

RESEARCH ARTICLE

Characterization and mapping of sensilla on the head appendages of noterid larvae (Coleoptera: Noteridae), and development of a preliminary biometric method for taxa delimitation

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Abstract

Larvae of the burrowing water beetle family Noteridae are distributed worldwide and are often abundant in a broad range of aquatic habitats, playing an important role in structuring freshwater communities, yet they have remained among the most poorly studied groups of aquatic beetles. Studies on sensillar equipment of aquatic insect larvae are largely lacking, despite their potential use in phylogeny and biometric identification methods. In this article, the external morphology and distribution of sensilla on the head appendages of first instar larvae of selected genera of Noteridae were examined using scanning electron microscopy. Seven main types were distinguished based on their morphological structure: basiconica (3 subtypes), campaniformia (2 subtypes), chaetica (7 subtypes), coeloconica (6 subtypes), coniform complex (2 subtypes), placodea, and styloconica (3 subtypes). The apex of the labial palpus was found to be the most variable and informative region in regard to the number, relative position, and topology of sensilla. Fingerprint models were, therefore, generated for this region in each of the studied genera, allowing their identification.

KEYWORDS

antenna, burrowing water beetle, micromorphology, mouthparts, SEM

1 | INTRODUCTION

A significant part of the sensory system of insects consists of a large number of highly diverse organs called sensilla. According to their sensory modality, the sensilla of insects are classified into four main groups—olfactory, gustatory, mechanosensory, and those that have hygro- and thermoreceptors (Nowińska & Brożek, 2017). Sensilla can be named after the structure of their cuticular parts (Schneider, 1964; Zacharuk, 1985),

and we can distinguish trichoid sensilla with a long thin hair; chaetic sensilla with a long thick hair; basiconic sensilla with a usually shorter hair and a thin cuticle; styloconic sensilla showing a wide stylus bearing an apical sensory cone; coeloconic sensilla with a short peg-like structure usually situated in a pit; and placoid sensilla situated in a plate-like cuticular structure (Hallberg & Hansson, 1999; Zacharuk, 1985).

The sensilla system of insects shows a remarkable morphological diversity. This diversity probably reflects selection pressures for high

sensitivity, phylogenetic, and/or developmental constraints and the physical environment in which the evolution took place (Rebora, Salerno, & Piersanti, 2019). Detailed investigations on adult sensory structures, both morphological and physiological, are available in the literature. Knowledge of aquatic insect sensilla, however, is still poorly known. It is likely that the successful invasion of lotic and lentic freshwaters by terrestrial insects required physiological constraints regarding sensory systems, which accordingly influenced the external morphology and distribution of sensilla. We echo Rebora et al. (2019), therefore, saying that better knowledge of aquatic insect sensilla could play a significant role in unravelling insect perception and adaptability of species to different aquatic environmental conditions.

Nearly 100,000 species from 12 insect orders spend one or more life stages in freshwater (Dijkstra, Monaghan, & Pauls, 2014). Of a particular interest is the study of body sensory equipment in larval stages of Hydradephaga (Coleoptera), some of which are adapted to live in turbid, dark, highly complex habitat conditions with poor vision possibilities (Brönmark & Hansson, 2000; Wisenden, 2000). One aim of this study therefore was to investigate the diversity, external morphology, and distribution of sensilla on the head appendages of four species from three genera of the beetle family Noteridae. Noterids are distributed worldwide (Nilsson, 2011), and generally include small to medium sized aquatic insects commonly encountered in a broad range of aquatic habitats. Larvae of the species studied were recently described (Urcola, Alarie, Benetti, & Michat, 2019c; Urcola, Alarie, Benetti, Rodriguez, & Michat, 2019a; Urcola, Benetti, Alarie, Torres, & Michat, 2019b), or are being described using the chaetotaxy system designed for study of other Hydradephaga families namely Hygrobiidae (Alarie, Beutel, & Watts, 2004), Aspitydidae (Alarie & Bilton, 2005), Dytiscidae (summarized in Alarie & Michat, 2014), and Gyrinidae (Michat, Marinho Alvarenga, Souza Silva, & Alarie, 2016).

As a corollary to this study, an attempt was made to use selected sensillar patterns to develop a fingerprint identification method for the taxa studied. Fingerprint identification methods are the best-known biometric systems which refer to any measurable, robust, and distinctive physical, anatomical, or molecular trait that can be used to uniquely identify or verify a claimed identity (Barron, Butler, McDonnell, & Ward, 2009). We postulate that sensillar patterns can work as fingerprints assuming variation in the arrangement of sensilla amongst taxa. We therefore explored, in a preliminary way, sensillar patterns as a new diagnostic tool (i.e., fingerprint).

2 | MATERIAL AND METHODS

2.1 | Material examined

First instar larvae of four species from three different genera were examined: *Suphis cimicoides* (Aubé, 1837) ($n = 27$); *Hydrocanthus socius* (Sahlberg, 1844) ($n = 21$); *Hydrocanthus sharpi* (Zimmermann, 1928) ($n = 30$); and *Suphisellus rufipes* (Sharp, 1882) ($n = 9$). The reader is referred to Urcola, Alarie, Benetti, Rodriguez, and Michat (2019a);

Urcola, Benetti, Alarie, Torres, and Michat (2019b); Urcola, Alarie, Benetti, and Michat (2019c) for detailed descriptions of three of these species with a focus on chaetotaxy, and collecting and rearing techniques. The material is stored in the collection of the Laboratory of Entomology, Buenos Aires University, Argentina.

2.2 | Optic and scanning electron microscopy

Ten larvae of *S. cimicoides*, *H. socius* and *H. sharpi*, and seven larvae of *S. rufipes* were cleared in lactic acid, mounted on slides and observed (at magnifications up to $\times 1,000$) with an Olympus CX41 compound microscope. The remaining specimens were photographed using a Zeiss NTS SUPRA 40 scanning electron microscope (SEM) in the Centro de Microscopías Avanzadas, University of Buenos Aires. Previously to SEM analysis, larvae were superficially cleaned with a soft brush and sonicated for 4 min in a solution of warm water and detergent. To remove the remaining particles, the samples were sonicated for 4 min with commercial window cleaner. Specimens were then dehydrated in a graded series of ethanol, infiltrated with hexamethyldisilazane, and air dried overnight. The samples were mounted on stubs using copper tape and sputter-coated with gold-palladium.

2.3 | Sensilla analysis

The sensilla present on the surface of the antenna, mandible, maxilla, and labium were examined using optic microscopy (*S. cimicoides*: $n = 10$; *H. socius*: $n = 10$; *H. sharpi*: $n = 10$; *S. rufipes*: $n = 7$), and the number of sensilla for each structure (S_{sur} , which do not show intra-specific variation) was counted. On the other hand, the sensilla present on the apices of the antenna (gAN), galea, and maxillary (gMX), and labial (gLA) palpi were counted using SEM photographs (*S. cimicoides*: $n = 17$; *H. socius*: $n = 11$; *H. sharpi*: $n = 20$; *S. rufipes*: $n = 2$), and the maximum number of sensilla (S_{api}) was registered. Finally, the total number of sensilla for each species was calculated ($Stot = S_{sur} + S_{api}$).

Sensilla were classified according to the morphology of their cuticular parts and their position with respect to the cuticle. We defined the sensilla following the terminology of Snodgrass (1935), Schneider (1964), Zacharuk (1985), and Kapoor (1987): sensilla basiconica (BS, including subtypes BS1, BS2, BS3, and BS4), sensilla campaniformia (SCa, including subtypes SCa1 and SCa2), sensilla chaetica (ChS, including subtypes ChS1, ChS2, ChS3, ChS4, ChS5, and ChS6), sensilla coeloconica (CoS, including subtypes CoS1, CoS2, CoS3, CoS4, CoS5, and CoS6), coniform sensillar complex (CSc, including subtypes CSc1 and CSc2), sensilla placodea (PS), and sensilla styloconica (SS, including subtypes SS1, SS2, and SS3). The number of sensilla of subtypes CoS4, SS2, and SS3 could not be registered accurately on the apices of the maxillary and labial palpi due to the orientation of these structures in the SEM microphotographs. To facilitate interpretation of the types and subtypes of sensilla, SEM microphotographs were colored using Adobe Photoshop CC2015: green

(coniform sensillar complex); light blue (sensilla basiconica); orange (sensilla placodea); pink (sensilla campaniformia); red (sensilla chaetica); violet (sensilla coeloconica); and yellow (sensilla styloconica). The sensilla were labeled following the ground plan of chaetotaxy for the family Noteridae (see Urcola, Alarie, Benetti, & Michat, 2019c; Urcola, Alarie, Benetti, Rodriguez, & Michat, 2019a; Urcola, Benetti, Alarie, Torres, & Michat, 2019b).

3 | RESULTS

Eight types and 26 subtypes of sensilla were identified in first-instar larvae of the studied species (Tables 1–3). All types are present in the three genera examined (Figure 1). The genus *Hydrocanthus* presents the largest number of sensilla (at least 362 in *H. sharpi* and 340 in *H. socius*) and 21–22 subtypes. *Suphisellus rufipes* presents at least 264 sensilla and the largest number of subtypes (23), whereas *Suphis cimicoides* exhibits the lowest number of total sensilla and subtypes (238 and 18, respectively) (Figures 1 and 2).

Sensilla basiconica are cone shaped. Five subtypes were detected (Table 1), mainly on the tips of the antenna and maxillary and labial palpi (Figure 3). Sensilla campaniformia are characterized by a button-like knob with a small irregular surface emerging from an opening in the center of a domed, smooth, and circular cuticular structure. We identified two subtypes of sensilla campaniformia (Table 1), subtype SCA1 is not easily seen in SEM images (for a detail of the position of these sensilla [referred to as pores]; see Urcola, Alarie, Benetti, Rodriguez, & Michat, 2019a; Urcola, Benetti, Alarie, Torres, & Michat, 2019b; Urcola, Alarie, Benetti, & Michat, 2019c). Sensilla chaetica are clearly distinguishable by their elongate shape. They are represented by the largest number of subtypes (7) (Figure 2; Table 2). Sensilla coeloconica are characteristically sunken in deep pits. We found six subtypes of these sensilla (Table 3). Coniform sensillar complexes are distinguishable by their

digitiform projections in conical arrangement. We registered two subtypes of these sensilla (Table 3), which are located mainly on the apices of the maxillary and labial palpi (gMX and gLA) (Figure 3). Sensilla placodea were scarce in the studied larvae, present only on the apex of the maxillary palpus (Figure 3). They are multiporous, plate-like, and only one subtype was detected (Table 3). Sensilla styloconica are the most abundant type on the apices of the maxillary and labial palpi (Figure 3). These sensilla presented three subtypes (Table 3).

3.1 | Distribution of sensilla on the head appendages of first instar larvae of Noteridae

3.1.1 | Antenna

The antenna bears six types of sensilla: one subtype of sensilla basiconica (BS1), two subtypes of sensilla chaetica (ChS1, ChS2), two subtypes of sensilla coeloconica (CoS1, CoS2), one subtype of coniform complex (CSc1), two subtypes of sensilla campaniformia (SCa1, SCa2), and one subtype of sensilla styloconica (SS1) (Figs. 4b–d, 5b, 6a–d, and 7e). The subtypes ChS1, ChS11, CoS1, CoS2, SCa1, and SCa2 appear on the antennal surface. SCa1 is present in all genera, whereas CoS1 is present only in *Suphis* (Figure 4b,c), ChS2 and SCa2 only in *Hydrocanthus* (Figure 6a–d), and CoS2 only in *Suphisellus* (Figure 7e). The subtypes BS1, CSc1, and SS1 appear at the apex, two of them occur in *Suphis* (BS1 and CSc1; Figure 4d), and two occur in *Hydrocanthus* and *Suphisellus* (BS1 and SS1; Figures 5b and 6d).

3.1.2 | Mandible

The mandibles are characterized by the presence of three types of sensilla: chaetica subtype ChS1 (MN1), coeloconica subtype CoS3

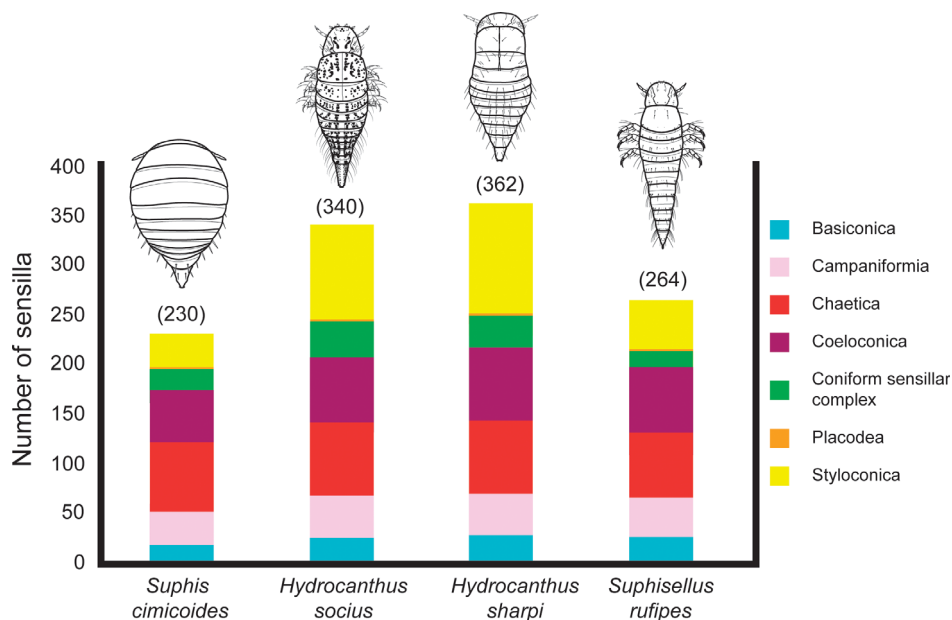


FIGURE 1 Total number (Stot) and types of sensilla present in first-instar larvae of the studied species

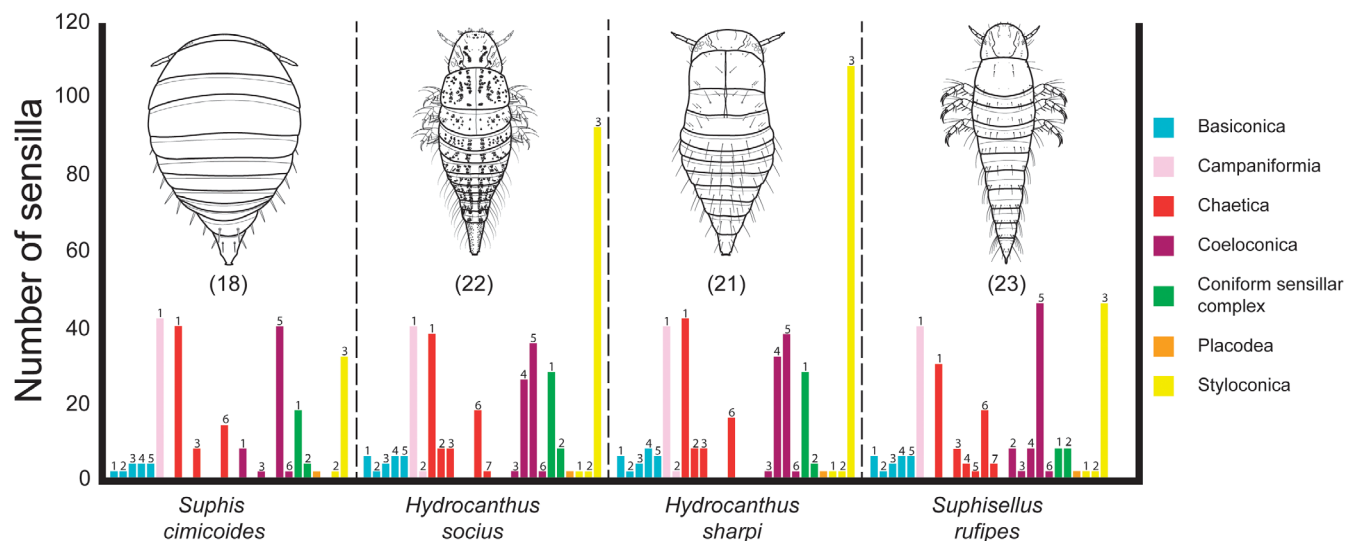


FIGURE 2 Number of sensilla per subtype present in first-instar larvae of the studied species

TABLE 1 Types and subtypes of sensilla basiconica and campaniformia present in first-instar larvae of the studied species

Type	Subtype	General shape	Tip	Surface	Pores	Socket	Figure
Basiconica	BS1	Medium-length, thorn-like, appreciably longer than wide	Truncate	Rough	Aporous	—	Figures 4d, 5b, and 6d
Basiconica	BS2	Small, subrectangular, laterally flattened, length similar to width	Rounded	Smooth	Aporous	—	Figures 5c, 6g, and 9b
Basiconica	BS3	Medium-length, peg-like	Rounded	Smooth	Uniporous	Flexible, wide, rounded, with a conspicuous ridge	Figures 5d, 6e,f, 7b, and 9c
Basiconica	BS4	Small, peg-like	With a terminal filament	Smooth	Aporous	Flexible, wide, rounded, not exceeding surface level	Figures 5e, 6j,f, 7d, and 9c
Basiconica	BS5	Small, peg-like	Rounded	Deeply grooved	Uniporous	—	Figure 5d,e
Campaniformia	SCa1	Oval or rounded, flat with gentle edges	—	Smooth	Uniporous	—	—
Campaniformia	SCa2	Small, pin-like shallow cuticular depression	—	Rough	Aporous	Shallow cuticular depression	Figure 6b

(MN2) (Figure 8c), and campaniformia subtype SCa1. Most of these sensilla are distributed along the outer margin.

3.1.3 | Maxilla

Seven types of sensilla were found on the maxilla. In addition, a great morphological diversity was observed with a maximum of 17 subtypes. The subtypes ChS1, ChS3, ChS4, ChS5, CoS4, and SCa1 are distributed on the maxilla surface. ChS1, ChS3, and SCa1 are present in all genera, whereas CoS4 is absent in *Suphis*, and ChS4 and ChS5 are present only in *Suphisellus*: ChS4 is restricted to the cardo (MX4, MX6) (Figure 8d,e), ChS5 is represented only by one seta located on the outer margin of the stipes (MX5) (Figure 8d), and few setae CoS4 are present on the third palpomere (Figure 7a). The subtypes BS2, CoS5,

and SS2 appear at the tip of the galea and are present in all genera. The sensilla of the subtypes SS2 (MX8) and BS2 (MX9) are located in the center of the apex surrounded by six sensilla of the subtype CoS5 (Figures 5c, 6g, and 9b). Ten subtypes were recognized on the apex of the palpus (gMX): BS3, BS4, BS5, ChS6, Co5, CSc1, CSc2, PS, and SS3. The arrangement of the sensilla differs among genera. In *Suphis* six subtypes occur on the apex of the maxillary palpus (gMX): five coniform complexes of the subtype CSc1 are located in the center of the apex, at least two ChS6, one PS, and two BS3 surrounding them, and seven Co5 and at least six SS3 in a more marginal position (Figure 9c). In *H. socius* nine subtypes of sensilla are found on the apex of the maxillary palpus (gMX) (Figure 5d): one PS and two CSc2 are located in central position, at least 21 SS3, three CSc1, four ChS6, and four sensilla basiconica (two BS3, one BS4, and one BS5) surrounding them, and at least five Co5 in a marginal position (Figure 5d). In

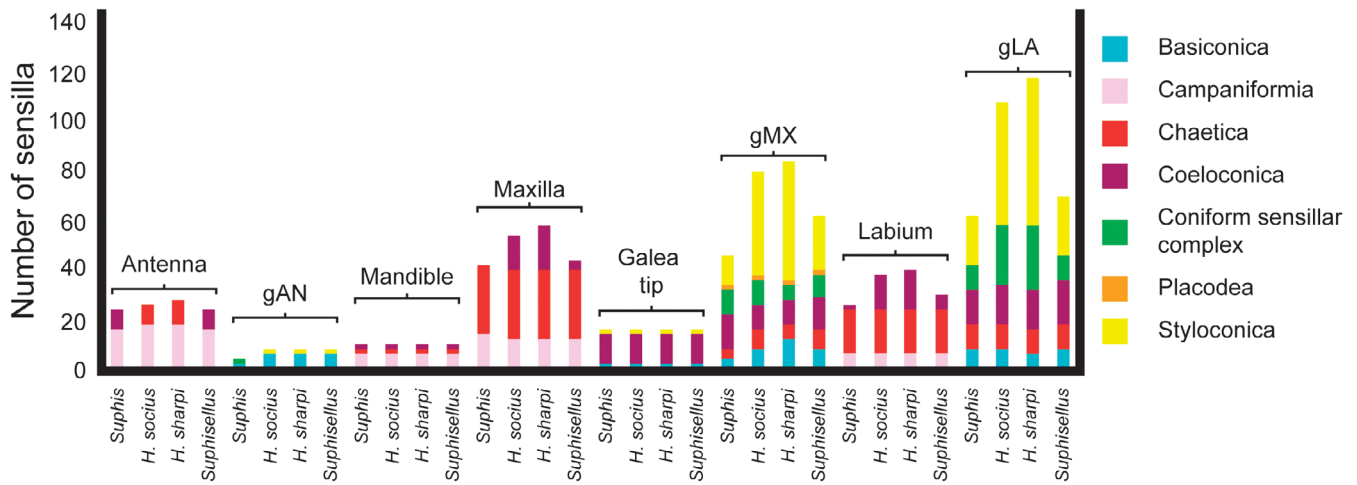


FIGURE 3 Number of sensilla on the antenna, mandible, maxilla, and labium, and maximum number of sensilla on the apices of the antenna (gAN), galea and maxillary (gMX), and labial (gLA) palpi by type of sensilla present in first-instar larvae of the studied species

TABLE 2 Types and subtypes of sensilla chaetica present in first-instar larvae of the studied species

Type	Subtype	General shape	Tip	Surface	Pores	Socket	Figure
Chaetica	ChS1	Long, hair-like	Sharp	Smooth	Aporous	Flexible, wide, rounded	Figures 4a, 8b, and 9a
Chaetica	ChS2	Small, hair-like	Sharp	Smooth	Aporous	Flexible, wide, rounded, depressed	Figure 6c
Chaetica	ChS3	Very small, spine-like	Sharp	Smooth	Aporous	Flexible, narrow, rounded	Figure 9a
Chaetica	ChS4	Rather long, hair-like, multifid	—	Smooth	Aporous	Flexible, narrow, cuticle raised covering the base of the shaft	Figure 8e
Chaetica	ChS5	Small, hand-like, multifid	—	Smooth	Aporous	Flexible, wide, rounded, depressed	Figure 8d
Chaetica	ChS6	Small, hair-like, branched from the base (multifid)	—	Smooth	Unknown	Flexible, wide, rounded, with a conspicuous ridge	Figures 5d,e, 6e,j, 7b,d, and 9d
Chaetica	ChS7	Long, hair-like	Sharp	Frayed	Aporous	Flexible, narrow, rounded	Figure 8b

H. sharpi eight subtypes of sensilla are found on the apex of the maxillary palpus (gMX) (Figure 6e,f): one PS is located in central position, at least 24 SS3, three CSc1, three ChS4, and six sensilla basiconica (two BS4, two BS5, and two BS7) surrounding them, and at least five CoS5 in a marginal position (Figure 6f). The subtype CSc2 was not registered in *H. sharpi* probably due to the orientation (Figure 6e) and dirtiness of the structure (Figure 6f). Finally, in *Suphisellus* nine subtypes of sensilla occur on the apex of the maxillary palpus (gMX): one PS located in central position, at least ten SS2, three coniform complexes (one CSc1 and two CSc2), four sensilla basiconica (two BS3, one BS4, and one BS5), and four ChS6 surrounding them, and at least eight CoS5 in a marginal position (Figure 7b).

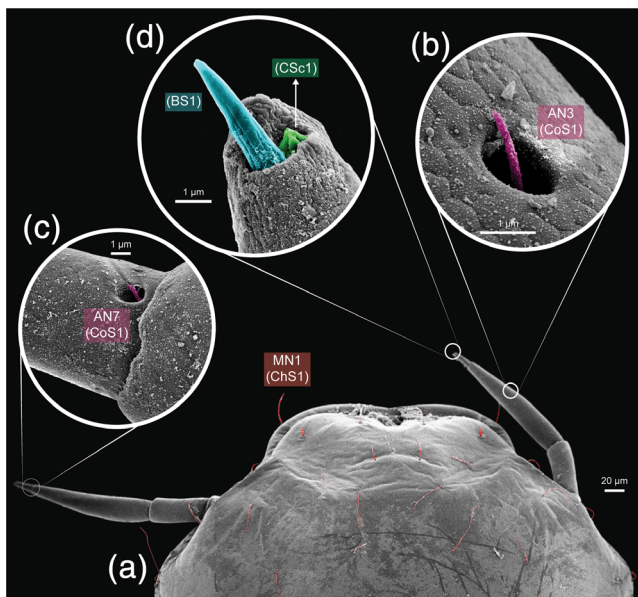
3.1.4 | Labium

The labium shows 13 subtypes of sensilla. The subtypes ChS1, ChS3, ChS7, CoS4, CoS6, and SCa1 are distributed along the surface (Figures 5a, 6h,i, 7a,c, 8a,b, and 9a). ChS1 and SCa1 are scattered in almost all regions, ChS3 is restricted to the postmentum and to the base of palpomere 1, and CoS6 is only represented by seta LAa

(misidentified as pore-like sensilla in Urcola, Alarie, Benetti, Rodriguez, & Michat, 2019a; Urcola, Benetti, Alarie, Torres, & Michat, 2019b; Urcola, Alarie, Benetti, & Michat, 2019c; Figure 8b). In *Hydrocanthus* and *Suphisellus* few minute sensilla of the subtype CoS4 appear on the second palpomere (Figures 6h,i and 7a,c), and a new subtype of sensilla chaetica (ChS7) is present on the prementum of *H. socius* (LA3) and *Suphisellus* (LA3 and LA6; Figures 8a,b and 7a). Seven subtypes were identified on the apex of the labial palpus (gLA): BS4, BS5, ChS6, CSc1, CSc2, CoS5, and SS3 (Figures 5e, 6j, 7d, and 9d). All subtypes are present in the three genera. The arrangement and number of sensilla differs between the genera. In *Suphis* five coniform complexes (three CSc1 and two CSc2) occur in the center of the apex, five sensilla chaetica (ChS6), and four sensilla basiconica (two BS4 and two BS5) are placed surrounding them, and seven CoS5 and 12 SS3 are located in a more marginal position (Figure 9d). In *Hydrocanthus* the sensilla of the subtype CSc1 are organized in two groups of five and six setae, respectively, separated by seven sensilla SS3 and two CSc2 arranged in a central line, five ChS6, four sensilla basiconica (BS4 and BS7) and at least 23 SS2 are located surrounding them (Figure 5e), and at least eight CoS5 are placed in a marginal position (Figure 6j). On the contrary, in *Suphisellus* three

TABLE 3 Types and subtypes of sensilla coeloconica, placodea, styloconica and coniform complex present in first-instar larvae of the studied species

Type	Subtype	General shape	Tip	Surface	Pores	Socket	Figure
Coeloconica	CoS1	Medium-length, slender	Rounded	Smooth	Aporous	Deep pit	Figure 4b,c
Coeloconica	CoS3	Short, peg-like	Rounded	Smooth	Aporous	Deep pit, surrounded by an inflexible cuticular ring	Figure 8c
Coeloconica	CoS4	Minute, peg-like	Split	Smooth	Aporous	Shallow pit	Figures 6i and 7a,c
Coeloconica	CoS5	Minute, knob-like	Rounded	Smooth	Aporous	Inflexible, wide, plate-like, with a deep pit on center	Figures 5d,e and 6g,j
Coeloconica	CoS2	Short, peg-like	Rounded	Smooth	Aporous	Shallow pit	Figure 7e
Coeloconica	CoS6	Minute, knob-like	Rounded	Smooth	Aporous	Inflexible, wide, dome-like, with a deep pit on center	Figure 8b
Coniform complex	CSc1	Rather large, with 12–15 digitiform projections in conical arrangement	—	Smooth	Multiporous	Inflexible, wide, rounded, with a conspicuous ridge	Figures 4d, 9d, 5d,e, and 6j
Coniform complex	CSc2	Small, with 4–5 subtriangular projections in conical arrangement	—	Smooth	Multiporous	Inflexible, wide, rounded, with a conspicuous ridge	Figures 9d and 10
Placodea	PS	Large, plate-like, rounded	—	Porous	Multiporous	—	Figures 6e,f,7b and 9c
Styloconica	SS1	Small, peg-like	Rounded	Smooth	Aporous	Inflexible, troncoconical	Figures 5b and 6d
Styloconica	SS2	Small, cone-like	Sharp	Smooth	Aporous	Inflexible, large, cylindrical	Figure 5c
Styloconica	SS3	Small, cone-like	Bifid	Smooth	Aporous	Inflexible, troncoconical	Figures 9c,d, 5d,e, 6e,j, and 7b,d

**FIGURE 4** *Suphis cimicoides* Aubé, 1837, SEM microphotographs of the cephalic capsule and antenna of first-instar larva. (a) Cephalic capsule, dorsal aspect; (b) seta AN3; (c) seta AN7; (d) apex of antenna. Green: coniform sensillar complex; Light blue: sensilla basiconica; Red: sensilla chaetica; Violet: sensilla coeloconica

sensilla CSc1 and two CSc2 occur in central position, five ChS6 and four sensilla basiconica (two BS4 and two BS7) are located surrounding them, and at least nine CoS5 and 12 SS3 are placed on the margin (Figure 10).

3.2 | Biometric analysis

The apices of the maxillary and labial palpi are the most variable and densely populated sensillar fields in noterid larvae (Figure 3). The fact that the apex of the labial palpus exhibits both the largest number and the highest diversity of sensilla of the studied structures makes this region a good candidate for the development of a biometric pattern (fingerprint). Each sensory organ represents a site that can vary in topology, position, and relative distance with respect to other sites, thus generating a fingerprint. Sensillar fingerprints are permanent morphological characteristics that are universally displayed throughout a study group (Kuhl & Burghardt, 2013), and provide measurable information that can be registered with a recording device (SEM). The identification of taxa is produced by matching the fingerprint models (Figure 10) with an image that captures the appearance of a phenotypic aspect, in this case the arrangement of sensilla on the apex of the labial palpus. Taxon recognition not only takes into account general information but also hot spots, that is, zones of high variation within the fingerprint that characterize each genus. For example, coniform complexes are arranged forming conspicuous groups. In *Suphis cimicoides* three CSc1 are located in the center forming a triangle and two CSc2 are placed forming a line from the center of the triangle. In *Suphisellus rufipes* the sensilla CSc1 also form a triangle but the CSc2 are not in touch and are located one in the center of the triangle and the other one outside it. On the contrary, in the genus *Hydrocanthus* the sensilla CSc1 are arranged in two large groups separated by sensilla of other types (Figure 10). In *H. sharpi*, in one of the groups the CSc1 are arranged linearly, whereas in *H. socius* none of the groups is

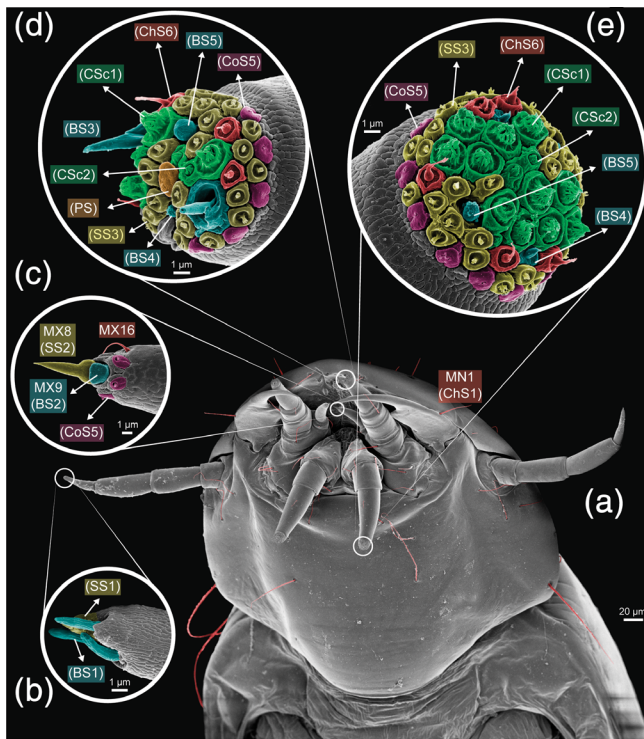


FIGURE 5 *Hydrocanthus socius* Sahlberg, 1844, SEM microphotographs of the cephalic capsule, antenna, mandible and labium of first-instar larva. (a) Cephalic capsule, ventral aspect; (b) apex of antenna; (c) apex of galea; (d) apex of maxillary palpus (gMX); (e) apex of labial palpus (gLA). Green: coniform sensillar complex; Light blue: sensilla basiconica; Orange: sensilla placodea; Red: sensilla chaetica; Violet: sensilla coeloconica; Yellow: sensilla styloconica

linearly distributed. The fingerprint models of *Suphis* and *Suphisellus* are evidently more similar to each other with respect to those of the *Hydrocanthus* species, which in turn are very similar to each other (Figure 10).

4 | DISCUSSION

In the body plan of insects, sensory structures vary in number and shape and their density is closely related to the corresponding behavior (e.g., predation, reproduction, habitat choice and intraspecific communication) of the species (Giglio et al., 2008a). The density of sensory organs in larvae of the noterid genera studied was higher on the apices of maxillary and labial palpi, followed by the apices of galea and antenna. The topology of the cluster of sensilla on the tips of maxillary and labial palpi was very similar (Table 4), in accordance with previous observations in other beetle families (Alekseev, Sinitsina, & Yu Chaika, 2006; Zacharuk, 1985). Also, the number of sensilla was similar in both structures in *Suphisellus* and *Suphis* as recorded in other beetle species (e.g., Rosciszewska, 1981; Tomkovich & Chaika, 2001). In *Hydrocanthus*, however, the number of sensilla was much higher on the labial palpus than on the maxillary palpus. According to Alekseev

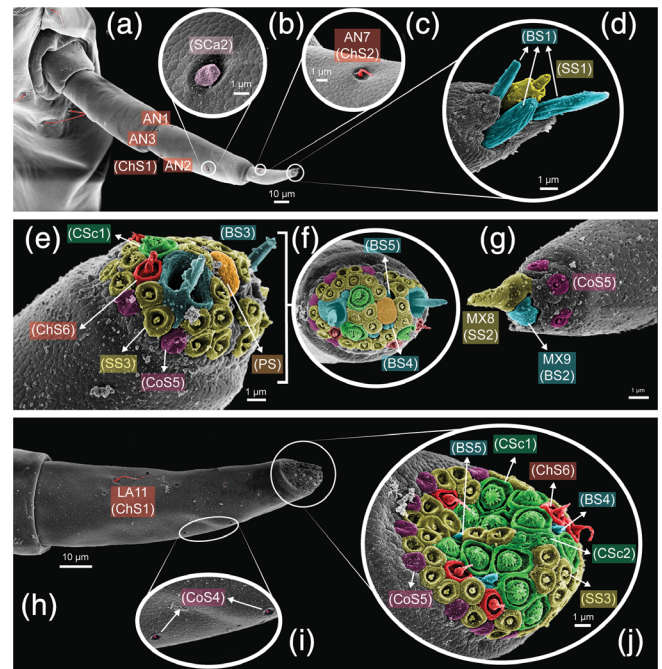


FIGURE 6 *Hydrocanthus sharpi* Zimmermann, 1928, SEM microphotographs of the antenna, maxilla and labium of first-instar larva. (a) Antenna, ventrolateral aspect; (b) detail of portion of antennomere 3, ventrolateral aspect; (c) seta AN7; (d) apex of antenna; (e–f) apex of maxillary palpus; (g) apex of galea; (h) labial palpomere 2, dorsal aspect; (i) detail of portion of labial palpomere 2, dorsal aspect; (j) apex of labial palpus. Green: coniform sensillar complex; Light blue: sensilla basiconica; Orange: sensilla placodea; Pink: sensilla campaniformia; Red: sensilla chaetica; Violet: sensilla coeloconica; Yellow: sensilla styloconica

et al. (2006), an increase in the quantity of sensilla in the palpal receptor groups was observed in some predaceous forms of the families Elateridae and Coccinellidae.

In larvae of aquatic insects it is very difficult to test the response of sensilla to specific stimuli (Gaino & Rebora, 1999). Therefore, the study of the microstructure provides relevant information to assign tentative functions to sensilla given the lack of electrophysiological data in the literature. Sensilla chaetica and campaniformia were found in all the studied structures and turn out to be the most abundant types in the head appendage of noterid larvae (except on the apices). Sensilla chaetica are usually considered mechanoreceptors but in some cases they have a bimodal function, sensing mechanical and chemosensory stimulation (Daly & Ryan, 1979; Zacharuk, 1985). This type of sensilla has proven to be the most variable with seven subtypes recognized. Campaniform sensilla are considered exclusively mechanoreceptors (Moran, Chapman, & Ellis, 1971; Zacharuk, 1985) and only three subtypes were identified. They were absent from the apices of the head appendages. The most abundant type of sensillum on the apices of the antenna, galea, and maxillary and labial palpi was the sensilla styloconica. In spite of their abundance, only three subtypes were distinguished. The sensilla styloconica are well studied and several functions have been assigned to them in different insects:

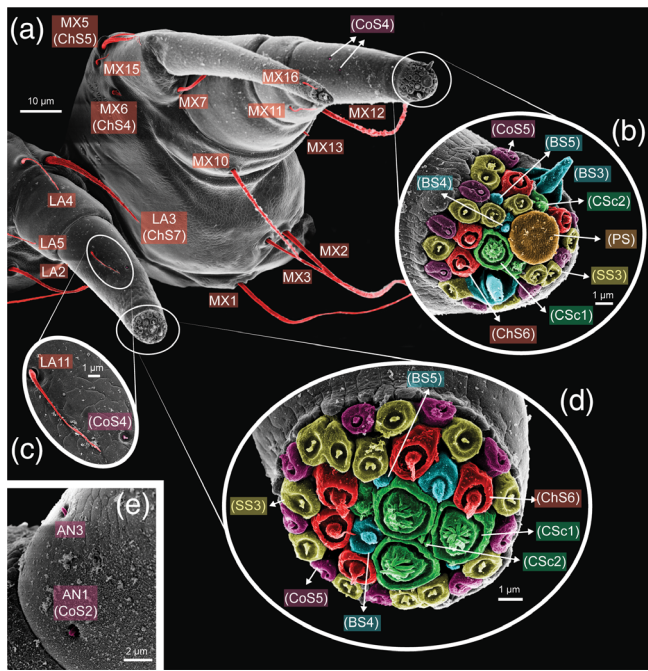


FIGURE 7 *Suphisellus rufipes* (Sharp, 1882), SEM microphotographs of the antenna, maxilla and labium of first-instar larva. (a) Maxilla and labium, frontal aspect; (b) apex of maxillary palpus; (c) detail of portion of palpomere 2, dorsal aspect; (d) apex of labial palpus; (e) setae AN1 and AN3. Green: coniform sensillar complex; Light blue: sensilla basiconica; Orange: sensilla placodea; Red: sensilla chaetica; Violet: sensilla coeloconica; Yellow: sensilla styloconica

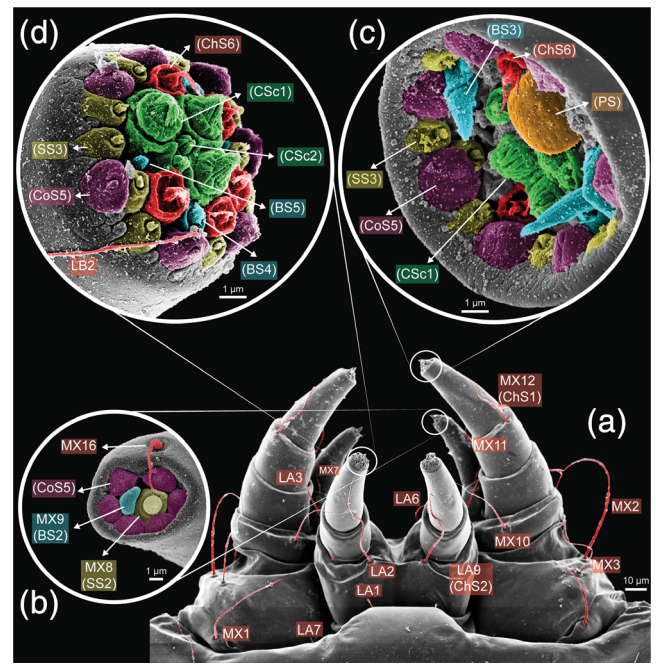


FIGURE 9 *Suphis cimicoides* Aubé, 1837, SEM microphotographs of the maxilla and labium of first-instar larva. (a) Maxilla and labium, ventral aspect; (b) apex of galea; (c) apex of maxillary palpus (gMX); (d) apex of labial palpus (gLA). Green: coniform sensillar complex; Light blue: sensilla basiconica; Orange: sensilla placodea; Red: sensilla chaetica; Violet: sensilla coeloconica; Yellow: sensilla styloconica

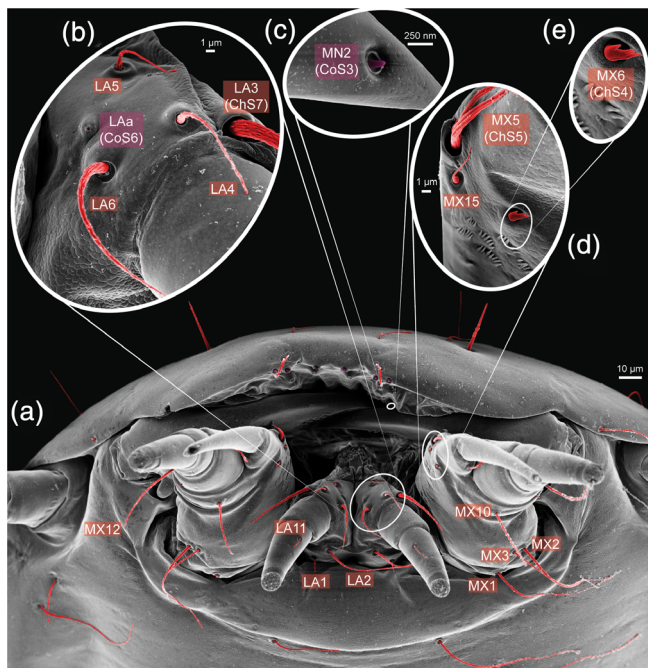
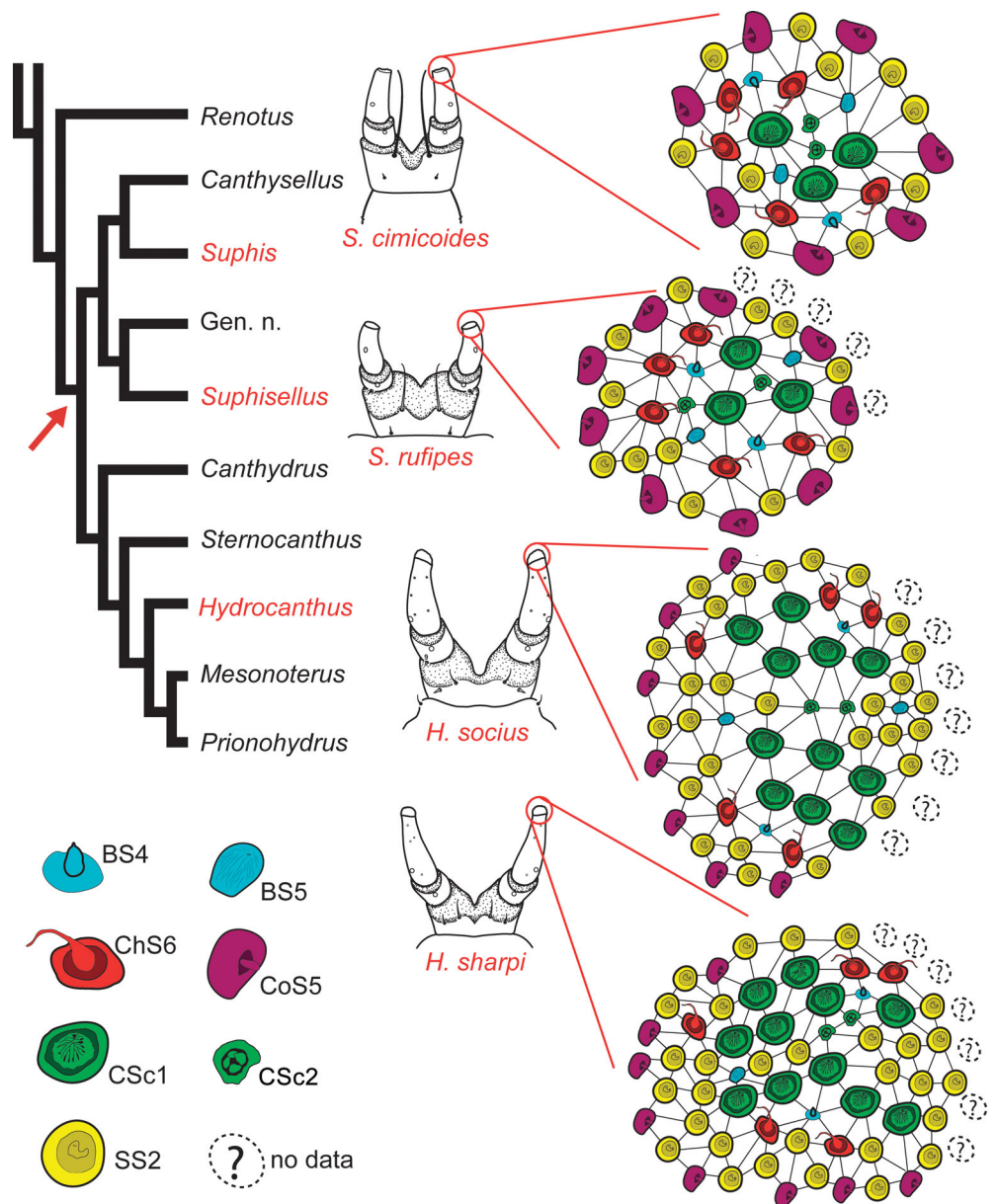


FIGURE 8 *Suphisellus rufipes* (Sharp, 1882), SEM microphotographs of the cephalic capsule, mandible, maxilla, and labium of first-instar larva. (a) Frontal aspect; (b) prementum, dorsal aspect; (c) apex of right mandible; (d) stipes, dorsal aspect; (e) seta MX6. Red: sensilla chaetica; Violet: sensilla coeloconica

mechano-, chemo-, hygro-, and/or thermoreceptors (Bissoto de Oliveira, Redaelli, & Santana, 2011; Schneider, 1964; Shields, 2009; Steinbrecht, 1998; Zacharuk, 1985). In aquatic insects sensilla styloconica are reported mainly as chemosensory organs, although little information is available (Gaino & Rebor, 1999). On the other hand, sensilla coeloconica were proven to be either hygro- or thermosensitive (Giglio et al., 2009; Ruchty et al., 2009; Zacharuk, 1985) or chemosensitive (Altner & Prillinger, 1980; Hunger & Steinbrecht, 1998; Zacharuk, 1985). Giglio, Perrotta, Talarico, Zetto Brandmayr, and Ferrero (2013) found this type of sensilla forming clusters around sensilla basiconica in the maxillary and labial palpi of some carabid larvae. In noterid larvae, sensilla coeloconica are abundant on the maxilla and labium, although they were also found at the tips of mandible and antennae, and on the anterior margin of the frontoclypeus. Coniform sensillar complexes were observed in Plecoptera (Kapoor, 1987), and within Coleoptera on the apex of the labial palpus in Nitidulidae (Ortloff et al., 2014), classified by these authors as sensilla styloconica (labeled St2). Within aquatic beetles, these complexes were so far observed on the antenna and on the apices of the maxillary and labial palpi in noterid larvae (this study), and on the apices of the maxillary and labial palpi in Hydrophilidae. Sensilla basiconica are usually considered chemosensitive, although thermosensitive and hygro- or thermosensitive functions have also been assigned to this type of sensory structures (Gaino & Rebor, 1999; Giglio, Ferrero, Perrotta, Tripepi, & Zetto Brandmayr, 2003; Zacharuk, 1985). In noterid larvae they are usually present on the apices of the antenna,

FIGURE 10 Portion of the phylogenetic tree in Baca, Toussaint, Miller, and Short (2017) depicting generic relationships within Noteridae, and the evolution of the distribution and number of sensilla on the apex of labial palpus (fingerprints) in the studied genera. Black lines indicate sensilla in contact with each other



galea, and maxillary and labial palpi. These sensilla were morphologically diverse with five subtypes identified in our study, ranging from small cone-like structures to large pegs with or without socket. Alekseev et al. (2006) found that in larval Coleoptera there are always one to several digitiform sensilla placodea on the lateral side of the last maxillary palpomere. In Noteridae these sensilla placodea are lacking, although a large plate-like sensillum placodeum was observed on the apex. Like most sensilla of this type (Song et al., 2017), they have a large number of pores on the surface, as is the case of noterid larvae. This kind of multiporous sensilla were associated with an olfactory function and more specifically with perception of CO₂ (Giglio et al., 2008b; Keil, 1996; Kim & Leal, 2000).

Representing and matching aspects of the phenotype in a quantifiable way is one of the main foundations of biometric methods. The selection of clusters of sensilla as biometric entities have proven to

successfully represent aquatic beetle appearance. The fingerprint models generated from the sensilla of the apex of the labial palpus allow an efficient identification of the Noteridae genera studied by using as parameters the types and subtypes of sensilla, and their relative position and arrangement. Giglio et al. (2003) examined the maxillary and labial palpi of larvae of 22 species in 16 genera of Carabidae and demonstrated that the number and location of sensory structures vary among species. In that sense, we have also found some differences between the two studied species of *Hydrocanthus*, both in the number (SS3) and distribution (CSc1) of sensilla (Figure 10; Table 4). In the future, it would be interesting to analyze in greater detail the variation of sensillar patterns in different noterid species to test the specificity of the method. This new biometric method is simple, easy to interpret, and not necessarily requires full SEM microphotographs, since an incomplete microphotograph would be sufficient to obtain a

TABLE 4 Number of sensilla in the body regions of first-instar larvae of the studied genera

	BS										Sca	ChS										CoS	CSc	PS	SS														
	1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	6	7	8	9	10																		
Antenna	Suphis									16																													
	<i>H. socius</i>									16	2	8																											
	<i>H. sharpi</i>									16	2	2	8																										
	<i>Suphisellus</i>									16																													
gAN	<i>Suphis</i>									2																													
	<i>H. socius</i>									6																													
	<i>H. sharpi</i>									6																													
	<i>Suphisellus</i>									6																													
Mandible	<i>Suphis</i>									6	2	2																											
	<i>H. socius</i>									6																													
	<i>H. sharpi</i>									6																													
	<i>Suphisellus</i>									6																													
Maxilla	<i>Suphis</i>									14	24	4																											
	<i>H. socius</i>									12	24	4																											
	<i>H. sharpi</i>									12	24	4																											
	<i>Suphisellus</i>									12	18	4	4	2																									
Galea tip	<i>Suphis</i>									2																													
	<i>H. socius</i>									2																													
	<i>H. sharpi</i>									2																													
	<i>Suphisellus</i>									2																													
gMX	<i>Suphis</i>									4																													
	<i>H. socius</i>									4	2	2																											
	<i>H. sharpi</i>									4	4	4																											
	<i>Suphisellus</i>									4	2	2																											
Labium	<i>Suphis</i>									6	14	4																											
	<i>H. socius</i>									6	12	4																											
	<i>H. sharpi</i>									6	14	4																											
	<i>Suphisellus</i>									6	10	4																											
gLA	<i>Suphis</i>									4	4																												
	<i>H. socius</i>									4	4																												
	<i>H. sharpi</i>									4	2																												
	<i>Suphisellus</i>									4	4																												

Abbreviations: BS, sensilla basiconica; ChS, sensilla chaetica; CoS, sensilla coeloconica; CSc, sensilla coeloconica; CSc, coniform sensillar complex; PS, sensilla placodea; Sca, sensilla campaniformia; SS, sensilla styloconica.

partial fingerprint. Identification from partial fingerprints may still be possible as the fingerprints have hotspots, that is, areas of complexity and variation that generate characteristic micropatterns for each taxon.

The study of these fingerprints allows not only to differentiate genera more efficiently, but also provides a new set of characters that can be used for phylogenetic analyses. In a very preliminary way, the fingerprint models generated in this article support current classification of Noteridae (Baca et al., 2017), as the fingerprint pattern is more similar in *Suphis* and *Suphisellus* as compared with the *Hydrocanthus* species, which in turn are very similar to each other (Figure 10). In fact, whereas the general pattern exhibits only minor differences in *Suphis* and *Suphisellus* (they vary mainly in the number and arrangement of subtypes SS2 and CoS5), in *Hydrocanthus* not only the general pattern is different but also the number of sensilla (*Hydrocanthus* has 108–118 sensilla compared with 62–70 in *Suphis* and *Suphisellus*). Based on current information, the presence of five sensilla chaetica of subtype ChS6, four sensilla basiconica (two each of subtypes BS4 and BS5), and the subtypes CoS5, CSc1, CSc2, and SS2 are putative synapomorphies of the largest clade including the three studied genera (marked with an arrow in Figure 10).

5 | CONCLUSION

The misidentification of insects impacts negatively in many areas of biological research, such as biodiversity, ecological, limnological, and phylogenetic studies, among others. The identification of an organism using traditional methods is often difficult and requires specialists in the subject (especially in immature insects). In this sense, the fingerprint identification method developed in this study, which allows identification of first instars of three noterid genera, should be viewed as a preliminary approach to introduce an alternative to more traditional identification tools. It would be interesting in future studies to assess in greater detail if the number of sensilla on the apex of labial palpus (selected fingerprint) presents both intraspecific and interspecific variations, and if the number of sensilla remains constant throughout the ontogeny of species.

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AUTHOR CONTRIBUTIONS

Juan Urcola: Conceptualization; data curation; formal analysis; investigation; methodology; resources; visualization; writing-original draft. **Cesar Benetti:** Resources; supervision; validation; writing-review and

editing. **Yves Alarie:** Investigation; resources; supervision; validation; writing-review and editing. **Georgina Rodriguez:** Conceptualization; formal analysis; investigation; resources; visualization; writing-original draft. **Mariano Michat:** Data curation; funding acquisition; investigation; project administration; resources; supervision; validation; writing-review and editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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