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Near-infrared spectroscopy and microstructure of the scales of *Sabethes* (*Sabethes*) *albiprivus* (Diptera: Culicidae)

Betina Westphal-Ferreira, Mario Antônio Navarro-Silva*

Universidade Federal do Paraná, Departamento de Zoologia, Laboratofio de Entomologia Médica e Veterinária, Curitiba, PR, Brazil

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ABSTRACT

Near-infrared spectroscopy and microstructure of the scales of *Sabethes* (*Sabethes*) *albiprivus* (Diptera: Culicidae). *Sabethes* (*Sabethes*) *albiprivus* Theobald individuals vary considerably in size and color of the reflections of the scales on their thorax, abdomen, antepronotal lobes and occiput. The goal of this study was to investigate and to characterize the differences in the color of the scales among preserved specimens and to analyze the differences in the microstructures of the scales that cover their bodies using near-infrared spectroscopy, and to evaluate whether the latter is efficient in distinguishing the populations. A total of 201 adult females were analyzed for the characterization of color patterns. In addition, absorbance spectra and scanning electron microscope images were obtained from them. As a result of color analysis, two variations were identified, one represented by specimens with yellow or green scales and the other with blue or purple scales. The same two variations were corroborated using NIRS. Analysis of the microstructure of the scales lining the mesonotum, occiput and antepronotal lobes resulted in the same variations. The three methodologies, near-infrared spectroscopy, scanning electron microscopy and coloration of the reflections of the scales revealed two variations within *Sa. albiprivus*.

Introduction

Sabethes (*Sabethes*) *albiprivus* Theobald, 1903 is restricted to South America. According to the original description of the adult, the coloration of the abdominal scales of this species is interrupted by irregular lines, and tufts of long scales resembling a paddle are present on the tibiae and tarsi (Lane and Cerqueira, 1942).

The yellow fever virus (YFV) was first isolated from *Sa. albiprivus* in 2012, in a population from Misiones, Argentina. It is possible that this population serves as a reservoir for VFA, although studies are needed to determine its vector competence (Goenaga et al., 2012).

Morphological analysis of *Sa. albiprivus* specimens from the type locality (São Paulo – Brazil) performed by Costa-Lima (1931), indicate that this species varies in body size (4–5 mm) and in the coloration of the scales that line the thorax and abdomen. However, such patterns of variation, as well as differences in the shape of scales and their utility in differentiating species groups have not yet been investigated in detail.

The color patterns of the scales that line the body of certain Culicidae are often used to distinguish among species or higher taxa (Wilkerson and Peyton, 1990). In *Sabethes*, characters such as the presence of white spots on the tarsi and coloration of the antepronotal lobes of the mesonotum (varying between yellow, green, blue and purple) are usually used to distinguish species, for instance *Sa. albiprivus*, *Sa.* (*Sabethes*) *cyaneus* (Fabricius, 1805), *Sa.* (*Sab.*) *quasicyaneus* Peryassu, 1922, *Sa.* (*Sab.*) *tarsopus* Dyar & Knab, 1908 and *Sa.* (*Sab.*) *bipartipes* Dyar & Knab, 1906 (Lane and Cerqueira, 1942; Harbach, 1991).

Although scale color is an important character to distinguish different species and subgenera of Culicidae the coloration of these cuticular structures and their association with the chemical composition of the exoskeleton has received little attention from researchers.

Near-infrared spectroscopy (NIRS) has the potential to detect differences in the chemical composition of the exoskeleton through the stretching and bending of functional groups CH, NH and OH of organic molecules (Pasquini, 2003). NIRS measures the amount of near-infrared energy absorbed at specific wavelengths by biological materials (Mayagaya et al., 2009). Early studies using NIRS in culicids were conducted by Micks and Benedic in 1953.

The application of NIRS is fast, non-invasive and does not cause damage to the specimens (Pasquini, 2003). Due to these desirable characteristics, NIRS has expanded its use to various fields of knowledge.

In Culicidae, NIRS has been used to identify species in the *Anopheles gambiae* Giles, 1902 species complex and to determine

* Corresponding author.
E-mail: mnavarro@ufpr.br (M.A. Navarro-Silva).

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Table 1
Specimens of *Sabethes albiprivus* analyzed for scale reflections, institution of origin, and collection data.

Locality	No. of specimens	Institute	Latitude	Longitude
Assaí – PR	1	DZUP	23°38′19″ S	50°84′26″ O
Barracão – PR	2	DZUP	26°23′73″ S	53°63′80″ O
Campina da Lagoa – PR	1	DZUP	24°58′53″ S	52°82′52″ O
Campo Mourão – PR	1	DZUP	24°07′23.5″ S	52°18′07.6″ O
Carlópolis – PR	2	DZUP	23°15′24.1″ S	50°57′55.9″ O
Cianorte – PR	1	DZUP	23°84′81″ S	52°71′65″ O
Chopinzinho – PR	3	DZUP	25°53′16.0″ S	52°20′23.8″ O
Cornélio Procópio – PR	1	DZUP	23°14′03″ S	50°69′21″ O
Diamante D'Oeste – PR	1	DZUP	24°92′95″ S	54°13′27″ O
Foz do Iguaçu – PR	34	DZUP	24°44′11.0″ S	54°55′49.3″ O
Guaíra – PR	1	DZUP	24°08′69″ S	54°28′10″ O
Inajá – PR	62	DZUP	22°39′25.8″ S	52°15′15.8″ O
Jaguariaíva – PR	1	DZUP	24°22′43.6″ S	49°39′38.0″ O
Lapa – PR	1	DZUP	25°76′83″ S	49°80′23″ O
Maringá – PR	57	DZUP	23°43′00.6″ S	52°33′11.8″ O
Mariópolis – PR	19	DZUP	26°16′08″ S	52°33′53″ O
Palmas – PR	6	DZUP	26°27′58″ S	51°58′58″ O
Paranaguá – PR	1	DZUP	25°54′44.9″ S	48°56′31.2″ O
Ponta Grossa – PR	1	DZUP	25°09′27″ S	50°17′45″ O
São Matheus do Sul – PR	2	DZUP	25°51′20″ S	50°19′08″ O
Sertaneja – PR	2	DZUP	22°54′12.0″ S	50°48′24.5″ O
Três Barras do Paraná – PR	6	DZUP	25°26′22″ S	53°09′34″ O
Tomazina – PR	1	DZUP	23°77′40″ S	49°93′97″ O
União da Vitória – PR	1	DZUP	26°24′53″ S	51°10′56″ O
Manaus – AM	3	INPA	22°97′04″ S	43°22′39″ O
Presidente Figueiredo – AM	2	INPA	02°01′02″ S	59°04′36″ O
Rio de Janeiro – RJ	2	INPA	22°97′04″ S	43°22′39″ O
Anápolis – GO	2	INPA	16°31′78″ S	48°97′56″ O
Itaituba – PA	2	INPA	01°27′43″ S	49°04′29″ O

the amount of blood ingested by adults of *Aedes aegypti* (Linnaeus 1762) and *Aedes sierrensis* (Ludlow 1905), without the need to destroy the specimens (Hall et al., 1990; Mayagaya et al., 2009).

Considering the lack of detailed descriptions of the color variations of the scales of *Sa. albiprivus*, the aim of this study was to analyze these variations, together with the patterns of microstructures found in these epidermal structures, and to evaluate the utility of the NIRS for differentiating the chemical composition of the exoskeleton of specimens from populations collected from different habitats and seasons.

Material and methods

Source of specimens and morphological study of scales

We examined a total of 201 adult females, obtained from institutions including the *Coleção Entomológica Padre Jesus Santiago Moure-UFPR* (DZUP) and the *Instituto Nacional de Pesquisas da Amazônia* (INPA) (Table 1). The specimens were analyzed under white light source, using a Zeiss Stereo Discovery V 2.0 microscope with an Axiocam ERc5s camera, to evaluate scale reflections and how they vary among specimens. Species identification was performed using dichotomic keys available in Costa-Lima (1931), Lane and Cerqueira (1942) and Forattini (2002). The mosquito anatomical terminology follows Harbach and Knight (1980, 1981).

Near-infrared spectroscopy (NIRS)

Specimens of *Sa. albiprivus* and *Sa. cyaneus* were analyzed by near-infrared spectroscopy (NIRS). They were collected from 18 locations in four different types of forests, during the four seasons and from two strata, canopy and ground (Table 2).

In our study we attempted to evaluate differences in the pattern among specimens collected from different types of forest: Ombrophilous Dense Forest (Atlantic Rain Forest), Ombrophilous Mixed Forest (Forest with Araucaria), Semi-deciduous Seasonal Forest (Seasonal Forest) and Deciduous Seasonal Forest, where they are exposed to different conditions such as temperature, humidity, larval habitats and blood sources.

The spectra were obtained from a 700-FTIR spectrophotometer housed at the Infrared Laboratory, Departamento de Química, Universidade Federal do Paraná. We used a fiber optic probe with a 2 mm viewpoint. The resulting spectrum is related to the near-infrared energy absorbance of the material. On average, 16 spectra were obtained from each specimen mesonotum on a 90° incidence angle of the fiber optic, positioned over a mirror about 2 mm below the probe.

The data were first processed using the Savitzky–Golay 1st derivative (Savitzky and Golay, 1964). Noise regions were detected and eliminated by viewing the spectra prior to statistical analysis. All analyses were performed in Unscrambler® 9.5 software (Camo, ProcessAS, Norway). The same strategy was used for all groups analyzed: multivariate models were constructed using principal component analysis (PCA).

Scanning electron microscopy (SEM)

The microstructure of the scales located on the mesonotum, occiput and antepronotal lobes of dry-preserved *Sa. albiprivus* adult females were observed using SEM (Table 3).

Preparation for viewing included gluing the specimens on double-sized carbon tape, depositing them on aluminum stubs, coating them with gold and analyzing them in a JEOL JSM-6360LV scanning electron microscope located at the *Centro de Microscopia Eletrônica/Setor de Ciências Biológicas/Universidade Federal do Paraná*.

The distance between the longitudinal ridges of 20 scales (4 measurements per scale) with different reflecting patterns was measured. The measurements were obtained at 10,000× magnification from the central point of scales, which is located halfway between the pedicel and the apex of the scale (Fig. 1).

Table 2
Localities, forest types, dates and strata where specimens subjected to NIRS were collected.

Locality	No. of specimens	Season	Canopy/ground	Forest type
Barracão – PR	1	13/05/2005 Autumn	Canopy	SSF
Barracão – PR	1	26/11/2004 Spring	Canopy	SSF
Campo Mourão – PR	1	03/10/2005 Spring	Canopy	OMF
Carlópolis – PR	1	23/02/2006 Summer	Ground	OMF
Carlópolis – PR	1	03/08/2005 Winter	Ground	OMF
Chopinzinho – PR	3	24/08/2004 Winter	Canopy	SSF
Foz do Iguaçu – PR	15	01/06/2004 Autumn	Ground	SSF
Foz do Iguaçu – PR	2	16/03/2004 Summer	Canopy	SSF
Foz do Iguaçu – PR	13	15/03/2004 Summer	Canopy/ Ground	SSF
Foz do Iguaçu – PR	1	12/07/2004 Winter	Canopy	SSF
Foz do Iguaçu – PR	2	12/04/2004 Autumn	Canopy	SSF
Inajá – PR	1	07/07/2004 Winter	Canopy	SSF
Inajá – PR	2	27/04/2004 Autumn	Canopy/ Ground	SSF
Inajá – PR	7	28/09/2004 Spring	Canopy/ Ground	SSF
Inajá – PR	1	01/03/2005 Summer	Canopy/ Ground	SSF
Inajá – PR	22	07/07/2004 Winter	Canopy/ Ground	SSF
Inajá – PR	6	28/09/2004 Winter	Canopy/ Ground	SSF
Inajá – PR	11	07/07/2004 Winter	Canopy/ Ground	SSF
Inajá – PR	11	01/03/2005 Summer	Canopy/ Ground	SSF
Inajá – PR	2	27/04/2004 Autumn	Ground	SSF
Jaguariaíva – PR	1	10/03/2005 Summer	Canopy	OMF
Maringá – PR	24	08/02/2012 Summer	Ground	SSF
Maringá – PR	30	23/02/2005 Summer	Ground	SSF
Mariópolis – PR	18	25/11/2004 Spring	Ground	OMF
Paranaguá – PR	1	20/09/2004 Winter	Canopy	ODF
São Matheus do Sul – PR	2	09/03/2005 Summer	Ground	OMF
Sertaneja – PR	1	15/10/2004 Spring	Ground	OMF
Sertaneja – PR	1	26/07/2005 Winter	Ground	OMF
Três Barras do Paraná – PR	6	23/11/2004 Spring	Canopy/ Ground	OMF
Manaus – AM	6	01/08/2012 Winter	Ground	ODF
Anápolis – GO	2	01/01/1938 Summer	Ground	DSF
Itaituba – PA	1	01/03/1938 Summer	Ground	ODF
Rio de Janeiro – RJ	3	01/06/1938 Autumn	Ground	ODF
Witmarsum – SC	1	30/12/2012 Summer	Ground	OMF

ODF, Ombrophilous Dense Forest; OMF, Ombrophilous Mixed Forest; SSF, Semideciduous Seasonal Forest; DSF, Deciduous Seasonal Forest.

Table 3
Specimens of *Sabethes albiprivus* deposited in the *Coleção Entomológica Pe. Jesus Santiago Moure*, used for SEM and collection data.

Specie	Locality	Latitude	Longitude
Sabethes albiprivus	Barracão – PR	26°23′73″ S	53°63′80″ O
	Carlópolis – PR	23°15′24.1″ S	50°57′55.9″ O
	Foz do Iguaçu – PR	24°44′11.0″ S	54°55′49.3″ O
	Inajá – PR	22°39′25.8″ S	52°15′15.8″ O
	Maringá – PR	23°43′00.6″ S	52°33′11.8″ O
	Manaus – AM	22°97′04″ S	43°22′39″ O
	Três Barras do Paraná – PR	25°26′22″ S	53°09′34″ O

Results

Variation in the reflection of scales

According to the redescription of *Sa. albiprivus* proposed by Lane and Cerqueira (1942), the mesonotum is covered with purple-blue metallic scales that become purple-golden on the prescutellum. The abdominal scales are blue, with purple reflections on the terga, gold reflections on the sterna, and golden lateral scale patches in the lateral margin of the terga. The vertex and antepronota are covered with metallic-blue scales with violet reflections, becoming silvery laterally.

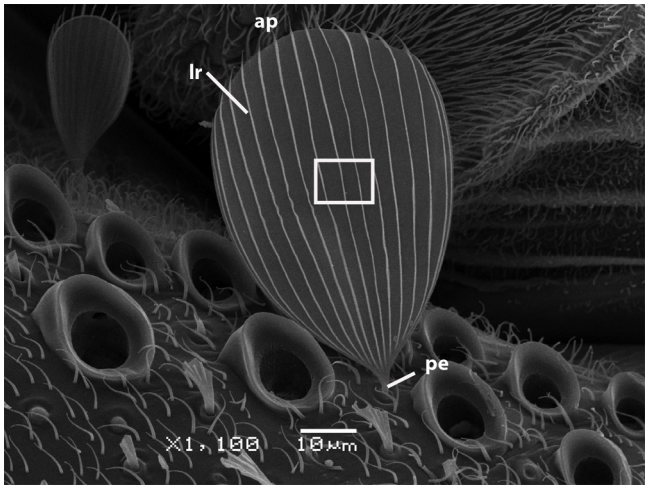

Fig. 1. Scanning electron micrograph of a scale on the antepronotum of *Sabethes albiprivus*. The rectangle indicates where the images were obtained at 10,000× magnification. Legends: ap, apex; lr, longitudinal ridge; pe, pedicel.

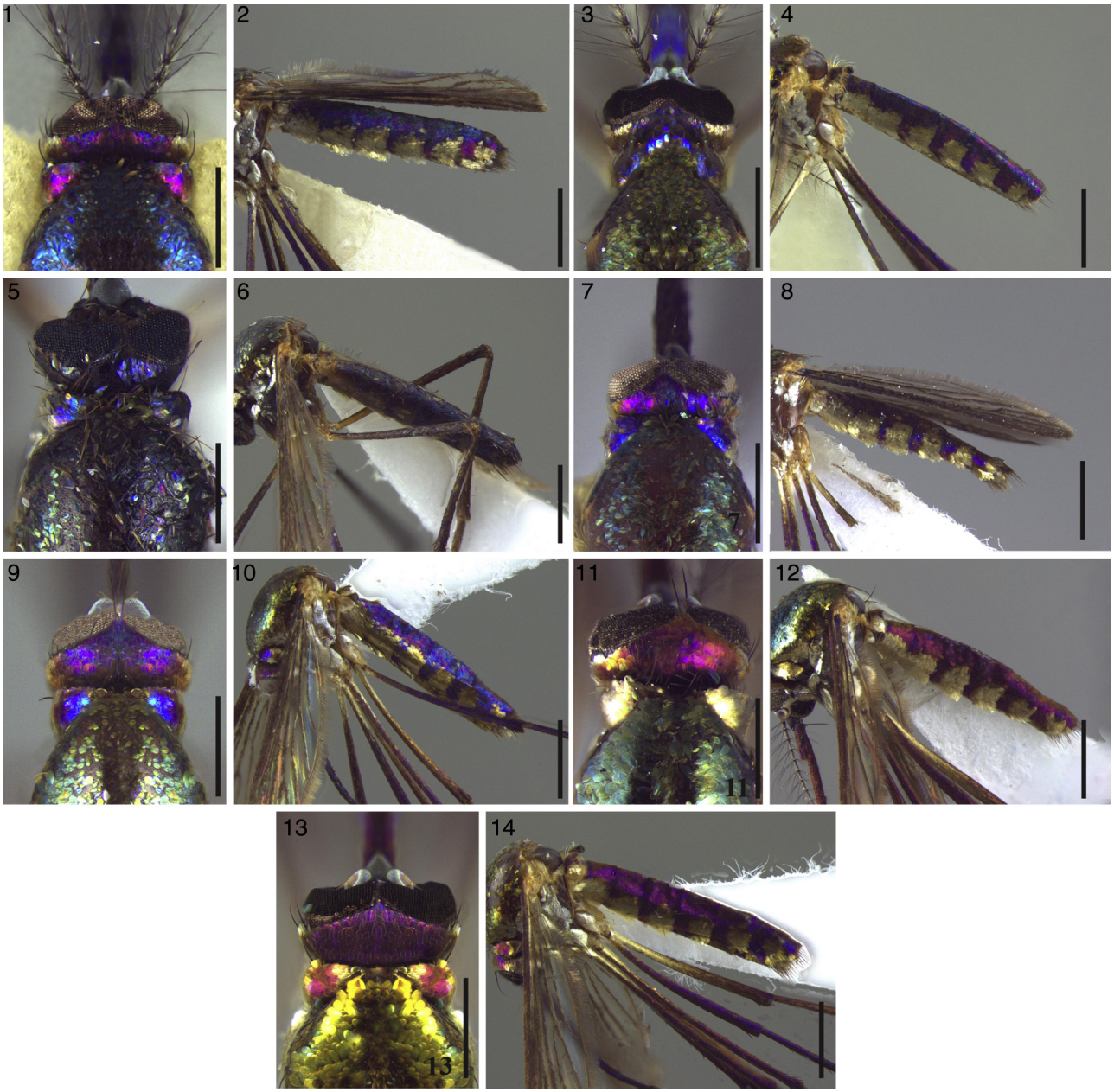

Fig. 2. 1–10: Specimens with scales that reflect blue, purple and green; 11–14: Specimens with scales that reflect golden and silver. The scale bars of the dorsal view images (1, 3, 5, 7, 9, 11, 13) represents 1 mm and of the side views of the abdomen (2, 6, 8, 10, 12, 14) indicate 0.5 mm.

Sabethes albiprivus vary in the color of the scales covering the vertex, antepronota and mesonotum (scutum). The reflections of the scales found on the mesonotum are blue, green, gold and purple (Fig. 2: 3, 9 and 11). In addition, reflections of two different colors sometimes occur on the mesonotum (Fig. 2: 1 and 13).

Two colors of reflections are often present on the vertex and antepronota: silver or gold laterally, and purple or bluish dorsally, respectively. However, the scales on the antepronota of some specimens (Fig. 2: 11) have only gold reflections, whereas in other specimens they have purple and golden reflections (Fig. 2: 13).

Abdominal reflections are only purple and blue, and reduced reflection is observed in this region in some specimens (Fig. 2: 6). These specimens also have the most contrasting scales in the specimens analyzed, being opaque on the abdomen and vertex, and with little purple or silvery reflection on the mesonotum and antepronota (Fig. 2: 5 and 6).

Two varieties of *Sa. albiprivus* were identified according to the color of the scales lining the vertex, mesonotum and antepronota. The first includes specimens with purple, blue and green scales. These features were observed in the lectotype, which was analyzed through photographs sent by the Natural History Museum, London. The second is characterized by scales that reflect silver and gold.

Near-infrared spectroscopy (NIRS) analysis

The relevant spectral range for analysis, after eliminating the noise, was 1000–2500 nm (4000–10,000 cm^{-1}). When evaluating the chemical composition patterns in the spectra from specimens of *Sa. albiprivus* mesonotum, two non-overlapping groups are discriminated, with 93% (89% + 4%) of the total information of the spectra (Fig. 3).

The assembly represented by number 1 (Fig. 3) includes specimens with blue, green and purple mesonotum, antepronota and

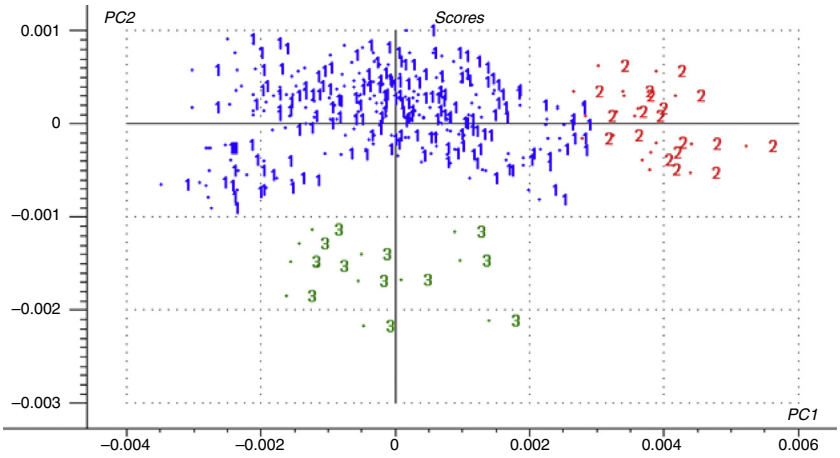

Fig. 3. Principal component analysis based on near-infrared Spectroscopy of *Sabethes albiprivus* (1 and 2) and *Sa. cyaneus* (3) mesonotum.

vertex scales reflections (Fig. 2: 1, 3, 5, 7 and 9). The second group is composed of specimens with silver or golden-reflecting scales covering the mesonotum, antepronota and vertex (Fig. 2: 11 and 13).

When the closely related species *Sa. cyaneus* is included in the analysis, the identity of each species is easily determined (*Sa. albiprivus:* groups 1 and 2; *Sa. cyaneus:* group 3). This corroborates the two groups of *Sa. albiprivus*, indicating variation in the chemical composition of the exoskeleton, with the two patterns detected by NIRS (Fig. 3).

Specimens of *Sa. albiprivus* analyzed in this study vary in the color of reflections of their scaling and some mosquito species can vary in color depending on the season they were collected. The specimens analyzed here were collected all year long, and the group 1 specimens (with blue, green and purple scales covering the body) comprised of 170 specimens have the following temporal distribution: spring (12.94%), summer (47.65%), autumn (12.35%) and winter (27.06%). The group 2 specimens, with gold or silver scales (19 individuals) were collected during spring (78.95%), summer (15.79%) and winter (5.25%).

Specimens with different color patterns were collected in the same locality and date of collection (Fig. 4). In the cities of Anápolis – GO, Foz do Iguaçu – PR, Inajá – PR, Manaus – AM, Maringá – PR and Mariópolis – PR, we found specimens that belong to groups 1 and 2. Therefore, groups 1 and 2 were not exclusively composed of specimens from the same locality. The group 1 specimens were also collected in Barracas – PR, Campo Mourão – PR, Carlópolis – PR, Chopinzinho – PR, Inajá – PR, Jaguariaíva – PR, Itaituba – PA, São

Fig. 4. Localities were the *Sabethes albiprivus* specimens were collected.

Matheus do Sul – PR, Rio de Janeiro – RJ, Sertaneja – PR, Três Barras do Paraná – PR and Witmarsum – SC. The group 2 specimens also occur in Paranaguá – PR.

The specimens belonging to group 1 were collected in greatest abundance in the Semi-deciduous Seasonal Forest (82.94%), followed by Ombrophilous Mixed Forest (10.00%), Ombrophilous Dense Forest (5.88%) and Deciduous Seasonal Forest (1.18%). The specimens of group 2 were mostly collected in the Ombrophilous Mixed Forest (63.16%), Semi-deciduous Seasonal Forest (26.32%), Deciduous Seasonal Forest (5.26%) and Ombrophilous Dense Forest (5.26%).

Both groups were collected at ground level and in the canopy: group 1 – canopy (51.43%) and ground (48.57%); group 2 canopy (47.37%) and ground (52.63%).

Scanning electron microscopy of the scales

The coloration of the metallic reflections of the scales of *Sa. albiprivus* are not derived from pigments. Instead, they are structural in origin (Ghiradella, 1998). The scales of *Sa. albiprivus* are spatulate (Fig. 5: 1, 2, 5 and 6) and may feature silver, gold, bluish, greenish and purple reflections.

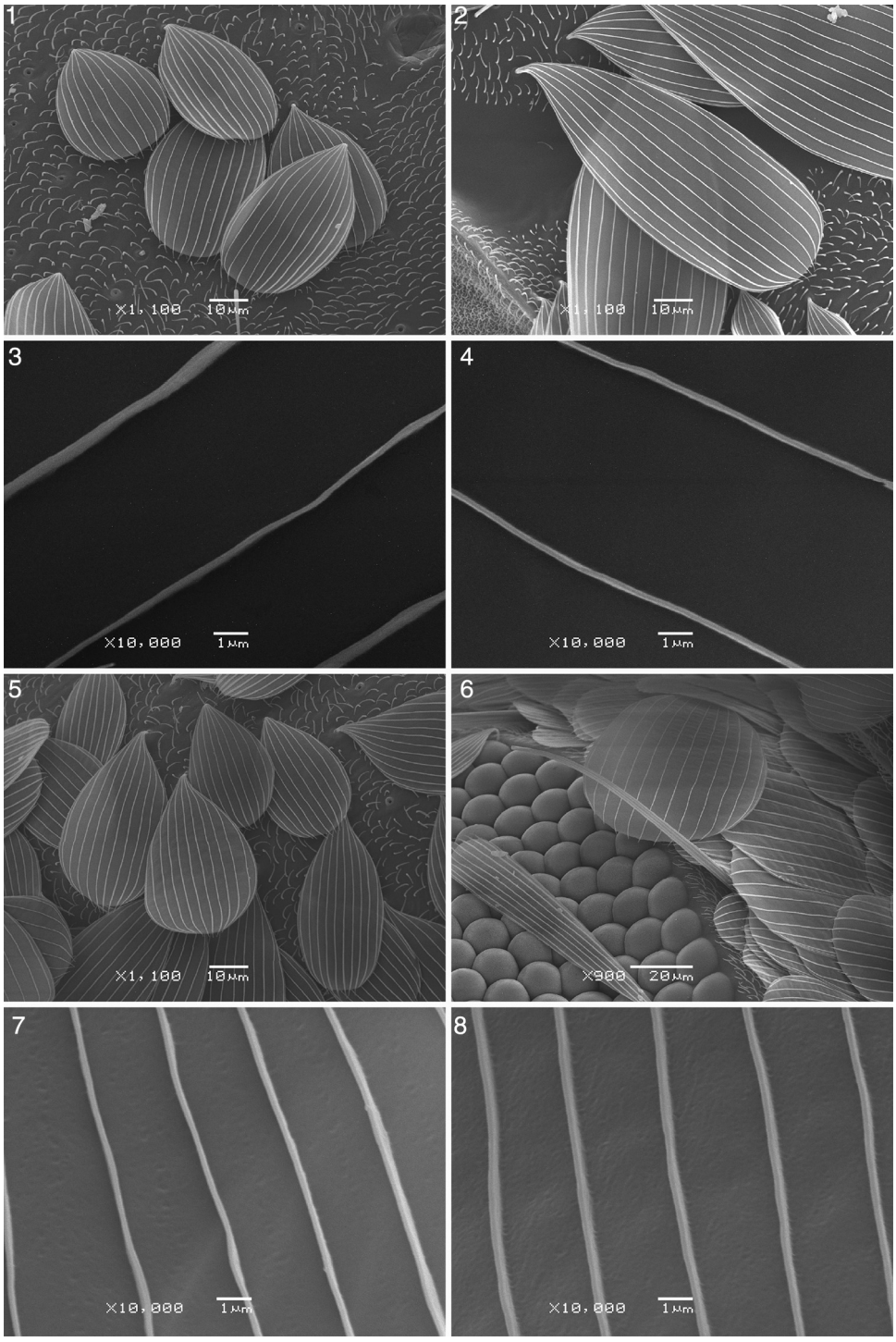

Fig. 5. Mesonotum scales of *Sabethes albiprivus*. 1, 2, 3 and 4: specimens of Group 1; 6, 7 and 8: specimens of Group 2.

The surface of the blue, green or purple scales is smooth between the longitudinal ridges (Fig. 5: 1–4). The distance between the ridges is 4.1–5.4 μm (mean 4.7 ± 0.43 μm). The specimens discriminated by NIRS in Group 1 have the mesonotum, antepronota and vertex coated in this type of scale.

The scales of the specimens that have gold or silver reflections have the longitudinal veins close together (Fig. 5: 5 and 6), only 2.1 to 3.2 μm (average 2.7 μm ± 0.36 μm) apart, and the surface between them is slightly rough (see Fig. 5: 7 and 8). These scales are found in NIRS Group 2.

Discussion

Specimens of *Sabethes* (*Sabethes*) can be identified based on the color variations of the antepronota and mesonotum, presence or absence of a row of tarsal white scales, and coloration of the mesepimeral setae. In addition, a complete or incomplete lateral line of pale scales on each abdominal tergum separates the terga from the sterna, the former, appearing as a complete straight lateral line, is present in *Sa. cyaneus*, whereas – line interrupted on each tergum – is present in *Sa. albiprivus* (Lane & Cerqueira 1953).

Because the species of this subgenus are distinguished by the color patterns of their scales, *Sa. albiprivus* can be separated into at least two variations. The specimens belonging to one group have scales that reflect purple, green and blue. Specimens in the second group have scales with gold and silver reflections.

The same two variations were also determined for *Sa. albiprivus* by the characterization of the variations in the chemical composition of the scales (NIRS) and the nanostructures present on them (SEM).

In specimens with scales ranging from green to blue and purple, the region between the longitudinal ridges is smooth. This contrasts with the situation found in specimens that reflect gold and silver, in which the space between the ridges is more irregular, thus diffusing the light that is reflected. The variation observed in the reflections is a function of the wavelengths that are reflected.

The influence of seasonal variation on physical aspects is well documented for various insect groups. In Culicidae, color differences are also be observed in *Culex apicalis* Adams, 1903 and *Culex nigripalpus* Theobald, 1901. Specimens collected during the winter in the United States are darker than the ones collected during the summer (Michner, 1945). The specimens analyzed in the present study were collected all year long, so the color variation observed cannot be attributed to a certain season.

Additionally, adaptations to temperature and other climatic factors linked to different latitudinal gradients are well documented for *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1720), *Aedes albopictus* (Skuse, 1894) and *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldon, 1941 (Rueda et al., 1990; Hribar, 1994; Alto and Juliano, 2001; Armbruster and Conn, 2006). The two variations observed in *Sa. albiprivus* were not exclusive for one locality. Specimens with different color patterns were collected in the same locality, date of collection and type of forest.

Different stratification has been found in Culicidae, particularly in forests. Species such as *Ae. aegypti*, *Ae. africanus* (Theobald, 1901) and *Culex* (*Melanoconion*) *sacchetae* Sirivakan & Jacob, 1982 are usually collected at the ground level, contrasting with *Sa.* (*Sabethoides*) *chloropterus* (von Humboldt, 1819) and *Sa. cyaneus*, which occur in higher vertical stratification levels (Amerasinghe and Alagoda, 1984; Gomes et al., 1987). The *Sa. albiprivus* specimens analyzed were collected at ground and canopy levels. Each group marked by color variation in this study was composed of specimens collected in more than one forest type. This shows that the observed color variation is not related to environmental variation and locality.

Sabethes albiprivus has an interspecific variation considering that the structural coloration is generated from the physical interaction between light and the microstructures located on the surface of the scales (Kynoshita et al., 2008). Differences in coloration found in specimens are derived from the presence or absence of microstructures on the surface of the scales and the variations occurs in sympatry.

Pedro et al. (2008) had mentioned the existence of two species that are commonly confused under the same name of *Sa. albiprivus*. Molecular evidences suggested that the museum voucher specimens of *Sa. albiprivus* differ from those analyzed in the study. It seems to be possible that the two color varieties presented here, based on scale morphology (SEM), color variation and NIRS, might represent these two species.

Given the variation in color pattern observed in *Sa. albiprivus*, it is important to check how much this variation could be associated with other morphological differences. Analyses should be undertaken, especially on immature stages and the male genitalia of adults, to search for other characters that facilitate recognition of the interspecific variation.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Alto, B.W., Juliano, S.A., 2001. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): implications for range expansion. J. Med. Entomol. 38 (5), 646–656.

Armbruster, P., Conn, J.E., 2006. Geographic variation of larval growth in North American *Aedes albipictus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 99 (2), 1234–1243.

Amerasinghe, F.P., Alagoda, T.S.B., 1984. Mosquito oviposition in bamboo traps, with special reference to *Aedes albopictus*, *Aedes novalbopictus* and *Armigeres subalbatus*. Insect Sci. Appl. 5 (6), 493–500.

Costa-Lima, A., 1931. Sobre as espécies do gênero Sabethes e Sabethoides. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, xxv+20 pp.

Forattini, O.P., 2002. Culicidologia Médica Identificação, Biologia, Epidemiologia. Editora da Universidade de São Paulo-Edusp, São Paulo, i+860 pp.

Goenaga, S., Fabri, C., Dueñas, J.C.R., Gardenal, C.N., Rossi, G.C., Calderon, G., Morales, M.A., Garcia, J.B., Enria, D.A., Levis, S., 2012. Isolation of yellow fever virus from mosquitoes in Missiones province, Argentina. Vector Borne Zoonotic Dis. 12 (11), 986–993.

Ghiradella, H., 1998. Microscopic Anatomy of Invertebrates. Wiley-Liss, New York, i+493 pp.

Gomes, A.C., Forattini, O.P., Natal, D., 1987. Composição e atividade de mosquitos Culicidae. Emprego de armadilha CDC no Vale do Ribeira, Estado de São Paulo, Brasil. Revista de Saúde Publica 21 (5), 363–370.

Hall, M.H., Dutro, S.M., Klowed, M.J., 1990. Determination by near-infrared reflectance spectroscopy of mosquito (Diptera: Culicidae) bloodmeal size. J. Med. Entomol. 27, 76–79.

Harbach, R.E., Knight, K.L., 1980. Taxonomist's Glossary of Mosquito Anatomy. Plexus Publishing, Incorporated, Marlton, NJ, USA, 414 pp.

Harbach, R.E., Knight, K.L., 1981. Corrections and additions to *Taxonomist's Glossary of Mosquito Anatomy*. Mosq. Syst. 13 (2), 201–217.

Harbach, R.E., 1991. A new subgenus of the genus *Sabethes* (Diptera: Culicidae). Mosq. Syst. 23, 1–9.

Hribar, L., 1994. Geographic variation of male genitalia of *Anopheles nuneztovari* (Diptera: Culicidae). Mosq. Syst. 26 (3), 132–144.

Kynoshita, S., Yoshioka, S., Miyazaki, J., 2008. Physics of structural colors. Reports on Progress in Physics, i+31 pp.

Lane, J., Cerqueira, N.L., 1942. Os sabetíneos da América (Diptera, Culicidae). Arquivos de Zoologia do Estado de São Paulo, São Paulo, 849 pp.

Mayagaya, V.S., Michel, K., Benedict, M.Q., Killeen, G.F., Wirtz, R.A., Ferguson, H.M., Dowell, F.E., 2009. Non-destructive determination of age and species of *Anophe-*

les gambiae s. l. using near-infrared spectroscopy. Am. Soc. Trop. Med. 81 (4), 622–630.
Michner, C.D., 1945. Seasonal variations in certain species of Mosquitoes (Diptera: Culicidae). J. N. Y. Entomol. Soc. 53 (4), 293–300.
Micks, D.W., Benedict, A.A., 1953. Infrared spectrophotometry as a means for identification of mosquitoes. Proc. Soc. Exp. Biol. Med. 84, 12–14.
Pasquini, C., 2003. Near infrared spectroscopy: fundamentals practical aspects and analytical applications. J. Braz. Chem. Soc. 14, 138–219.
Pedro, P.M., Sallum, M.A.M., Butlin, R.K., 2008. Forest-obligate Sabethes mosquitoes suggest palaeocological perturbations. Heredity 101, 186–195.
Rueda, L.M., Patel, K.J., Axtell, R.C., Stinner, R.E., 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Entomol. Soc. Am. 27 (5), 892–898.
Savitzky, A., Golay, M.J.E., 1964. Smoothing and differentiation of data by simplified least squares procedures. Anal. Chem. 36, 1627–1639.
Theobald, F.V., 1903. A Monograph of the Culicidae of the World. British Museum of Natural History, Londres, 391 pp.
Wilkerson, R.C., Peyton, E.L., 1990. Standardized nomenclature for the Costal wing spots of genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27 (2), 207–224.