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FROM PARATAXONOMY TO MOLECULAR DATA: THE CASE OF RHAGIDIIDAE (ACARI) FROM BELGIAN SOILS

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ABSTRACT — Two Rhagidiidae, *Brevipalpia minima* Zacharda, 1980 and *Hammenia macrostella* Zacharda, 1980 were retrieved from forests of Belgium. This gave us the opportunity to assess the role of parataxonomy and molecular data in identifying soil mites and to emphasize the importance of morphological characters.

KEYWORDS — morphospecies; morphology; taxonomy; picture; drawing; species delineation; Belgium

Systematics can be considered to have two major goals: (1) to discover and describe species and (2) to determine the phylogenetic relationships of these species... species delimitation, the process by which species boundaries are determined and new species are discovered, may finally be emerging as a major topic in modern systematics.

Wiens, 2007

INTRODUCTION

Soils are believed to be exceptionally diverse parts of ecosystems (Fitter, 2005; Coleman, 2008). Among the soil dwellers, the bacteria, fungi and protists are spectacularly speciose (Hawksworth, 2001 ; Finlay, 2004 ; Hong *et al.*, 2006) and play pivotal roles in ecology (Kirk *et al.*, 2004).

Soil mites are also diverse (Wallwork, 1976; Lebrun, 1979; Coleman, 2001) and form "hyperdiverse" assemblages (St. John *et al.*, 2006), difficult to study. Among soil mites, the cosmopolitan Rhagidiidae are morphologically rather uniform and their representatives frequently differ only in subtle structural characters. They live in different soil compartments: litter, mineral soil, caves, talus voids, etc.

Two Rhagidiidae, *Brevipalpia minima* Zacharda, 1980 and *Hammenia macrostella* Zacharda, 1980 were retrieved from forests of Belgium. The first species was collected in hemiorganic horizons in the Nature Reserve of Lauzelle (Hesbaye) by Ducarme *et al.*, (2004a) as well as in deep soils (15–20 cm) located in Rochefort (Calestienne, Belgian karst area) by Ducarme (2003). The second species was found only in Rochefort (Ducarme *et al.*, 2004b). Both species were described from litter in Bo-

hemia (Czech Republic). Each of them belong to a monospecific genus, erected by Zacharda (1980). They are common soil dwellers, which might be confused by parataxonomists and soil ecologists not prepared to the study of so small mite species.

MATERIAL AND METHODS

The terminology follows that of Lindquist and Zacharda (1987) and Baker (1990).

Because Rhagidiidae are minute soft body mites, phase contrast microscope is necessary, and identification and morphologic study are helped by microphotography. The photographs were taken with a Leica TC200 digital camera mounted on a Leica DM LB phase contrast microscope. Most photographs were combined with the AUTOMONTAGE program (version 5.00.0777 by Synoptics Ltd) as explained in André and Ducarme (2003). This program automatically combines the in-focus regions from a series of source images, each of them taken at a different point of focus, to generate a single montaged image, which is completely in focus.

This assemblage helped greatly, by the quality of documents provided, for both chaetotaxy and morphology.

The characterization of the distribution of eupathidias is essential for rhagidiids: the eupathidiotaxy relies in the original definition of Grandjean (1943), i.e. the hollowness of the shaft and widely open base. Eupathidia may also exhibit differences in ornamentation. Nevertheless, these characters have never been used for practical identification of rhagidiid species because of the fragility of these organs in the collected specimens and the difficulties of their reliable identification in standard light microscopy.

RESULTS

Comparison of *B. minima* and *H. macrostella*

The two species belong to the Rhagidiidae. They are both minute soft-bodied mites which are collected in similar habitats. *B. minima* is generally longer than *H. macrostella*, total idiosomal length 338-380

µm vs. 241-309 µm (Zacharda, 1980; Ducarme *et al.*, 2004b) (Fig. 1, 2). Contrary to many other species (Fig. 1), both mite species have prodorsal trichobothria clavate, but the density of barbles covering the trichobothria is different in the two species. The trichobothrias arise from a sclerite which extends from the naso to the anterior part of the opisthosoma (Fig. 3).

The microsculpture of this sclerite differs between the two species (Fig. 3). Besides, a nodular microstructure is observed on the integument between chelicerae and on the paraxial faces of palps and legs (Fig. 3, 4), the shape and density of nodules are characteristic of the species.

The other major distinctive characters between *B. minima* and *H. macrostella* rely mostly on the unique diversity of forms of the subcapitulum, the chelicerae, the palps, the tarsi I, the rhagidial organs and the leg chaetotaxy as described hereafter.

Brevipalpia minima (Figs 1A, 2A, 3A, 4, 5A-C, 7A-B)

The rhagidial organ on tarsus I is composed of two grooves, the paraxial groove receives two recumbent solenidia while the antiaxial shelters only one. Between the two grooves arises the stellate organ, *e*. On tibia I, the recumbent solenidion and its groove are flanked antiaxially by a distal seta, *k*", and a second solenidion which is as small as *k*" and erected more or less behind the first one (Fig. 7A, B).

On tarsus II, the rhagidial organ comprises three grooves, each with a recumbent solenidion. Tibia II has a distal *k*" and a recessed solenidion, both located antiaxially along seta *d* Fig. 7D, E.

Formulae are as follow.

Epimera: 3-1-4-3.

Legs:

I (17(3)-10(1)-11-7-1 with (*ft*), (*tc*), (*it*) and (*p*) as tarsal eupathidia,

II (14(3)-5(1)-7-9-1 with (*tc*), (*it*), (*p*) as tarsal eupathidia and a recessed solenidion on tibia,

III (12-5-6-(4-3)-1) with (*it*), (*p*) as tarsal eupathidia,

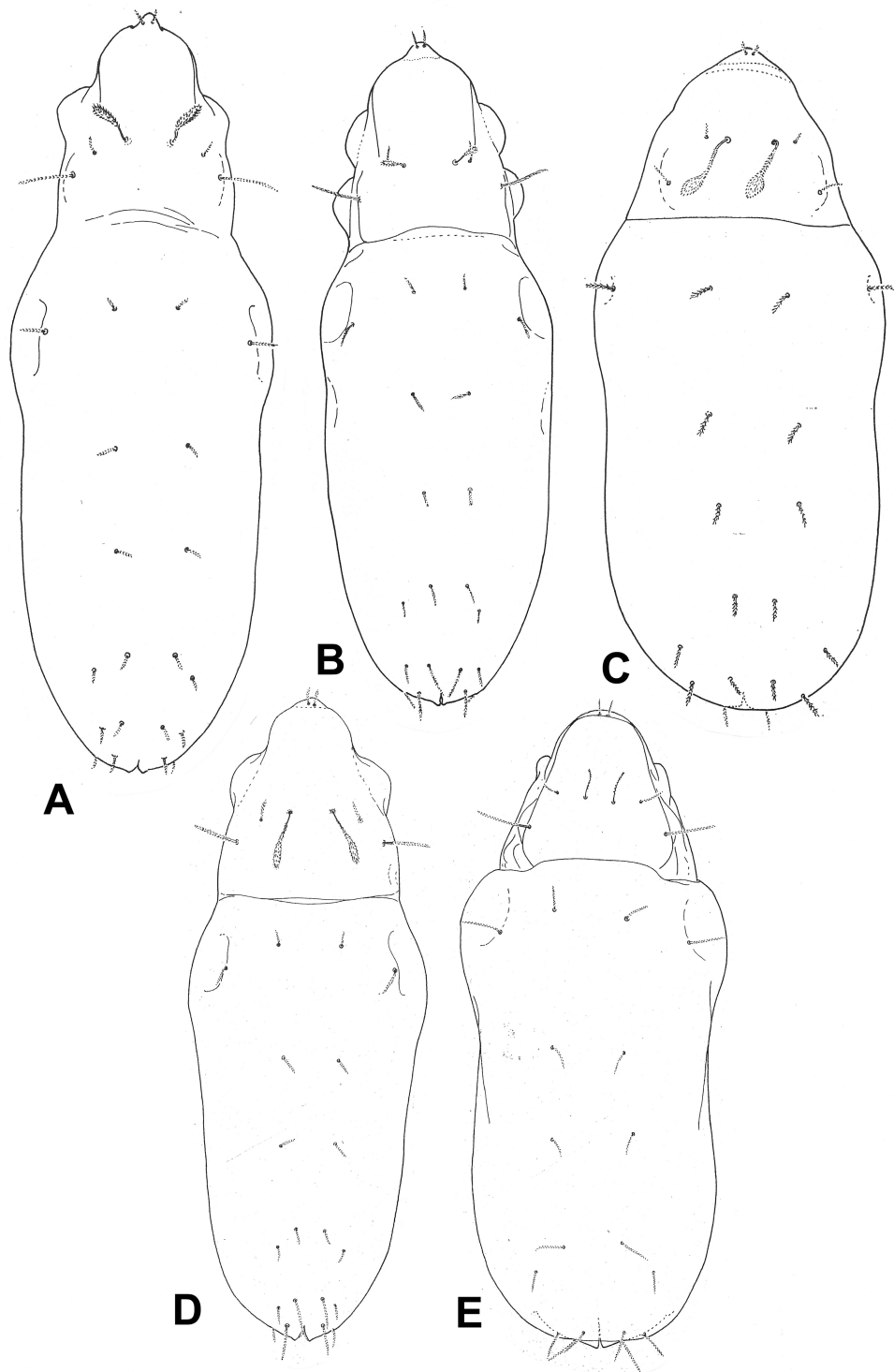


FIGURE 1: Dorsal aspect of different soil Rhagidiidae. A – *Brevipalpia minima* Zacharda, 1980; B – *Coccorhagidia clavifrons* (Canestrini, 1886); C – *Hammenia macrostella* Zacharda, 1980; D – *Parallelorhagidia evansi* (Strandtmann and Prasse, 1976); E – *Crassocheles virgo* Zacharda, 1980 (from Zacharda 1980).

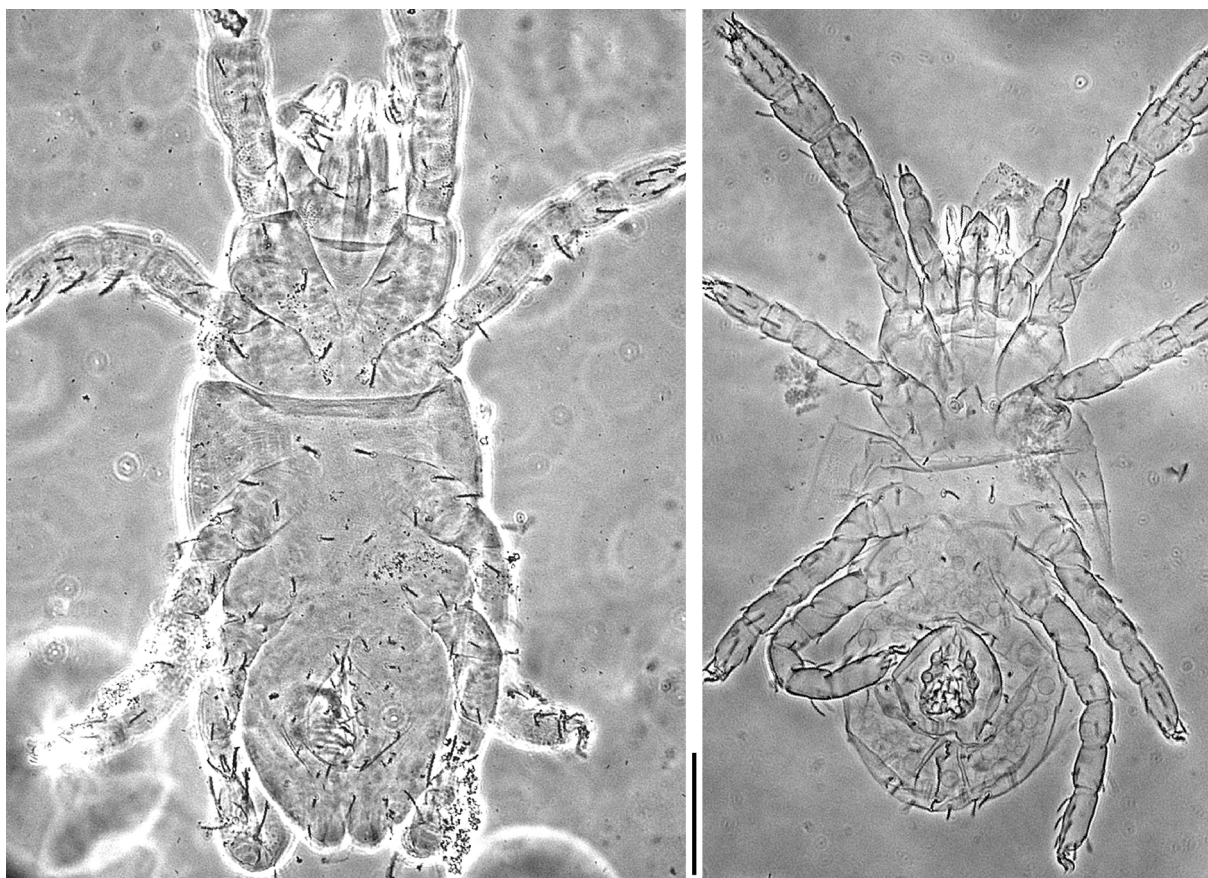


FIGURE 2: *Brevipalpia minima* and *Hammenia macrostella* in ventral view. Scale bar = 50 μ m.

IV (12-6-5-(3-3)-1) with *ft''*, (*tc*) and *p'* as tarsal eupathidia.

Palp: 9-1-2-0.

Hammenia macrostella
(Figs 1C, 2B, 3B, 5D-E, 6, 7C-D)

The rhagidial organ on tarsus I is composed of four parallel grooves, each receiving a recumbent solenidion. The stellate organ, *e*, is particularly developed, hence the name of the species. On tibia I, the recumbent solenidion and its groove are flanked paraxially by the dorsal seta which is eupathidial, *d* (Fig. 4C).

On tarsus II, the rhagidial organ comprises three grooves, each with a recumbent solenidion. Tibia II has a single groove with a recumbent solenidion (Fig. 4F).

Formulae:

Epimera: 3-1-4-3.

Legs:

I (21(2)-8(1)-7-(9-1)-1) with (*ft*), (*tc*), (*it*), (*u*), (*p*) as tarsal eupathidia,

II (15(2)-5(1)-5-8-1) with (*tc*), (*it*), (*p*) and proximal pairs of (*v*) as tarsal eupathidia

III (12-4-5-(4-2)-1 or 2) with *tc'*, (*it*), (*p*) as tarsal eupathidia,

IV (11-4-4-(3-2)-1) with (*tc*) and (*p*) as tarsal eupathidia.

Rhagidiidae

Although the genera were collected in different places (Canada, Japan by Nakamura *et al.*, 2006), both species belong to monospecific genera erected by Zacharda (1980) and were described from litter in Bohemia (Czech Republic). The two species are

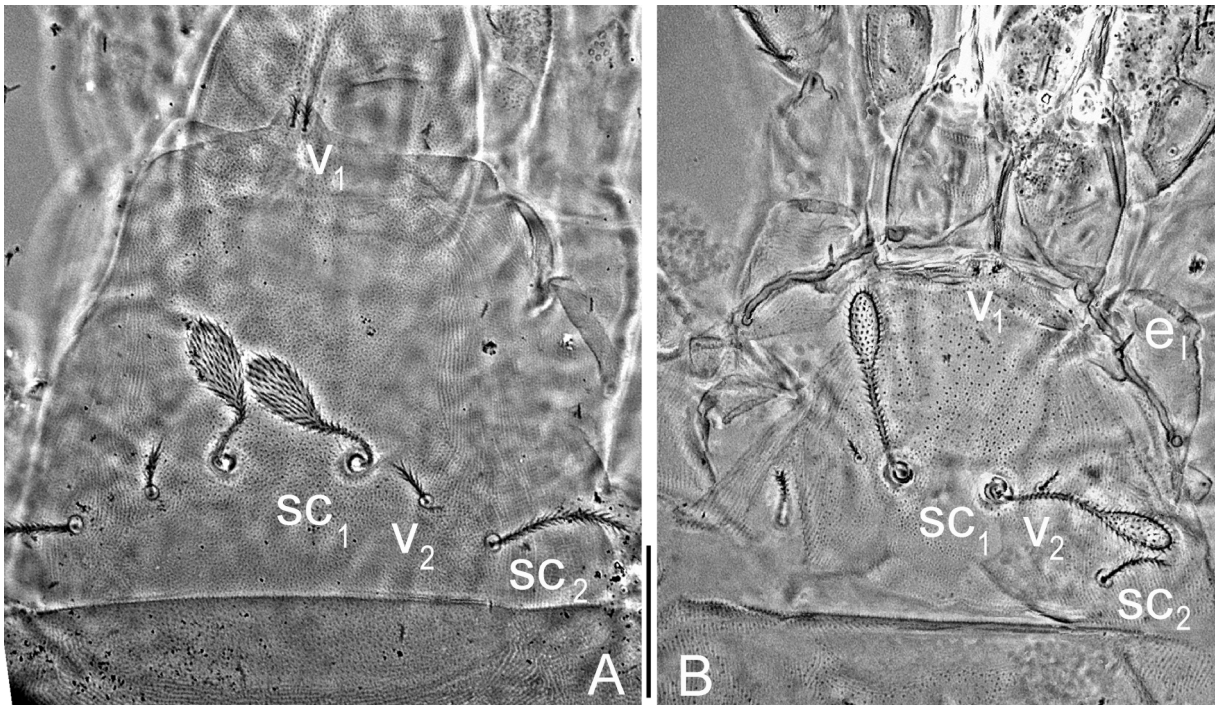


FIGURE 3: Prodorsum of *Brevipalpia minima* and *Hammenia macrostella*. Scale bar = 25 μ m.

identified not only by meristic characters such as presence/absence of setae and solenidia but also by morphometric characteristics and the unique diversity of forms of subcapitulum, chelicera, palp, tarsus I and dorsal setae. Other characters imply the integument and local ornamentations. The first definitions of the two mite species rely thus on morphological characters.

In addition to morphological data, the two Rhagidiidae are characterized by the habitat. Both mites are soil dwellers and present many edaphomorphisms, e.g. clavate trichobothria, underlined by Zacharda (1980). *H. macrostella* is even recognized as an indicator species of deep soil habitats after a statistical analysis by Ducarme *et al.* (2004b). Of particular interest is the absence of both species from cave samples, even from nearby caves (Ducarme *et al.*, 2004b; Vermandere and Lebrun, 2005, 7 caves representing 221 samples were prospected). In this case, ecological traits support morphological characters. Rhagidiidae remain however neglected like most Prostigmata which are studied in less than 11 % of the synecological papers

published in soil ecology (André *et al.*, 2002).

Line drawings and photographs

Line drawings of mites are interpretations of what acarologists see and observe. (Coineau, 1982; Coineau and Legendre, 1997).

Photographs show the integument of the two species, particularly the nodules and the microsculpture that can hardly be observed with a standard light microscope (i.e. with no phase contrast). They also reveal the presence of a prodorsal sclerite, a feature already recorded in Eupodidae (Baker and Lindquist, 2002) but only seldomly seen in Rhagidiidae, (see an example in *Coccorhagidia pittardi* Strandtmann, 1971; Zacharda, 1980: 709). The presence and number of sclerites is a key character to discriminate genera in other mites such as Stigmaeidae (Summers, 1966). Last, photographs show the details of the chaetotaxy, especially the structure and shape of eupathidia which clearly differ from normal setae.

Scientific pictures here presented are far from the strict positivist position of considering cameras to

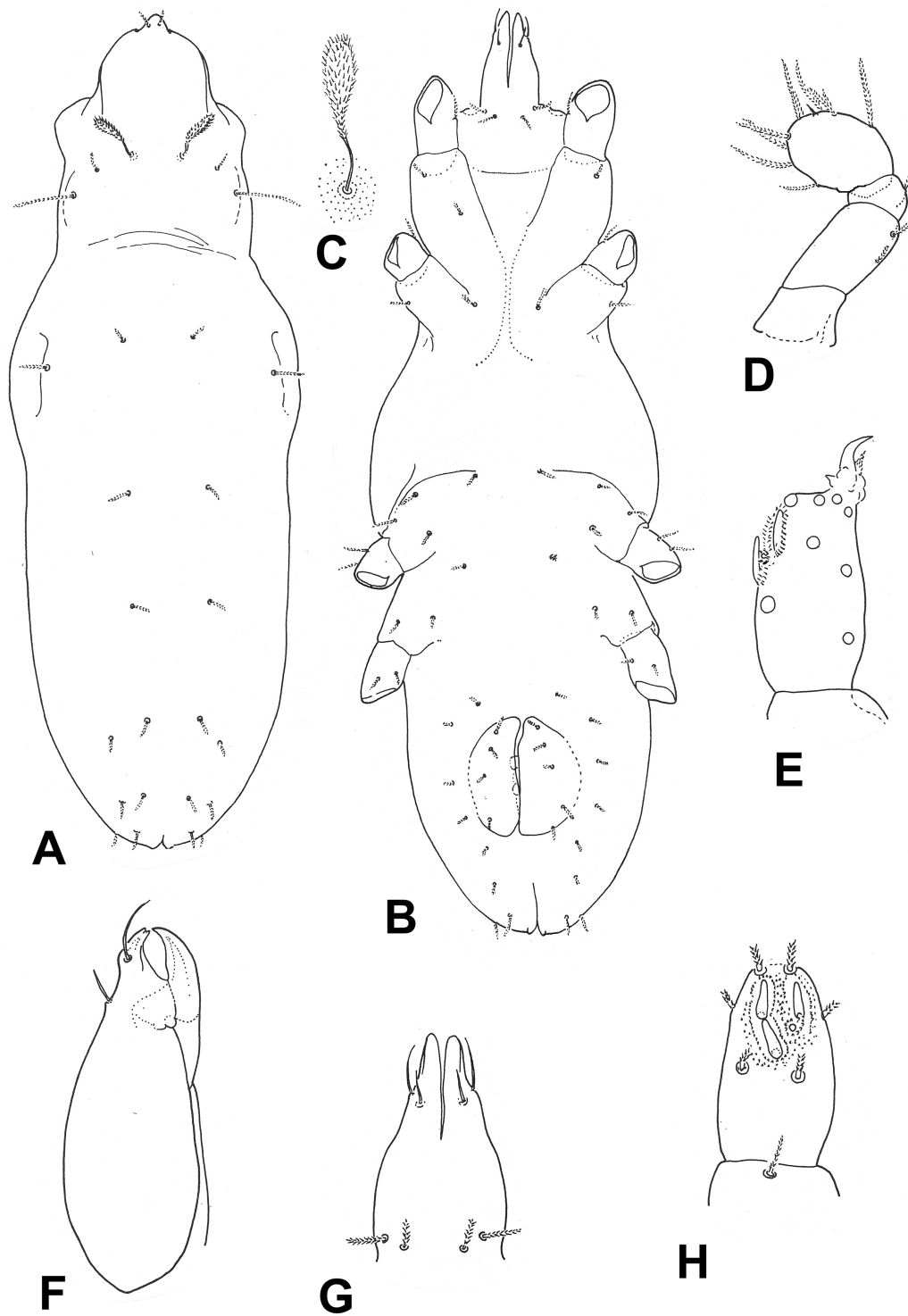


FIGURE 4: *Brevipalpia minima* Zacharda, 1980: A – dorsum, B – venter, C – trichobothrium, D – palp, E – tarsus I in lateral aspect, F – chelicera, G – subcapitulum, H – rhagidial organ I (from Zacharda 1980).

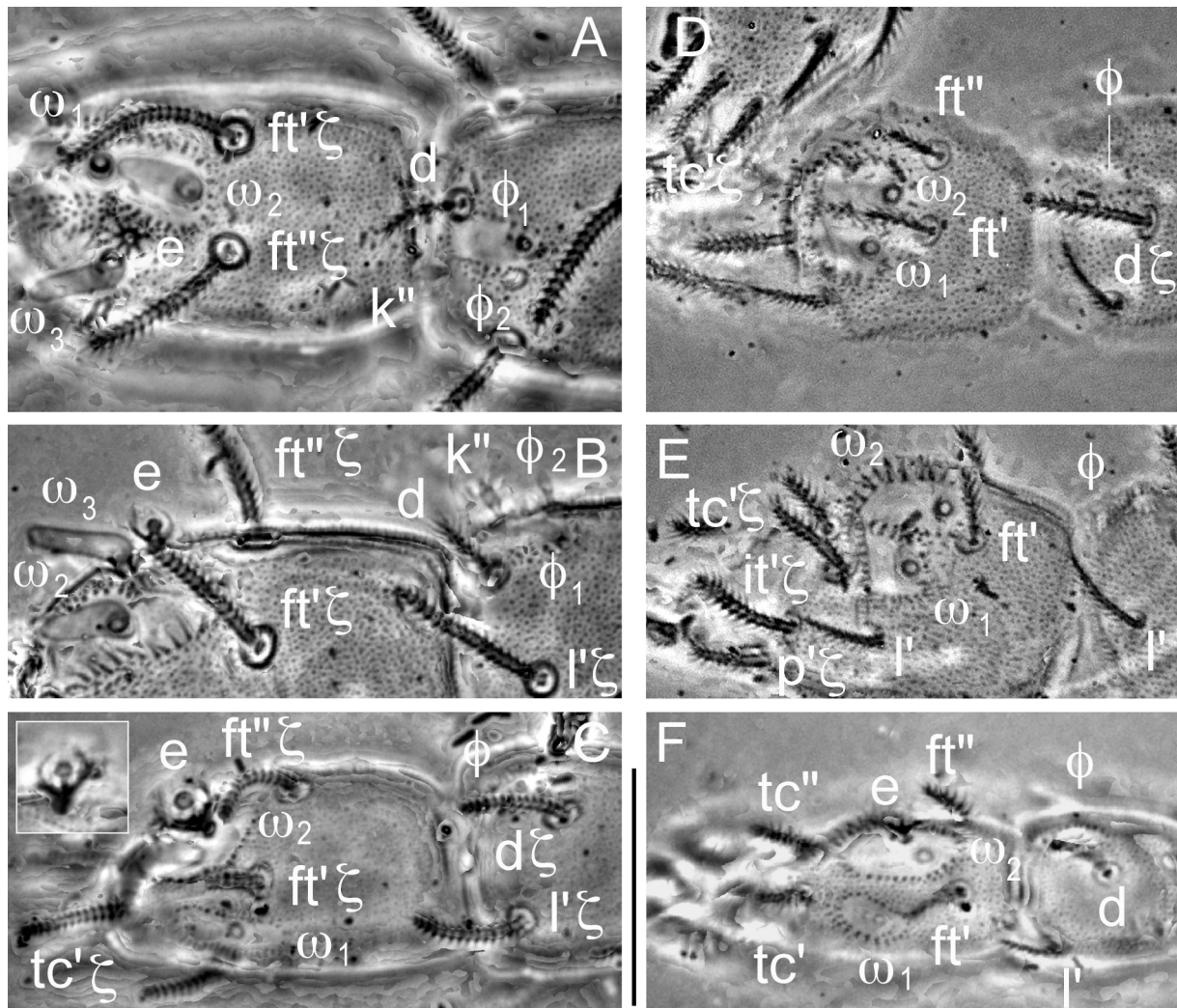


FIGURE 5: Rhagidial organs I in dorsal (A, D) and lateral (B, E) views and rhagidial organs II in latero-dorsal view (C, F) of *Brevipalpia minima* (A–C) and *Hammenia macrostella* (D–F). Insert of famulus in C. Scale bar = 100 μm .

be "pencils of nature" as did Talbot in his famous book published in 1844 (The Metropolitan Museum of Art, 2000). The images are manipulated. Manipulation *sensu* Gordon (2003) involves cropping, color balancing, contrast adjustment, burning and dodging. Not only, the images are manipulated, but they are combined to generate a single montaged image. Last, the software default options are not necessarily appropriate for a particular organ.

For example, this is the case of solenidion on tarsus I. In lateral view, it is difficult to see it as it tends to be hidden by fastigial setae which are more contrasted and thus selected at the time of the mon-

tage. A manual effect, i.e. an additional image editing, is sometimes necessary to select the appropriate source image(s) when a structure is transparent or little contrasted (cf. Fig. 5).

DISCUSSION

Soil mites are diverse as well as Rhagidiidae which live in different soil compartments: litter, mineral soil, caves, etc... But the 'morphospecies' is merely a first step in erecting the taxonomy of mites, and it is expected to become more meaningful in the light

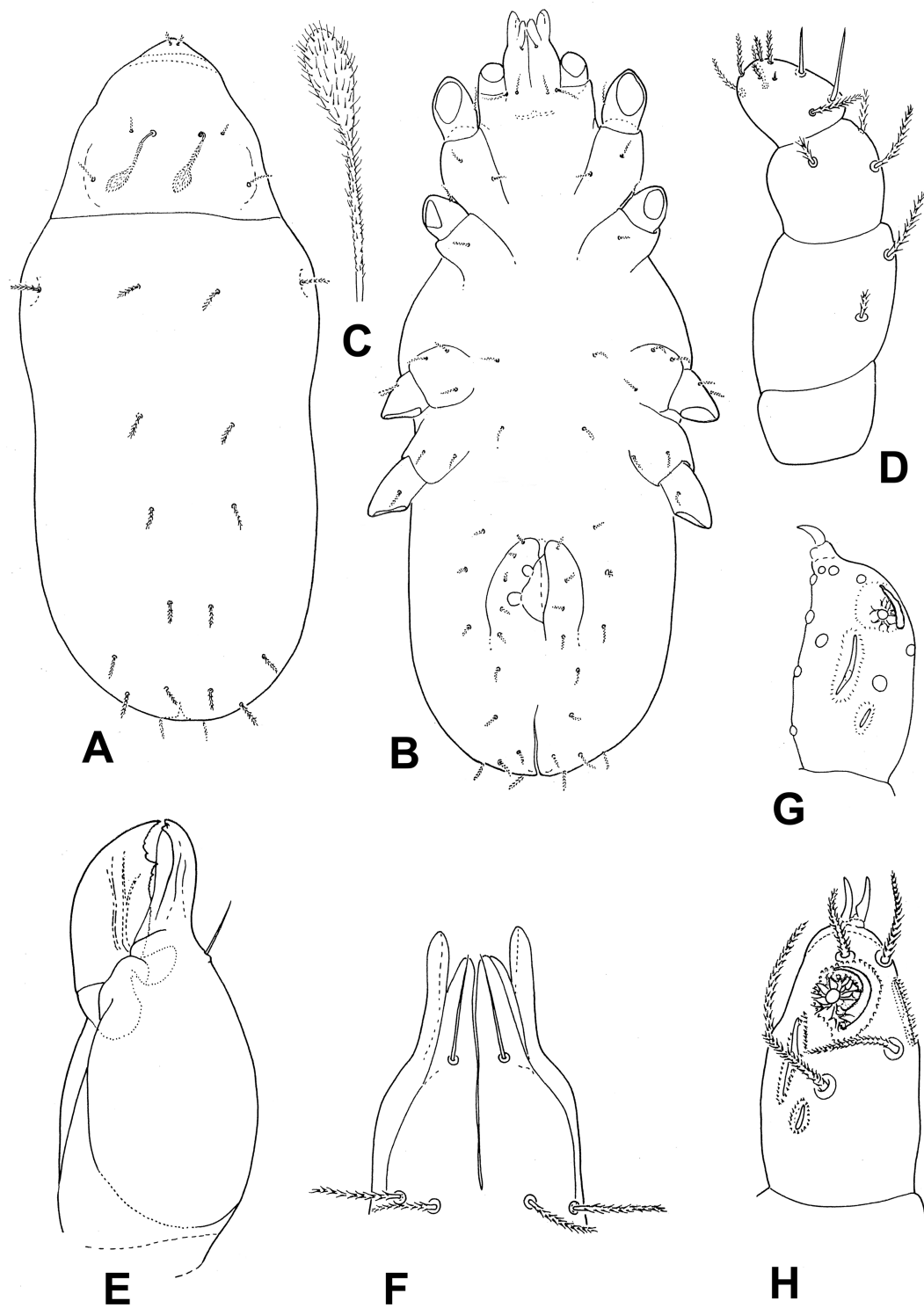


FIGURE 6: *Hammenia macrostella* Zacharda, 1980: A – dorsum, B – venter, C – trichobothrium, D – palp, E – chelicera, F – subcapitulum, G – tarsus I in lateral aspect, H – rhagidial organ I (from Zacharda 1980).

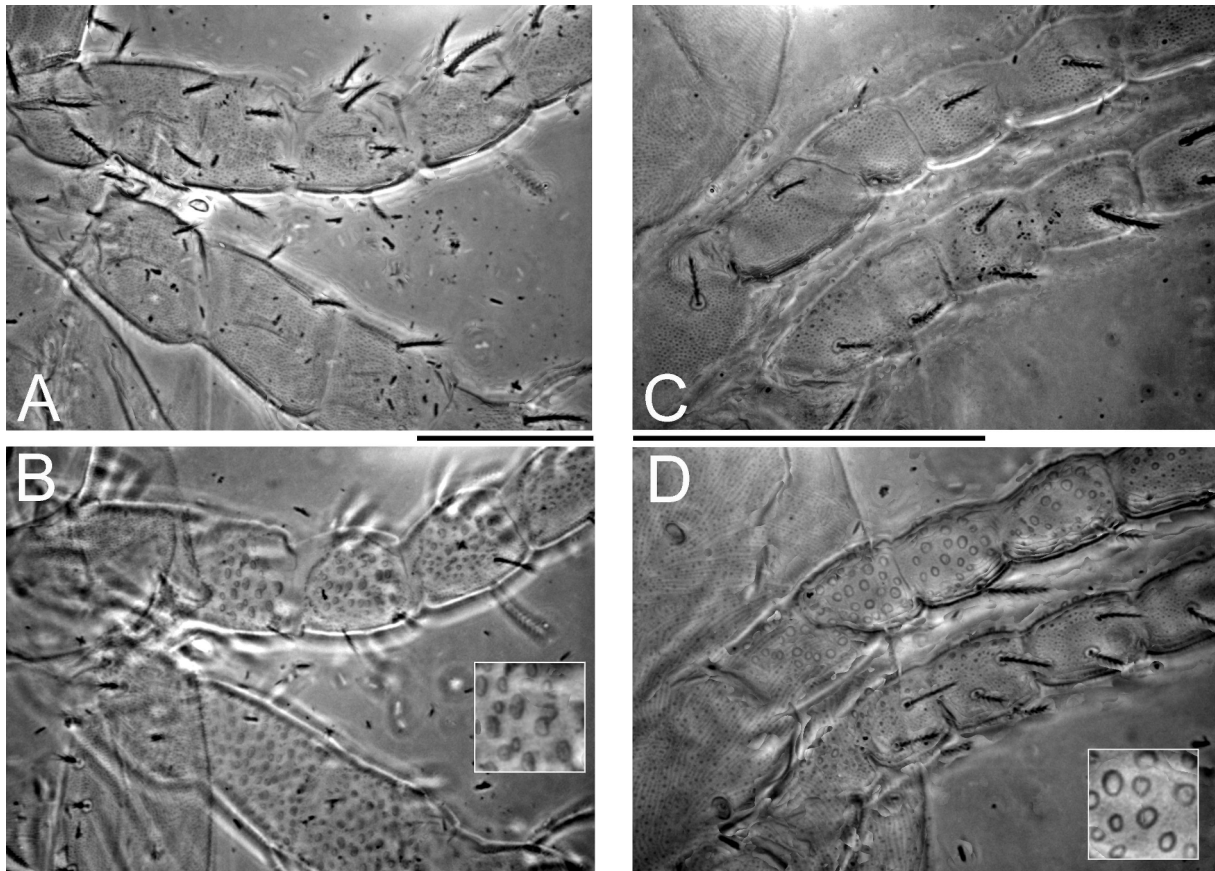


FIGURE 7: Legs III and IV in antiaxial (A, B) and paraxial (C, D) views of *Brevipalpia minima* (A-B) and *Hammenia macrostella* (C-D). Insert showing the microsculpture in B and D. Scale bar = 100 μm .

of genetic, physiological and ecological research in the near future.

As already claimed by Darwin (1859), "no one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species." Despite many years of discussion, the species problem has still not been adequately resolved (Reydon, 2004). However, the half-century of controversy tends to vanish when the species concept is separated from the issue of species delimitation (de Queiroz, 2007). This distinction is useful as it refrain scientist meetings from degenerating into endless disagreements (Wiens, 2007) and allows systematists to develop operational methods of delimiting species and other taxa. Species delimitation is essential because species are used as basic units of biology (Dobzhansky, 1951) and analysis in several areas of biogeography and

ecology, e. g. for global biodiversity assessments in conservation programs.

However, it is not by chance that a major omission in the symposium introduced by Wiens (2007) is the lack of papers on methods for delimiting species using morphological data. The literature on the methodology of species delimitation remains meager, especially if it is contrasted with publications on the theory and methods of phylogenetic analysis (Wiens and Penkrot, 2002).

Parataxonomy

Pictures might suggest that it is easy to discriminate soil mite species. Parataxonomy and the sorting of specimens to recognizable taxonomic units (RTU's) are common approaches to invertebrate biodiversity studies worldwide (Ward and Stanley, 2004)

and have been proposed recently to sort the springtail and mite specimens collected from the field (Alberta Biodiversity Monitoring Program, 2006). Digital photography greatly enhances the ability of parataxonomists to efficiently recognize morphospecies (Basset *et al.*, 2000). However, recognizing, naming, and identifying species is not an easy task, requiring experience or at least knowledge of all the taxon-specific pitfalls caused by variation and similarity (Krell, 2004). Mites and other organisms whose length is less than one millimeter do not escape from the difficulty and necessitate skilled eyes to go on studying the richness of the soil (André *et al.*, 2001). Species are sometimes similar and sibling species are not rare. Compare for instance *Tydeus bedfordiensis* and *T. stephani*, two prostigmatid mites meet on bark by André (1987). The problem is still greater when the ontogeny is considered. The *Oribatella* specimens collected in soil by Wauthy (e.g. Wauthy *et al.*, 1989) have nothing to do with the *Oribatella* observed on bark by André (1984), the adults -usually recorded by soil zoologists-seemed similar in every respect but the immatures were easily distinguished by different clavate sensilla. Last, the taxonomic level matters (Purvis and Agapow, 2002). Yet the taxonomic resolution used in soil studies does not improve in recent years (André *et al.*, 2002).

If parataxonomy does not fulfill the criteria of a scientific method as claimed by Krell (2004), it can be a heuristically valuable tool to find out strange specimens. The recent description of one eupodoid mite with idiosomal setae *h1* in the form of trichobothria offers a nice example; the litter mite was first observed by the parataxonomist staff of Project ALAS and then described by specialists (Baker and Lindquist, 2002).

Molecular data

Molecular data are used in Acarology for nearly 20 years (see the pioneer work by Navajas *et al.*, 1992). The two rhagidiid species presented here are characterized by morphological and ecological data. They represent merely a first step in erecting the taxonomy of Rhagidiidae, and are expected to become more meaningful in the light of genetic, physiological and ecological research in the near fu-

ture. Molecular techniques are routinely employed in soils (Gibb *et al.*, 2007) as well as in caves (Berry, 2005).

The molecuration of taxonomy (Lee, 2004), i.e. the analysis of DNA sequences to identify and delimit species in Rhagidiidae, maybe helpful (cf. Zacharda, 2000). However, disagreement between species boundaries inferred from different data types raises several important questions (Wiens and Penkrot, 2002; DeSalle *et al.*, 2005). The recent example of Hebert *et al.* (2004) which reveals ten "molecular" species in one cryptic species is reassessed by Brower (2006) who distinguishes at least three, but not more than seven mtDNA clades that may correspond to cryptic species and are supported by the evidence. Molecular data are also the subject of interpretations.

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