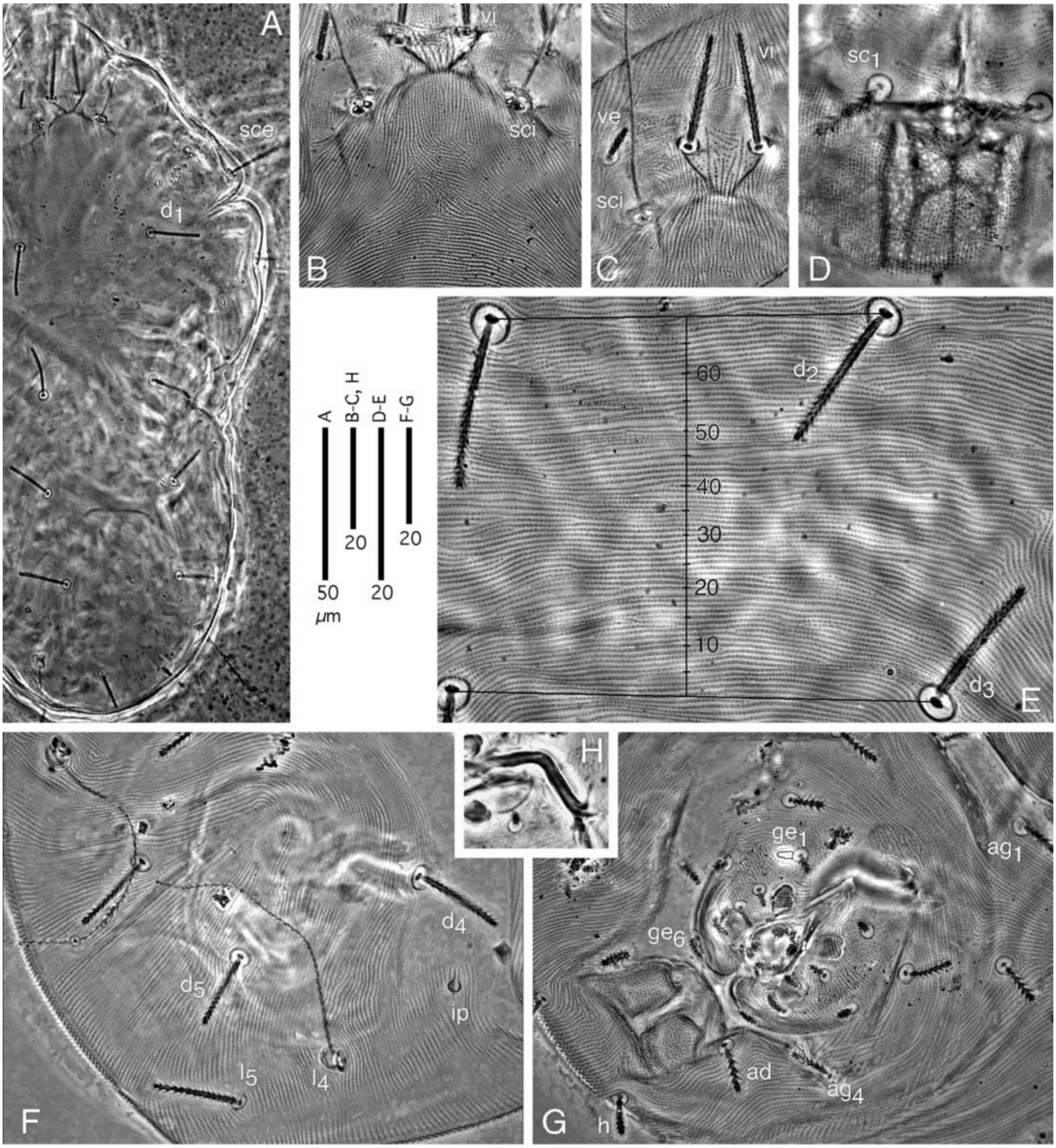

Figure 1. Idiosoma of *Hanriccardoella faini* n.gen., n.sp. adult. (A) Dorsal view. (B-C) Prodorsum. (D) Ventral ornamentation of gnathosoma. (E) Dorsal striation between (*d*2) and (*d*3). (F) Dorsal view of posterior opisthosoma. (G) Anal and genital areas. (H) Aedeagus-like structure. (A: holotype, B-H: paratypes)

Etymology: The name refers to that of the cave (Han) where the types were found. The cave is also the type locality of another Ereynetidae, *Neospeleognathopsis* (*Speleomyotis*) *bastini* (Fain 1958), parasitic in the nasal cavities of bats.

Type-species: *Hanriccardoella faini* n. sp.

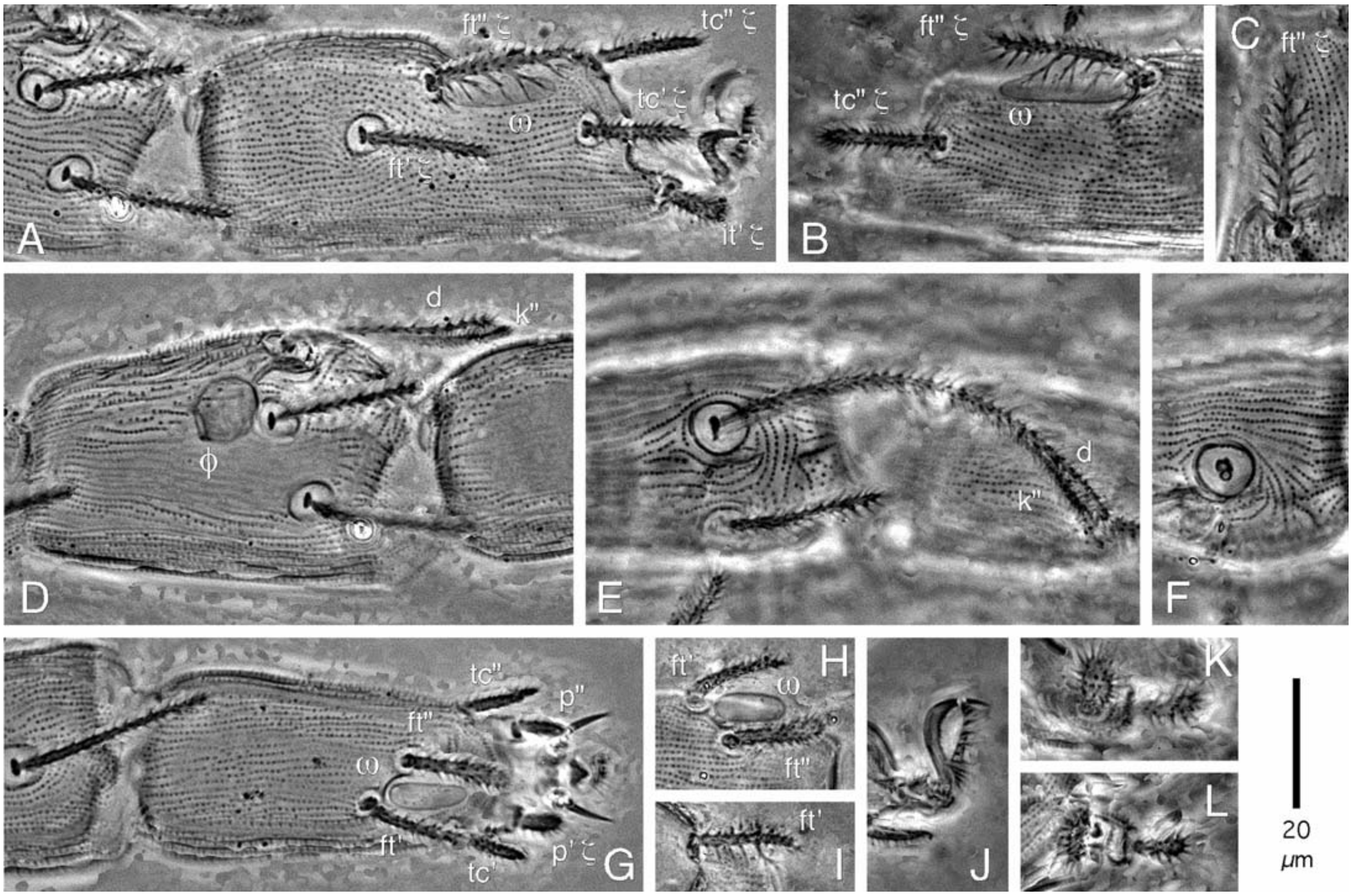

Figure 2. Legs of *Hanriccardoella faini* n.gen., n.sp. adult. (A-C) Paraxial (A), antiaxial (B) and dorsal views of tarsus I. (D-E) Tibia I with ereynetal organ (D) and duplex setae (E). (F) Same as (E) with broken setae. (G-I) Dorsal (G, I) and anti-axial (H) viewsTarsus II. (H) Apotele I. (I-J) Lateral (I) and end (J) views of palptarsus. (A-J: paratypes).

Hanriccardoella faini n. sp.

Presence of a x-shaped pattern between setae (*vi*) and (*sci*) (Fig. 1A-C); such a prodorsal ornamentation similar to the prodorsal shield observed in some *Ereynetes* has never been observed in the genera *Riccardoella* and *Austreynetes* Prodorsal striation longitudinal except in front of setae (*vi*), where striae form a rounded pattern (Fig. 1C). Gnathosoma with a 5-line pattern on the ventral face. Transverse striation on the opisthosoma (Fig. 1E), number of striae between (*d*2) and (*d*3): 69-70.

Guard setae with ramified barbules which overlie and seemingly protect solenidia ωI and ωII (Fig. 2A-C, 2G-I). Such ramifications have never been described in any ereynetid species. In *Riccardoella,* seta *ft'* moves distally beyond solenidion ωI (Fig. 4E, F) while it keeps its original location, behind solenidion ωI, in the new species (Fig. 2A).Tarsal solenidia recumbent and bent (Fig. 2H). Intertwined cluster on tibia I with *k*" as long as its guard seta, *d* (Fig. 2E). Famulus *k*" translucent and difficult to see; its base is however very distinct when the setae are broken (Fig. 2F). Empodium with 10-11 raylets (Fig. 2J).

Size: length of idiosoma: 358±19 in adults (*n*=3).

Etymology: The authors are pleased to name the new species after Prof. A. Fain who discovered and described so many ereynetid mites (111 out of 180 valid species).

Material examined: 3 females (2 with a single egg), 2 males, 5 trito-, 1 deuto- and 1 protonymphs, 1 larva. All mites were collected from the Han Cave (Rochefort, Belgium), at two sampling sites (Mamelons gallery and crossing of Mamelons gallery with “Fer de Lance”) on three dates (16 September 1999, May and November 2000).

Deposition: The holotype (female with no egg) and 4 paratypes (1 gravid female, 1 male, 1 trito- and 1 protonymph) are deposited at the “Institut royal des Sciences naturelles”, Brussels. Remaining specimens in André’s collection.

Ontogeny of *Hanriccardoella*

The earlier the stase, the more rounded is the body (body length/maximum width, L/W=1.95 in adults, 1.39 in deutonymphs (Fig. 3A) and 1.26 in larvae (Fig. 3B).

The ontogenetic bipartition of femur IV is similar to that observed in the meyerellid genus *Triophtydeus* (André 1985) but is delayed to the tritonymph.

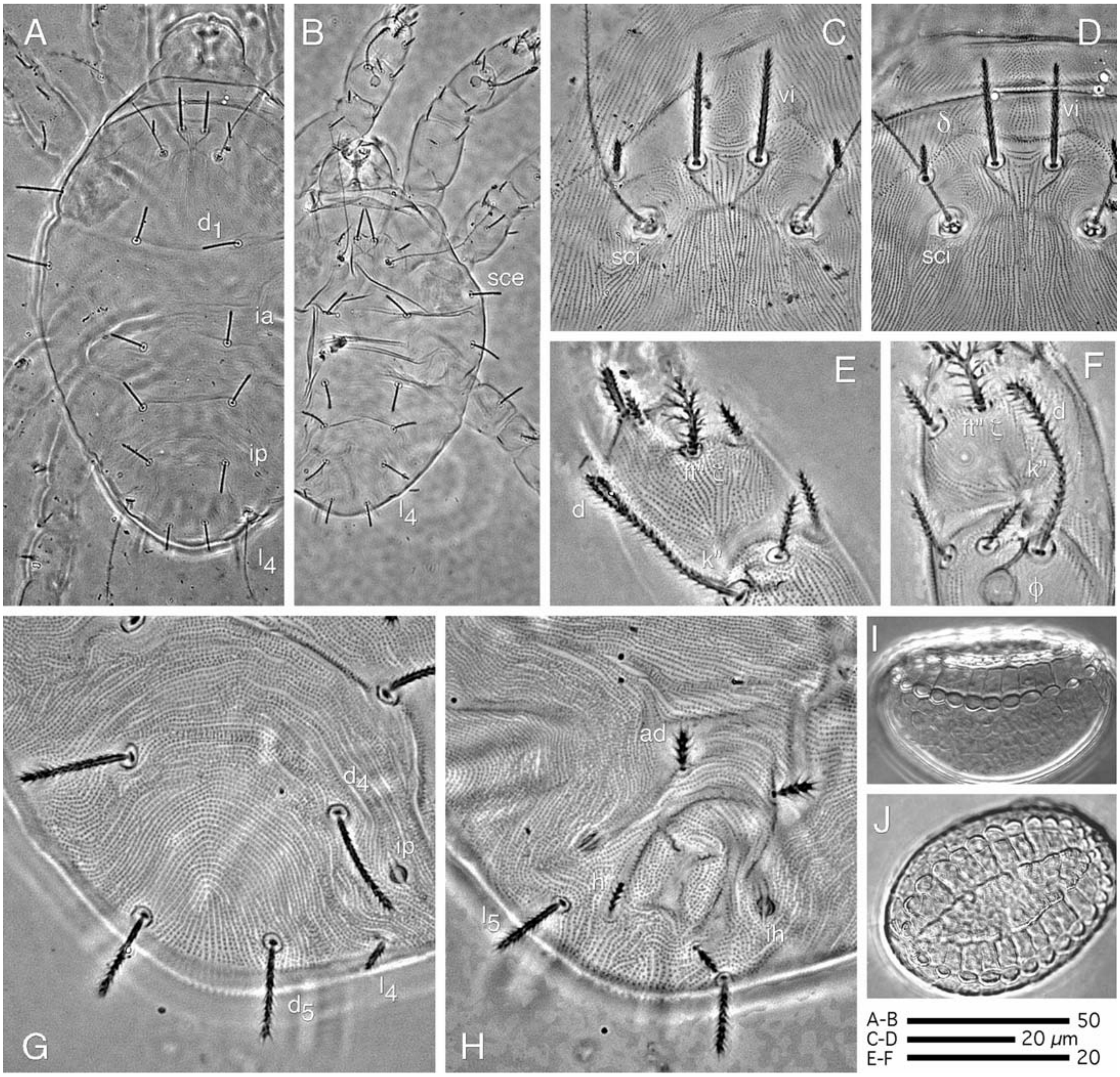

Figure 3. *Hanriccardoella faini* n.gen., n.sp. immatures. (A-B) Dorsal views of the tritonymph (A) and larva (B). (C-D) Prodorsum of the trito- (C) and deutonymph (D). (E-F) Tarsus and tibia I of the larva. (G) Dorsal view of posterior opisthosoma of the larva. (H) Anal and genital areas in the larva. (I-J) Ventral (I) and lateral (J) views of the egg. (A-J: paratypes).

The ontogeny of tarsal chaetotaxy is complex. On tarsi II to IV, tectals are present from the deutonymph while ventrals are observed only in adults. In larvae, tarsus I is regular in shape before the fastigial and tectal areas rises up to form the tarsal protuberances observed in nymphs and adult, the anabasis of tectals is simple and concerns the antiaxial seta. Translocation of *ft"* already present in the larva (Fig. 3E, F). Setae *ft'* and (*tc*) eupathidial in the protonymph and onwards. Iterals appear in the tritonymph and become eupathidial in the adult.

Trochanteral II is present in the deutonymph of *Hanricardoella* but not in that of *Riccardoella oudemansi*.

The genital chaetotaxy as detailed in the description of the genus. Posterior bothridia absent in the larva (Fig. 3H).

The egg presents ornamentation (Fig. 3I, J) contrary to that of other ereynetid species which are quite smooth.

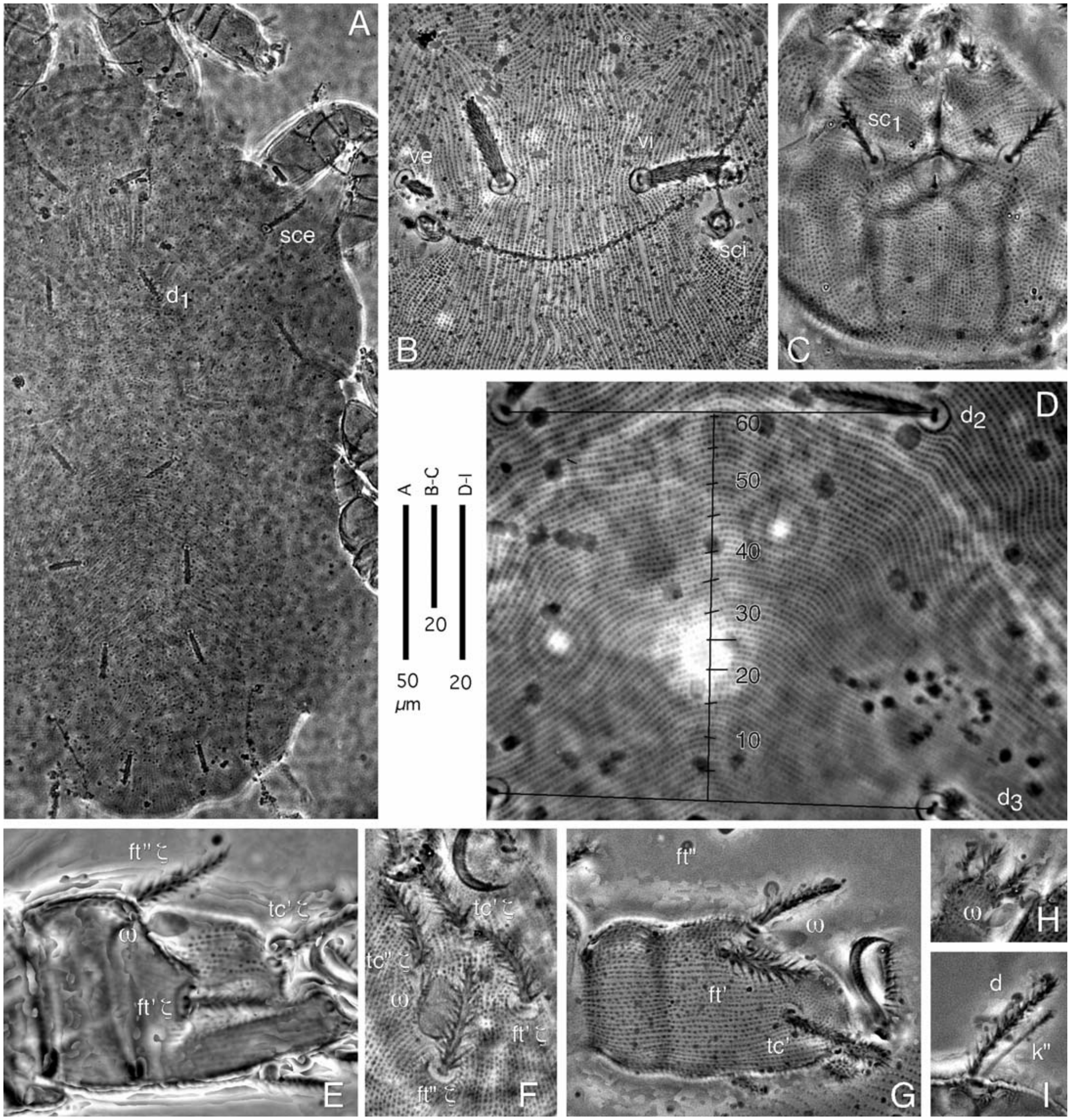

Figure 4. *Riccardoella zadeliensis* n. sp. adult. (A) Dorsal view. (B) Prodorsum. (C) Ventral ornamentation of gnathosoma. (D) Dorsal striation between (*d*2) and (*d*3). (E-F) Antiaxial (E) and dorsal (F) views of tarsus I. (G) Antiaxial view of tarsus II. (H) Palptarsus. (I) Duplex setae of tibia I. (A-I: paratypes).

Riccardoella zadielensis n. sp.

The new species displays the chaetotaxy of the subgenus *Proriccardoella* as defined by Fain and Van Goethem (1986). It is close to *R. triodopsis* as it also has four setae on the palptarsus (Fig. 4H) and a simple 2-line pattern on the ventral face of the gnathosoma (Fig. 4C). The tarsal solenidia of the new species are more or less ovoid (Fig. 4E-G) whereas they are elongate in *triodopsis*. The dorsal setae are rather wide (Fig. 4B, D) and similar in shape to those of *R. oudemansi*. The new species differs from the only other free-living *Riccardoella* by the shape of solenidion ωII, which is globulous in *R. canadensis*. Famulus *k*" is shorter than seta *d* (Fig. 4I). Striation pattern between setae (*d*2) and (*d*3) is inverse v-shaped and the number of striae is approximately 60 (Fig. 4D). Ereynetal organ is similar to that of *H. faini*. Empodium with 13-14 raylets (Fig. 4J).

The specimen from the Amateur's Cave identified as *R. oudemansi* by Zacharda (1978) appears to belong to the new species.

Size: length of idiosoma: 359±30 in adults (*n*=9).

Etymology: The name refers to the field collection site, the famous Zádiel Gorge.

Material examined: 11 females (2 with a single egg), 2 males, 2 larvae from the Zádiel Gorge, Zádiel (Slovakia), 2 June, 8 August, 14 October 1988; 1 male from Silická l'adnica Chasm, Silica (Slovakia), 16 September 1987; 1 male from Amateur's Cave (Czech Republic), 12 February 1974.

Deposition: The holotype (female) and 2 paratypes (1 female and 1 male) (all from the Zádiel Gorge) are deposited at the Slovak National Museum in Bratislava. Remaining specimens in André's collection.

DISCUSSION

Compared to *Riccardoella*, the new genus presents a mixture of plesiomorphic ("primitive") and apomorphic traits. Plesiomorphic features include the anterior position of setae (*vi*), proximal location of *ft* on tarsus I, presence of two setae on basifemur IV (vs one in *Riccardoella*) and of three setae on epimeron IV (vs two in *Riccardoella*). In contrast, apomorphic traits include the ornamentation of eggs, presence of an aedeagus-like structure (absent in *Riccardoella*), reduced chaetotaxy of the palptarsus, loss of lyrifissure *im*, and the development of ramified barbules on guard setae associated with tarsal solenidia. Actually, the last character is shared by two other species, *R. zadeliensis* and *canadensis*. Ramified barbules on guard setae associated to tarsal solenidia are thus adaptations associated with free-living species in contrast to parasitic species deprived of such associations and barbules (see recent SEM published by Ueckermann & Tiedt in 2003). The biological meaning of such an adaptation remains to be understood. It probably is related to life in the soil and soil-like material, more precisely to life in the porosphere, which is the substrate

Key to the genera of Ereynetinae (adults only)

1. Palp seemingly 5-segmented with a strong distal seta, opisthosoma with one pair of trichobothria, femur I with 7 setae .... *Ereynetes*
-. Palp with less than 5 segments, femur I with 6 setae or less............ 2.
2. 4-podomere palp, opisthosoma without trichobothria, tibia I with 5 setae............ *Pseudotydeus*
-. 3-podomere palp............ 3.
3. Opisthosoma without trichobothria, tibia I with 4 setae, all trochanters nude............ *Austreynetes*
-. Opisthosoma with trichobothria, tibia with 6 setae, trochanteral I present............ 4.
4. Lyrifissure *im* present, femur IV with 3 setae............ *Riccardoella*
-. Lyrifissure *im* absent, femur IV with 4 setae............ *Hanriccardoella*

Table 1. Distribution and habitats of free-living species of the *Riccardoella*-complex.

Species	**Habitat**	**Geology (1)**	**Location**
Hanriccardoella faini	Han Cave, sediment	Calestienne karst, DL	Belgium, Rochefort
Riccardoella canadensis	Mull, Farmington Loam	Limestone bedrock	Canada, Ste Anne de Bellevue
	Humus, Chernozem	$CaCO_3$ at depth	Ukraine, Askania-Nova
zadeliensis	Grassy soil	Slovak karst, DL	Slovakia, Zádiel Gorge, Zádiel
	Soil	Slovak karst, WL	Slovakia, Silická l'adnica Chasm, Silica
	Amateur's Cave, clay	Moravian karst, DL	Czech Republic, Suchy zleb-valley

(1) DL: Devonian limestone; WL: Wetterstein limestone.

region of water films occupied by bacteria, protozoa, and nematodes and of channels between aggregates occupied by microarthropods and the aerial hyphae (Vannier 1987, Beare *et al*. 1995). This is also supported by the presence of the same structure in the closely-related genera, *Ereynetes* (Grandjean 1939, Fig. 1B; Fain & Camerik 1994, Fig. 18) and *Pseudotydeus* (André & Ducarme 2003, Fig. 10) which are also soil-dwelling species.

Although the two new species were collected from karstic areas, their habitats differ markedly. *Riccardoella zadieliensis* turns out to be an edaphobite, *i.e*. a soil inhabitant that also occurs in caves. All specimens but one were found in grassy soil. The remaining specimen was collected in Amateur's Cave. In contrast, all specimens of *H. faini* were collected from the Han Cave. Of paramount importance is the absence of *H. faini* from the survey carried out at the soil surface overlying the cave (146 samples scattered over three seasons; Ducarme *et al*. 2005). As the species was collected from the cave from five different samples on three dates and it was represented by all stases from the larva to the adult, one can reasonably conclude that it is troglobitic.

Records of free-living *Riccardoella* and *Hanriccardoella* are rare, particularly if their distribution is compared to that of free-living species of the sister-genus *Ereynetes* (Fig. 5). Still more surprising is that their distribution is restricted to karstic and other calcareous-rich areas (Table 1). *Riccardoella canadensis* was first collected from mull under sugar maple (Marshall & McE. Kevan 1964). Yet, sugar maple is commonly associated with alluvial or calcareous soils (Pearson 1962, Fralish 1976, Great Plains Flora Association 1986) and occurs on soils with high exchangeable calcium (Bigelow & Canham 2002). Marshall (1963) added that these samples came from loam of the Ste-Rosalie Series. However, Christina Idziak, curator at the Morgan Arboretum, compared the sampling map in Marshall's (1963) thesis to the soil map of the arboretum and concluded that the mites came from Farmington Loam, a calcareous loam formed by the *in situ* weathering of limestone bedrock (C. Idziak, personal communication 2004). *Riccardoella canadensis* specimens recorded by Zabludovskaya (1995) were collected from humus under elder (*Sambucus*) at the Askania-Nova botanical garden. Zabludovskaya (personal communication 2004) reported to us that the soil consisted of chernozem. Chernozems, also called 'Calcareous Black Soils', are characterized by the presence of calcium carbonate at depth in the soil profile.

The hypothesized association between calcium and the *Riccardoella*-complex is all the more compelling if it is remembered that parasitic species of the complex are only known to occur on slugs and snails, obviously good reservoirs of calcium. However, the biological meaning of this relationship remains unclear. The richness of calcareous grasslands in both plant and animal species is well documented (Dolek & Geyer 2002, Kahmen *et al*. 2002, Wallis De ries *et al*. 2002) and the distribution of invertebrate species they shelter is usually explained through two factors. First, calcareous grasslands are known to support some plant-specific insects, like the Silver-spotted Skipper, *Hesperia comma* (L.), because its larvae feed on Sheep's Fescue, *Festuca ovina* L. (Thomas *et al*. 1986, Hill *et al*. 1996). Dependence on a food-plant does not apply to *Riccardoella* as the parasitic species are hematophagous (Baker 1970) and free-living forms are thought to be predators (Krantz 1978). Calcareous grasslands are also known to support thermophilic species, especially in butterflies (Thomas *et al*. 1986) and spiders (Hänggi 1996). This explanation does not clarify the distribution of mites of the *Riccardoella* complex living either in caves or in deep soils whose temperatures are low. A third explanation for the hypothesized association is that calcareous grasslands shelter a high diversity of bacteria (Clark 1967) that might account for the distribution of free-living *Riccardoella* if they were bacteriophagous. No data on the feeding habits in *Riccardoella* support this third explanation. Lastly, Norton & Behan-Pelletier's (1991) observation that calcium carbonate and calcium oxalate are cuticular hardening agents in oribatid mites does not apply to soft-bodied mites such as Ereynetidae.

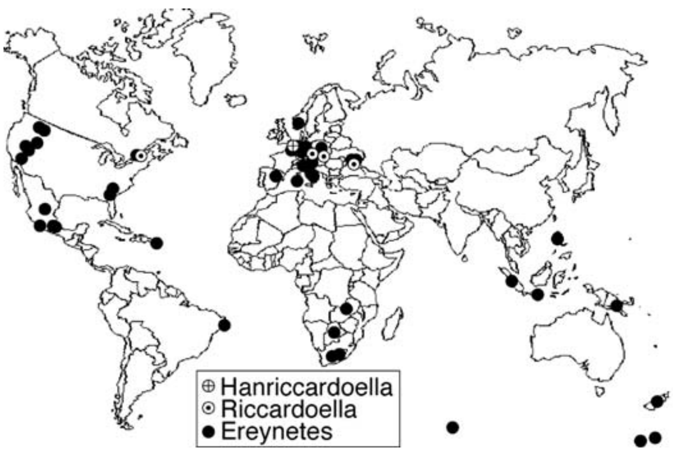

Figure 5. Distribution of free-living species of the genera *Hanriccardoella* and *Riccardoella* compared to free-living species of the sister-genus, *Ereynetes*.

Records of free-living *Riccardoella* are still rare, and we cannot exclude sampling bias as an alternative explanation for the apparent association with karst and other calcareous-rich areas. More research is thus needed to confirm that mites of the *Riccardoella*-complex are really associated with calcium salts and to elucidate the true nature of the association. As demonstrated by recent surveys (Welbourn 1999, Lewis *et al*. 2003, Ducarme et al. 2003), mites have been largely ignored in speleological studies, probably due to the lack of taxonomic expertise and adequate sampling methods. We hope this study will help raise interest of biospeleologists in mites, a highly speciose group including about 500,000 to 1,000,000 species.

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