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NEW TOPOLOGICAL AND STATISTICAL OBSERVATIONS ON THE MOULT AND SKIN OF TOKAY GECKOS

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Abstract. We report the experimental description of the entire (about 10 hours long) moulting process of a female tokay gecko (*Gekko gecko*) and the related observations on its skin topology. A statistical analysis on the skin tessellation has been performed. A detailed a detailed scanning electron microscopy (SEM)characterization of the complete adhesive system of the gecko foot, discovering new peculiarities, as well as of four samples of skin taken from the gecko back, upper tail, abdomen and upper head is carried out. The gecko skin shows unexpected complexity, e.g. it is covered by nanohooks (0.2-1.5 μm in length and ~30-50 nm in diameter) superimposed to the evident nearly circular tessellations (circularity ~0.9; area ~0.5 mm^2, perimeter ~3 mm). The connection area between next lamellae and the edge of the toe are both covered with nano-hairs (~2-5 μm in length and ~200 nm in diameter). The skin of the eye seems to be fully anti-adhesive.

1. INTRODUCTION

After the pioneering observation by Aristotele [1], biologists and material scientists posed their attention on the adhesive abilities of geckos and similar creatures [2-17] and are nowadays renewing their interest on tokay gecko (*Gekko gecko*) [18-28], which displays the strongest dry adhesion known in Nature. Technological applications are consequently envisioned and recently the feasibility of Spiderman Suits has been demonstrated [29,30]. Fracture Mechanics approaches, able to solve problems in extremely different contexts [31-35], are expected to play a fundamental role in better understanding the animal adhesion.

Different techniques based on electron focalization, SEM and Field Emission Scanning Electron Microscopy (FESEM), has brought about new opportunities to go under the limitation given by the wavelength of the visible light and to study the gecko micrometric and sub-micrometric hierarchical architecture of its toes, Fig. 1a. Gecko foot consists of five digits (Fig. 1a) covered with macroscopic hairy structures called lamellae (~0.5-3 mm in width and 200-500 μm in length, Fig. 1b). These lamellae are organized in a series of multi-arrays localized perpendicular to the longitudinal axis of each digit. Each lamella area is covered with several thousands of setae (10-130 μm in length and 3-10 μm in diameter, density of ~0.014 μm^{-2} [11,17], Figs. 1b and 1c), which in turn contain at their tips hierarchical substructures called spatulae (0.1-0.2 μm wide and 15-20 nm thick, Fig. 1d). Terminal claws are located at the top of each single toe (~500 μm in diameter and ~1 mm in length, Fig. 1a) and guarantee a secure mechanical interlocking on high rough surfaces, for which the diameter of the gecko's claw tip is smaller than roughness [22]. Furthermore, our present observations suggest new peculiarities of the gecko foot (Figs. 2 and 3): nanostructured hairy units (~2-5 μm in length and ~200 nm in diameter, Figs. 2c, 2d) have been identified on the connection

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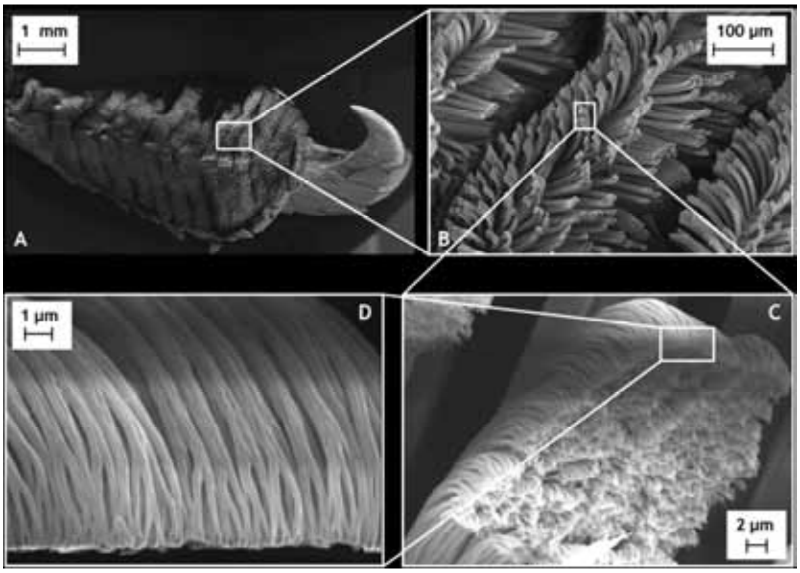

Fig. 1. Gecko adhesion system showed by FESEM (ZEISS SUPRA 40) (A, B) and by SEM (ZEISS EVO 50) (C, D). (A) Tokay gecko toe and FESEM micrograph of the setae (B). SEM micrograph of SEM micrograph of the setae (C) and nanoscale array of hundreds of spatula tips (D).

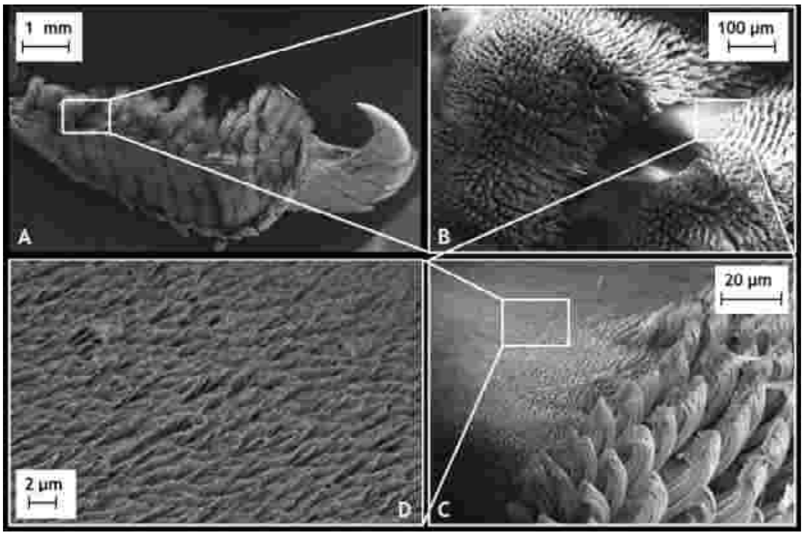

Fig. 2. Gecko adhesion system showed by FESEM (ZEISS SUPRA 40). (A) Tokay gecko toe; (B, C) The connection area between adjacent lamellae, localized perpendicular to the longitudinal axis of each digit, are covered by nanostructured hairy units; (D) at high magnification.

area between adjacent lamellae (Fig. 2c) and on the edge of each single digit (Figs. 3b, 3c, and 3d).

The epidermal adhesive layer, entirely covering the reptile, has a complex multi-structure: it consists of a new (inner) generation, formed beneath the older (outer) generation and each of them is constituted by six distinct layers. In the period between two next moults, the formation of a new inner generation initiates and goes on, so that the older generation is shed during the next moult. As a consequence, the gecko replaces the outer generation with a complete new one at each shedding cycle [23].

Casual observations reveal little about the mechanism and time scale of the moulting process. We know that although geckos are not known to groom their feet yet retain their stickiness and clearness for all the months between two next moulting processes: gecko setae become cleaner with repeated use, thus self-cleaning [24]. However, it was also showed that the gecko's adhesive ability of clinging to inclined surfaces decreased constantly over a period of one month after the molting process [12,24]. Thus the mechanism of the moulting process remains partially unclear.

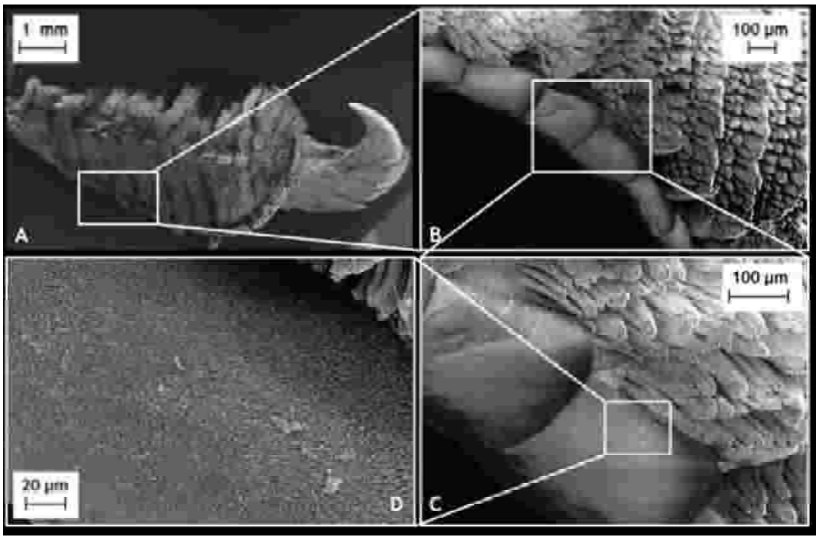

Fig. 3. Gecko adhesion system showed by FESEM (ZEISS SUPRA 40). (A) Tokay gecko toe. (B, C) The edge of gecko toe is covered by nanostructured hairy units; (D) at high magnification.

The aim of this paper is firstly to describe the entire moulting process of a female tokay gecko in terms of time and animal actions through a 16h-video recorded. Secondly, four pieces of the gecko old skin were taken from four different parts of the gecko body, FESEM-analyzed and characterized via a topological statistical analysis. A unexpected complexity is observed. The eye skin seems to be fully anti-adhesive.

2. MATERIALS AND METHODS

2.1. Moulting process

The entire moulting process, about 10 hours long, of a 50 g female tokay gecko (here called G1) was experimentally video recorded. The animal was left in its terrarium (a Poly(methyl methacrylate), i.e. PMMA, box of sizes 32x32x38 cm^3), provided with several air inlets and with the bottom covered with a natural reptile bedding (Repti Bark).

The considered gecko had been maintained in captivity prior to the analyzed moult and was in perfect healthy condition before, during and after the observation. Gecko was maintained in its terrarium at ~28 °C. The temperature of the room, in which the entire moulting process was observed, was ~22 °C. Gecko fed moths and water *ad libitum* and crickets two times per week. The gecko feeding was maintained always the same. The animal did not show any kind of discomfort, any symptoms of suffering or distress and, in addition, any lasting physical damages for the absolutely natural conditions of our observations.

2.2. Scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) new observations

We accurately observed the complete gecko adhesive system (Fig. 1), focusing the SEM and FESEM eye on particular new unexplored zones, i.e. the connection area between next lamellae (Fig. 2) and the edge of the gecko toe (Fig. 3). In addition, we performed detailed FESEM analyses of the gecko skin topology, naturally shed during the moulting process. We observed different gecko body parts: back and upper tail of G1 (Figs. 4a, 4c and 4b, 4d, respectively before and after specimen removal), abdomen and eyes of a 64 g male adult tokay gecko (here denoted by G2) (Figs. 4e, 4g and 4f, 4h, respectively before and after specimen removal).

Considering Figs. 1, 2, and 3, the investigations of the hierarchical structure of the gecko toe were possible by means of SEM (ZEISS EVO 50) equipped with a lanthanum hexaboride cathode and FESEM (ZEISS SUPRA 40) equipped with a field emission tungsten cathode. About SEM analysis, three frozen and formaldehyde fixed samples of toes retrieved from two geckos died naturally were unfrozen at room temperature, 5h-dehydrated with ethanol increasing its percentage at every next hour (10%, 30%, 50%, 70%, 100%). Thus, samples were fixed to aluminium stubs by double-sided adhesive carbon conductive tape (Agar Scientific), 30-min air-dried and gold-coated (approx. 40 nm) in a SCD 050 sputter coater (BalTec). About FESEM analy-

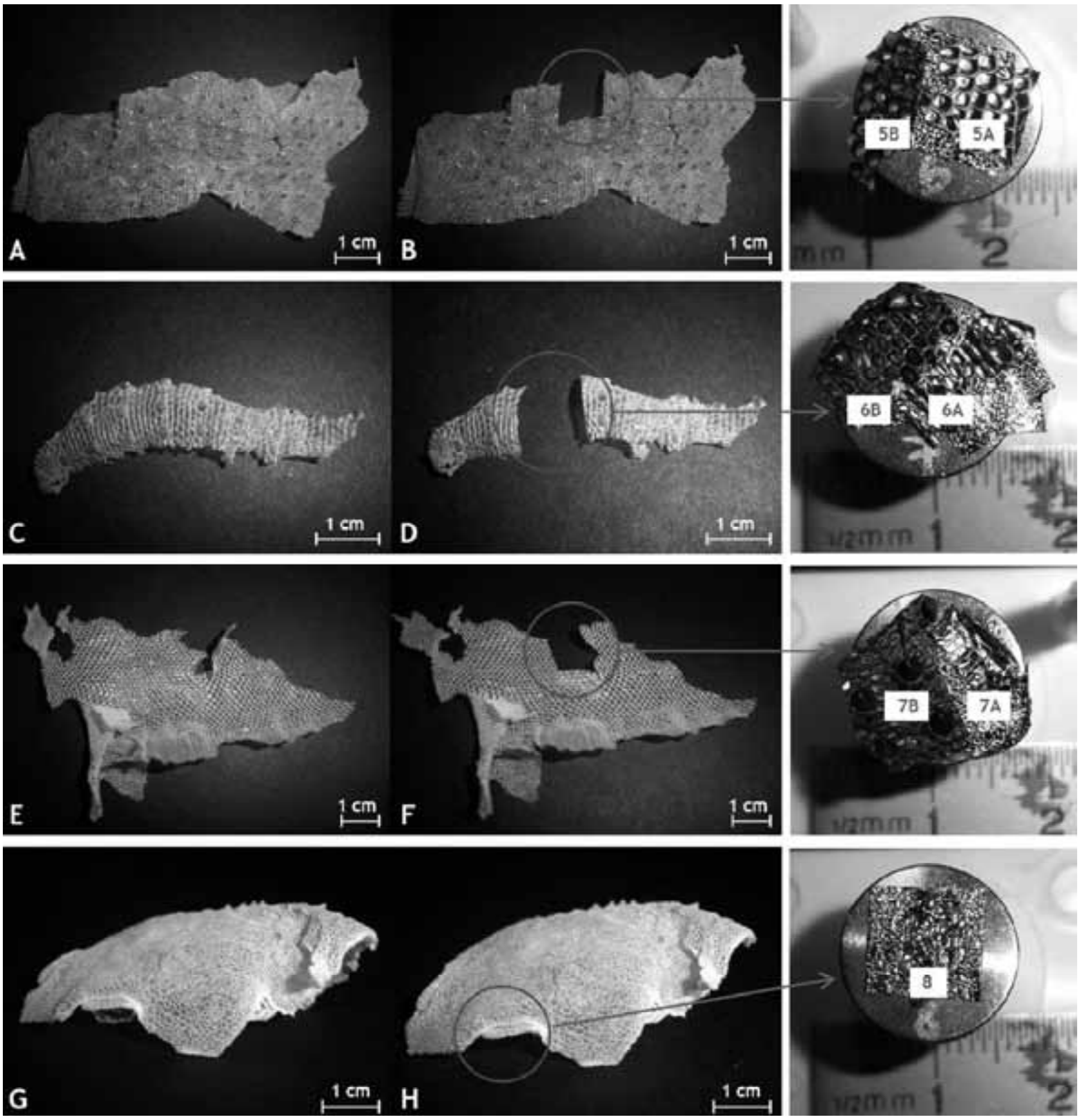

Fig. 4. Details of the gecko skin: back and upper tail of G1 (A, C and B, D respectively before and after specimen removal), abdomen and eyes of G2 (E, G and F, H respectively before and after specimen removal, photographed with Kodak V1003): the red circle individuates the specimen area. On the right, FESEM-samples (photographed with Kodak V1003): the red letters mark the corresponding samples analyzed in the next Figs. (5-8).

sis, the procedure of sample preparation is the same but the samples are chrome-coated (approx. 20 nm).

Referring to Figs. 5, 6, 7, and 8, the investigations of the three dimensional structures of the skin surface were carried out only with FESEM (ZEISS SUPRA 40). To avoid alteration of the thin superficial structures, no fixation procedure was applied to the gecko skin. Samples of about ~0.8 mm^2 were cut (see Fig. 4) and fixed to aluminium stubs by double-sided adhesive carbon tape (Nisshin EM Co. Ltd.), 6h air-dried and chrome-coated (approx. 25 nm).

2.3. Statistical topological analysis of the skin

The gecko skin was statistically analyzed using the software ImageJ 1.41o. The number of the evident roughly circular structures, their area, perimeter and circularity were quantified considering statistically representative areas of 5x5 mm^2. The unit scales of area and perimeter are mm^2 and mm, respectively. The values of the circularity vary in the range between 0 (straight line) to 1 (perfect circle).

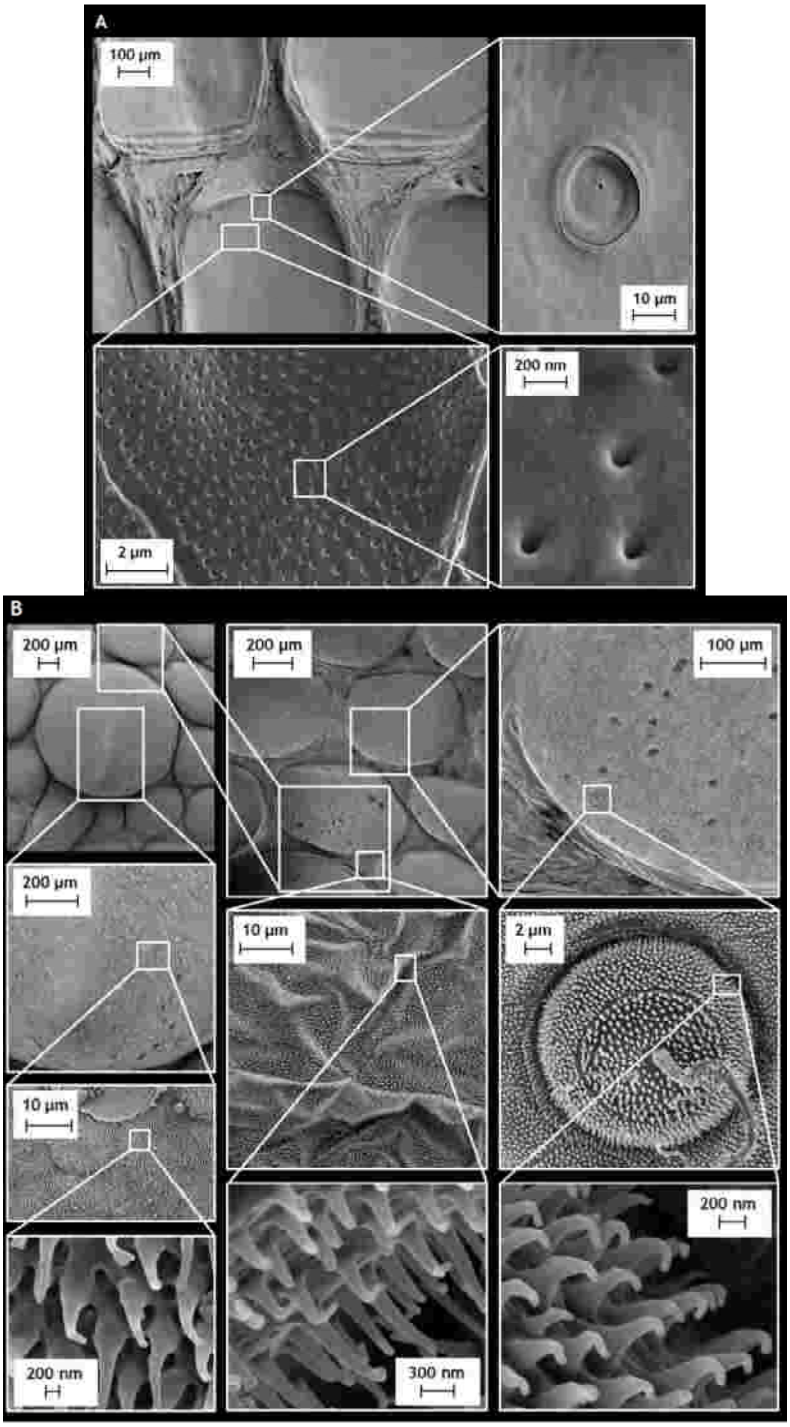

Fig. 5. Details of the skin of the tokay gecko G1, back (see red circle in Fig. 4B) showed by FESEM (ZEISS SUPRA 40): from down (A), from up (B).

3. RESULTS

3.1. Moulting process

The entire moulting process was recorded for 16 consecutive hours by DCR SR55E SONY digital video camera. Gecko started its effective moulting process at 12:30 a.m. and finished it at 10:30 p.m. (after 10 hours; we continued to record its movements until 4:30 a.m. of the day after). Significant events were then extracted using Nero Vision software (Fig. 9).

The camera was located out of the terrarium and the gecko was left alone, with the exception of the short (few minutes) operator presence when the camera orientation was changed from the lateral prospective to the frontal one (after 1h and 45 minutes

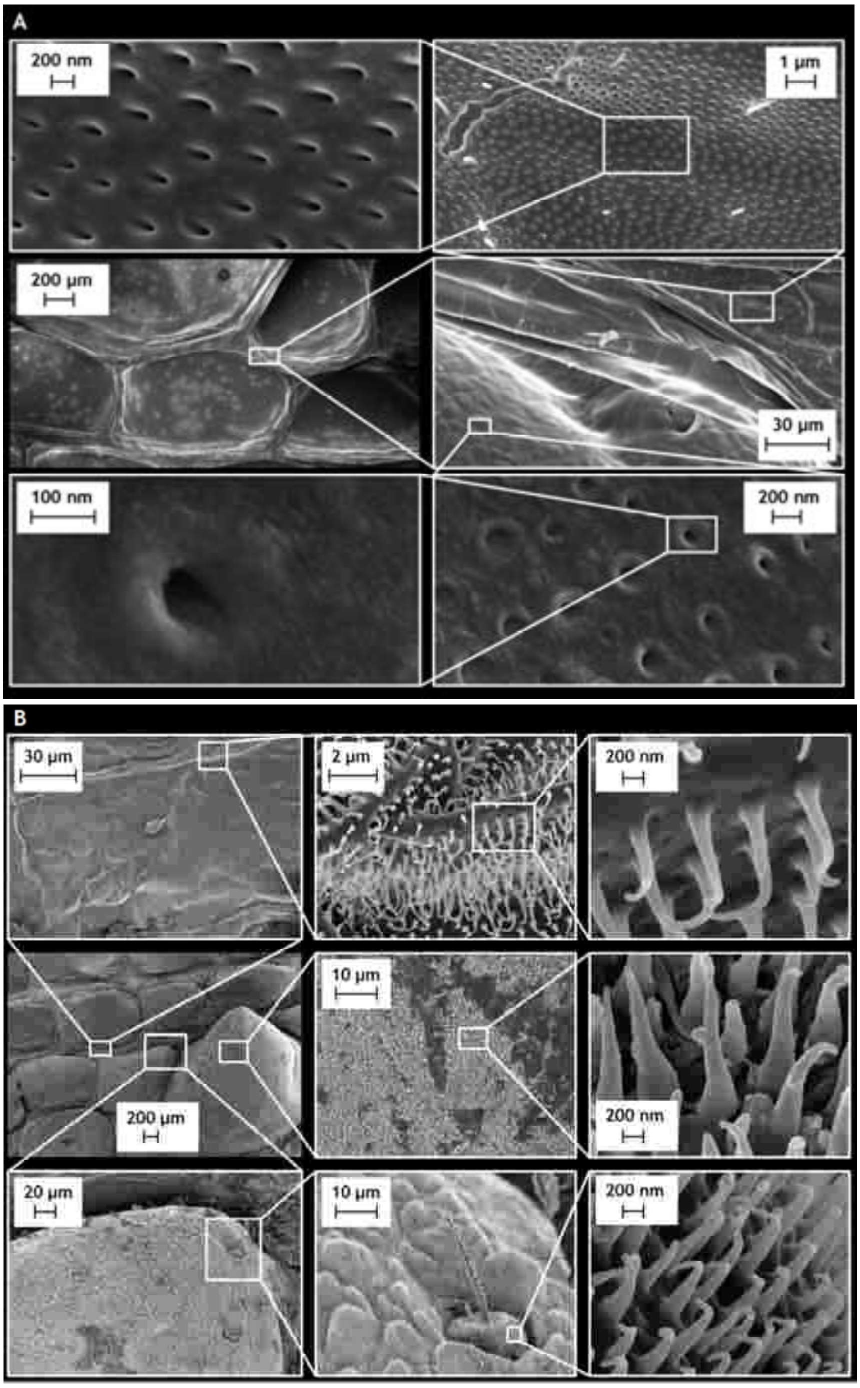

Fig. 6. Details of the skin of tokay gecko G1, upper tail (see red circle in Fig. 4D) showed by FESEM (ZEISS SUPRA 40): from down (A), from up (B).

from the beginning, see Fig. 9, snapshots 17, 18) and when the samples of the back and upper tail skin were collected (see Fig. 9, snapshots 35).

3.2. FESEM observations

SEM micrographs of the gecko skin (Figs. 5, 6, and 7) show that the skin of the back, upper tail and abdomen of the animal body is covered with nanostructured hairy units similar to those identified on the connection area between next lamellae (Fig. 2) and on the edge of each single toe (Fig. 3). From the micrographs, we can distinguish hairy units of ~0.5-1 μm in length and ~50 nm in diameter for the back skin, ~0.5-1.5 μm in length and ~30-50 nm in diameter for the upper tail skin and ~0.2-1 μm

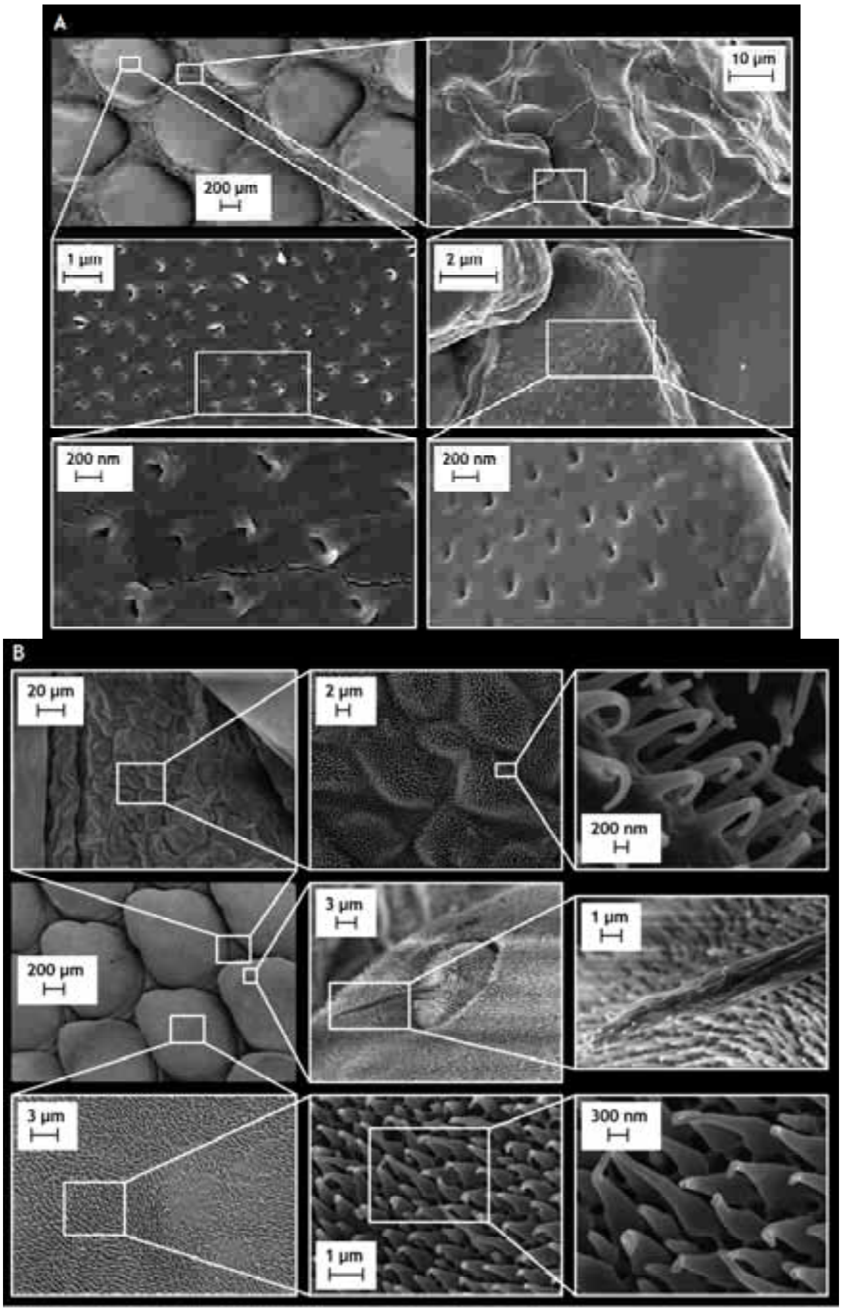

Fig. 7. Details of the skin of tokay gecko G2, abdomen (see red circle in Fig. 4F) showed by FESEM (ZEISS SUPRA 40): from down (A), from up (B).

in length and ~50 nm in diameter for the abdomen skin. Thus, comparing these dimensions with those of the hairy units discovered in the toe (~2-5 μm in length and ~200 nm in diameter), we conclude that the two hairy units are similar, but scaled by a factor of about 3. The inability of the metallization to adhere on the eye skin seems to preliminary suggest an anti-adhesive and thus self-cleaning surface, perhaps developed for maximizing the visual ability in critical conditions (e.g. as anti-fogging mechanism, as observed in other animals).

3.3. Statistical analysis

Tables 1, 2, 3, and 4 summarize the average parameters of area, perimeter, circularity of the skin structures. The standard deviation (SD), the maximum and minimum values are also reported. Note that samples A3 and B3 in Fig. 10 were double-

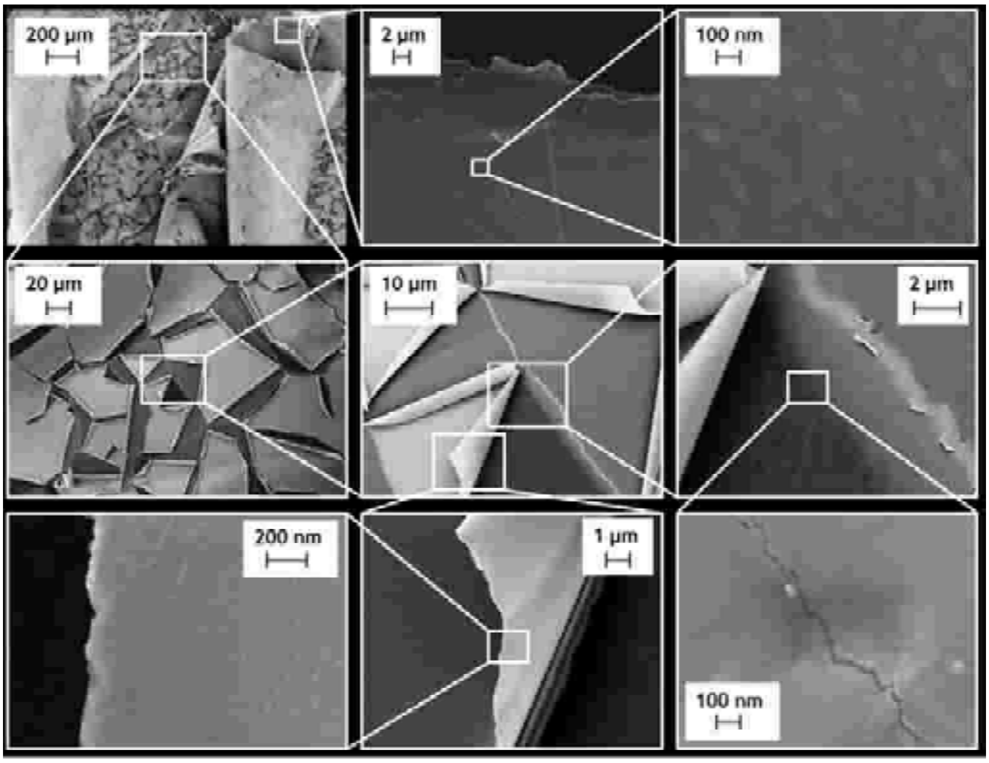

Fig. 8. Details of the skin of tokay gecko G2, eye (see red circle in Fig. 4H) showed by FESEM (ZEISS SUPRA 40). The inability of the metallization to adhere on the eye skin seems to preliminary suggest an anti-adhesive and thus self-cleaning surface, perhaps developed for maximizing the visual ability in critical conditions (e.g. as anti-fogging mechanism, as observed in other animals).

analyzed: in Table 1 B and 2 B, we isolated the calculation of the above mentioned parameters for specific 3D conical structures (coloured in light yellow in Fig. 10), found in the number of 2 within the 5x5 mm analyzed areas. The summary of the calculated parameters is shown in Fig. 11.

4. ADHESION AND ANTI-ADHESION

From one hand, it was demonstrated that the gecko's adhesive ability increased after the moult by a factor of about 10 (in terms of adhesion times) [22,25]. Noting that the van der Waals forces F_{vdW}, mainly responsible for the gecko adhesion, can be described as $F_{vdW} = k/s^3$, where k is a constant and s represents the contact separation, we here roughly estimate the role of the moult on the gecko adhesion. Even if the contact separation has to be of the order of the nanometer in order to match the observed setal forces, we simply assume here a value for s of the order of the thickness t of the new skin for the configuration just after the moult and of the order of $2t$ (new plus old skin) before the moult. As a consequence, the ratio of the adhesive forces just after and before the moulting process is predicted to be $F_{vdW}(s = t)/F_{vdW}(s = 2t) = 8$. Note that such a prediction is independent from the actual thickness t of the skin and suggests that the contact separation s is reduced by a factor of 2-3 after the moult.

On the other hand, we want here to stress again the inability of the metallization to adhere on the skin of the eye (Fig. 8), which seems to preliminary suggest an anti-adhesive and thus self-cleaning surface, perhaps developed for maximizing the visual ability in critical conditions.

5. CONCLUSIONS

We have reported the experimental description of the entire moulting process of a female tokay gecko and of the related observation on its skin topology. The gecko skin shows unexpected complexity, e.g. it is covered by nanohooks (0.2-1.5 µm in length and ~30-50 nm in diameter) superimposed to the evident nearly circular tessellations (for them the statistical analysis suggests values of circularity of ~0.9, mean area of ~0.5 mm^2 and mean perimeter of ~3 mm). The connection area between next lamellae and the edge of the toe are both covered with nano-hairs (~2-5 µm in length and ~200 nm in di-

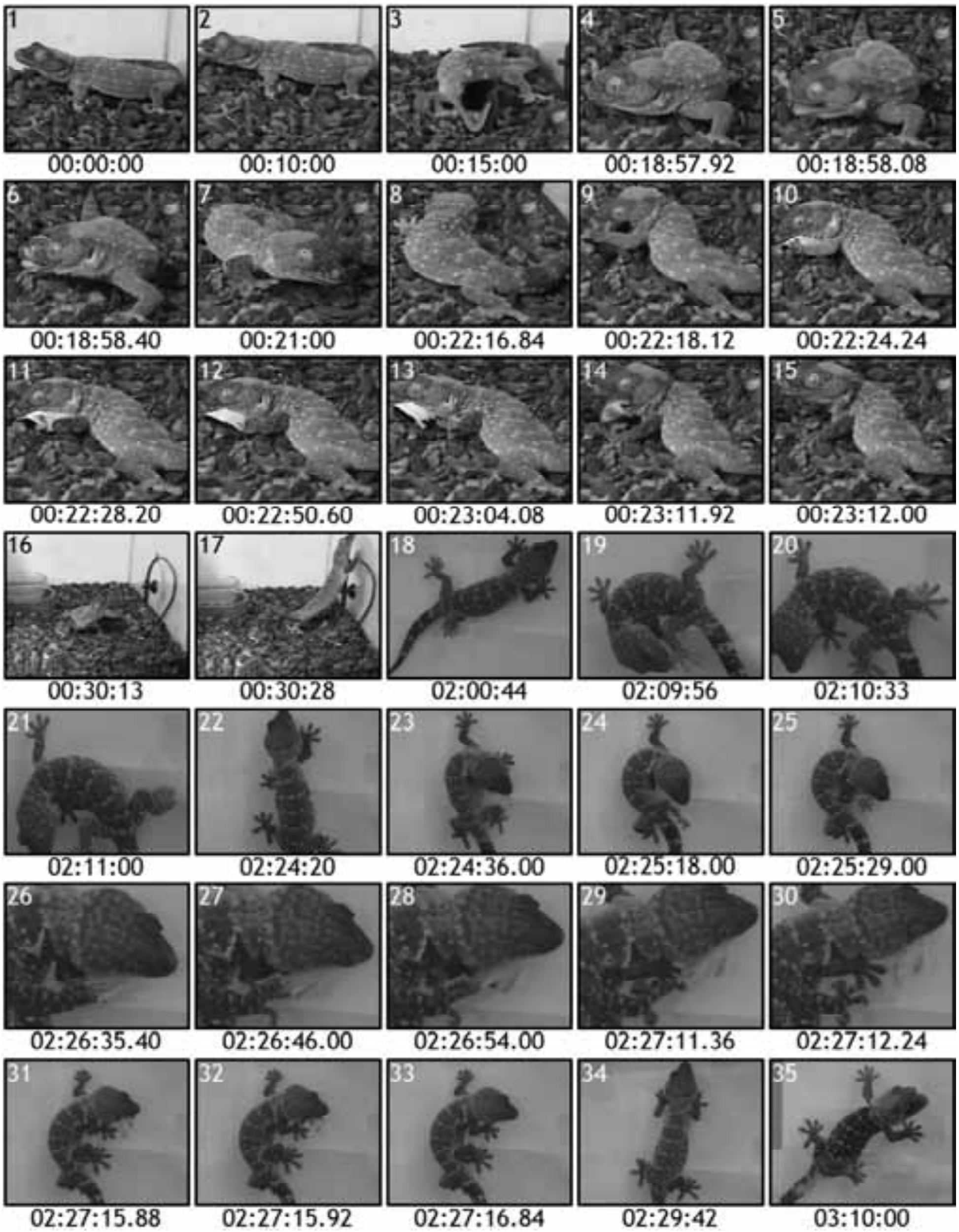

Fig. 9. Snapshots (ss) of the entire moulting process of the gecko G1. Staying on the bottom of its terrarium, the gecko begun its moulting process using its tongue to try to remove the older generation skin from its head and eyes (ss 1÷4): it opened wide its mouth in order to facilitate the detachment of the old skin from the new one (ss 3). After almost 20 min, gecko started to remove its old skin from the fore right foot (ss 5÷7): it put the toes in its mouth in order to easily remove its old skin. The old skin generation of its fore right foot was entirely eaten and the result is shown in ss 7 (note the clean fore right foot). After only 1 min, gecko proceeded with its fore left foot following the same technique: this phase is accurately recorded and ss 8÷15 show it step by step (note the clean fore left foot in ss 15). Afterwards, gecko left the bottom and came up to the vertical surface of the terrarium (ss 16, 17). The animal stayed on the vertical surface almost 45 min motionless and made only few steps on the bottom and again on the vertical surface during the following 45 min, reaching the position shown in ss 18. Thus, gecko tried to clean its hind left foot but its adhesion on surface was not sufficient to guarantee the gecko vertical stability: instead gecko slowly slipped down (ss 19÷21) and decided to clean its hind left foot positioning again on the bottom of the terrarium. Gecko spent less than 15 min for this operation and then returned on the vertical surface. The result is shown in ss 22 (note the clean hind left foot). After only few seconds, the procedure of removing the old skin from the last hind right foot was started: gecko begun with the skin of its right side (ss 23-24) and continued taking off the old skin from its hind right foot, as an adherent suit (ss 25÷33). (Fig. 9 continuance) The technique of taking off the old skin from the toes of the hind right foot

was highlighted in the snapshots 26÷30. As usual, at the end the gecko had eaten the old generation of its skin (ss 31÷33). The result is shown in ss 34 (note the clean hind right foot). After 3 hours, samples of the skin of gecko back and upper tail were taken and the result is shown in ss 35. No more snapshots were reported in Fig.10 due to the inappropriate location of the digital video camera focus with respect the new animal position.

After making clean all its feet, gecko stayed almost motionless for 70 min on the vertical surface. At 4 h and 20 min from the beginning, it came down to the bottom of the box and made its tail completely clean in 3 min using only its mouth. After 110 min almost motionless (so at 6 h and 30 min), gecko begun the procedure of making its head completely clean rubbing its head against the small pieces of bark the bottom is covered of. This procedure was stopped for about 145 min and then restarted (so 9 h from the beginning) following the same technique and with the final help of its hind foot in order to scratch out the last piece of old skin from its head (this happened after 10 h from the beginning). After making all its body clean, removing the skin of the old generation, the animal maintained its last position motionless on the bottom and nothing happens for the following 6 hours.

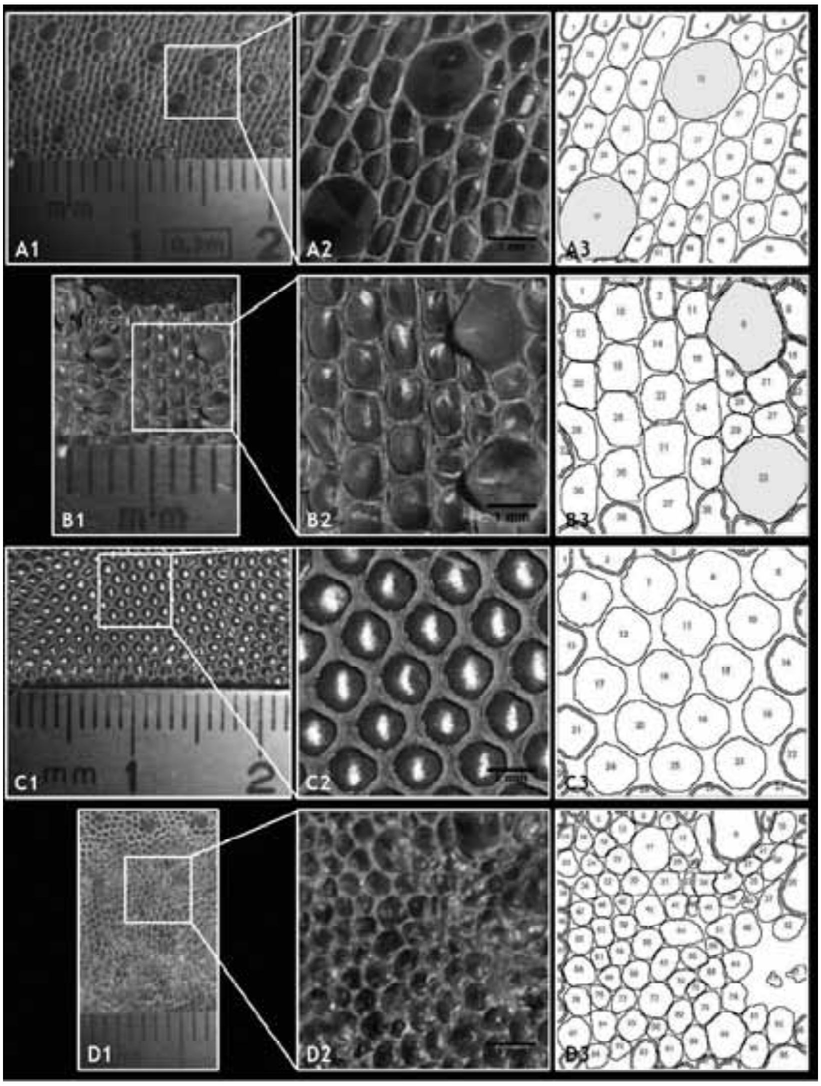

Fig. 10. Statistical topological analysis of the gecko skin (photographed with Kodak V1003 and acquired with the software ImageJ 1.41o): back (A1) and upper tail (B1) of G1, abdomen (C1) and head (D1) of G2. Photographs A1, B1, C1 and D1 refer to samples of Fig. 4 (A, C, E and G respectively). A2, B2, C2 and D2 insets show the grayscale image of the analyzed 5x5 mm areas. A3, B3, C3 and D3 insets present the final result of the image acquisition. Note that A2 and B2 insets show 3D conical structures, marked in light yellow in A3 and B3 insets and accordingly analyzed separately (Tables 1B and 2B).

Table 1. Statistical topological analysis of the back skin (G1). These data refer to Fig. 9, series A: the parameters of the white almost-circular structures are summarized in Table 1A; the parameters of the light yellow almost-conical structures are summarized in Table 1B.

	Table 1A.		
n° = 29	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	0.313	2.226	0.782
SD	0.07308	0.26154	0.04472
Max Value	0.435	2.636	0.856
Min Value	0.136	1.557	0.696
	Table 1B.		
n° = 2	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	1.840	5.164	0.867
SD	0.07707	0.12304	0.00495
Max Value	1.894	5.251	0.870
Min Value	1.785	5.077	0.863

Table 2. Statistical topological analysis of the upper tail skin (G1). These data refer to Fig. 9, series B: the parameters of the white almost-circular structures are summarized in Table 2A; the parameters of the light yellow almost-conical structures are summarized in Table 2B.

	Table 1A.		
n° = 29	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	0.472	2.698	0.788
SD	0.15956	0.50616	0.04584
Max Value	0.686	3.281	0.826
Min Value	0.100	1.239	0.643
	Table 1B.		
n° = 2	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	1.832	5.259	0.833
SD	0.11455	0.01202	0.04879
Max Value	1.913	5.267	0.867
Min Value	1.751	5.250	0.798

Table 3. Statistical topological analysis of the abdomen skin (G2). These data refer to Fig. 10, series C.

n° = 16	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	0.874	3.553	0.870
SD	0.04765	0.12104	0.01684
Max Value	0.947	3.722	0.903
Min Value	0.799	3.367	0.839

Table 4. Statistical topological analysis of the upper head skin (G2). These data refer to Fig. 10, series D.

n° = 75	Area (mm^2)	Perimeter (mm)	Circolarity
Average value	0.156	1.513	0.794
SD	0.08683	0.46079	0.10723
Max Value	0.474	3.038	0.902
Min Value	0.003	0.260	0.348

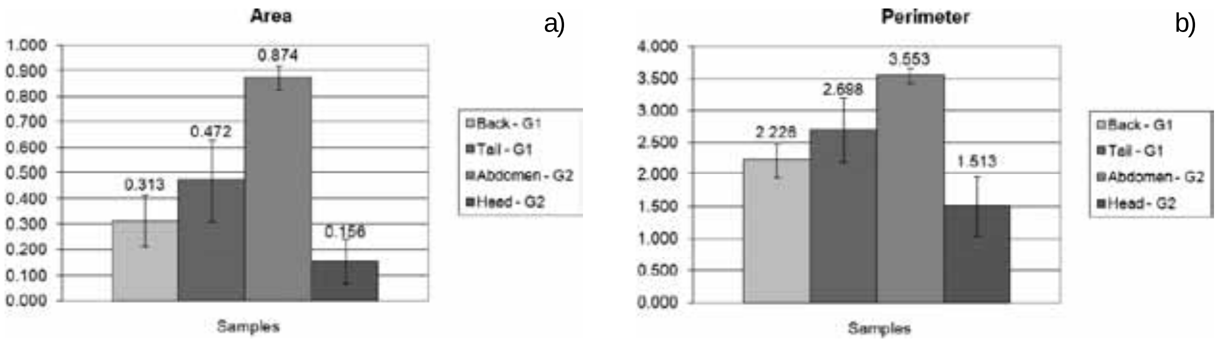

Fig. 11. Summary of the statistical analysis for both area and perimeter skin structures (the conical structures are not considered here).

ameter). The moult, a well-documented complex process about 10 hours long, increases the adhesion ability by a factor of ~10. The inability of the metallization to adhere on the eye skin seems to preliminary suggest an anti-adhesive and thus self-cleaning surface, perhaps developed for maximizing the visual ability in critical conditions (e.g. as anti-fogging mechanism, as observed in other animals).

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