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Glaucalges tytonis sp. n. (Analgoidea, Xolalgidae) from the barn owl *Tyto alba* (Strigiformes, Tytonidae): compiling morphology with DNA barcode data for taxon descriptions in mites (Acari)

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Abstract

Typical (re)descriptions of feather mite species are based on characteristics of external morphology and of internal sclerotized structures visible in cleared specimens. We propose extending this standard by including sequence data of the cytochrome oxidase subunit I gene fragment (DNA barcode region chosen by the Consortium for the Barcode of Life). We describe a method of nondestructive DNA isolation, which leaves the feather mite exoskeleton intact for subsequent morphological analysis. Description of a new feather mite species *Glaucalges tytonis* (Analgoidea, Xolalgidae) from the plumage of the barn owl *Tyto alba* (Scopoli, 1769) (Strigiformes, Tytonidae) is presented as an example of the new procedure that may be implemented both for feather mites as well as for other groups of Acari.

Key words: cryptic species, DNA barcoding, COI, molecular taxonomy, morphological description, new species

Introduction

Feather mites (Astigmata, Analgoidea + Pterolichoidea) are a vast group of parasitic and commensalistic mites associated with birds, mainly with the plumage. Their entire life cycle takes place on the host body and dispersal is almost exclusively via transfer between conspecific bird individuals (Dabert & Mironov 1999, Proctor 2003). This should result in a predominant pattern of single-host (monoxenous) host-parasite associations that evolved as a consequence of close cospeciation between feather mites and birds (Dabert 2005). However, a relatively large number of host-mite associations are classified as multi-host (oligo- or polyxenous) where morphologically undistinguishable mites inhabit a wide range of variously related host species.

An example of multi-host relationships is the xolalgid species *Glaucalges attenuatus* (Buchholz, 1869). The genus *Glaucalges* (Analgoidea, Xolalgidae) was established by Gaud (1980) for a single species *Dermaleichus attenuatus*. This species was primarily described and illustrated by Buchholz (1869) from long-eared owl *Asio otus* (L., 1758) (Strigiformes, Strigidae) and subsequently reported from a wide range of owl taxa (Tab. 1). Gaud and Atyeo (1981) have included in the genus *Glaucalges* a second species, *Glaucalges pteropus* (*Protalges pteropus* Gaud et Mouchet, 1959) described from great blue turaco *Corythaeola cristata* (Vieillot) (Musophagiformes: Musophagidae). Our present stage of knowledge of *Glaucalges* systematics cannot answer if the genus has an unusually wide host range or if the host record for *G. pteropus* is a case of accidental contamination.

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Extensive morphological investigations supported by biometric analysis may sometimes reveal phenotypic discontinuity between populations from various hosts that may be interpreted as cryptic species (Badek & Dabert 2005, 2006). However, the main limitation in applying morphometrical methods in practice is that they are extremely time consuming and require a large number of individuals for study. A new and very effective tool for analyzing the systematic status of multihost species in feather mites appeared with the development of DNA sequencing techniques and applying specific molecular markers (Dabert *et al.* 2005). Recently the DNA barcoding system based on a nucleotide sequence of a cytochrome oxidase subunit I (COI) gene fragment was proposed as a standard procedure for effective species determination in animals (Hebert *et al.* 2003). This marker has proven a high degree of taxonomical resolution in most Metazoa (for review see Waugh 2007).

In the present paper we use DNA barcoding as a complement to morphology-based taxonomy of feather mites. According to this approach we describe a new *Glaucalges* species from the barn owl *Tyto alba* (Scopoli, 1769) (Strigiformes, Tytonidae). This new species is a cryptic species closely related to *G. attenuatus* and until now was considered as conspecific to it. We extend the standard scheme of morphology-based description by DNA barcode data obtained from the type material of the new species.

Materials and methods

Animal material. The material used in the present study was collected from two owl species: *Asio otus*, freshly killed in an accident at Poznan, Poland, and frozen material from Osnabrueck, Germany, of this species and *Tyto alba*. Mites were preserved in 96% ethyl alcohol and, before mounting on microscopic slides, were subjected to DNA extraction. Vouchers, including type material (specimens and corresponding DNA samples) are deposited in the collection of the Faculty of Biology, Adam Mickiewicz University in Poznan, Poland.

Genetic analysis. Total genomic DNA was extracted from 4 to 13 whole specimens by using the DNAeasy Tissue Kit (Qiagen). Mites were transferred from 96% ethyl alcohol to 180 µl of ATL lysis buffer and overnight pre-incubated at 56°C with 450 rpm shaking in a thermomixer (Eppendorf). Next day, 20 µl of Proteinase K (Qiagen) was added to the sample and incubation was continued for 72 h at the same conditions. After digestion the sample was mixed by vortexing for 10 sec and spin down in a microcentrifuge. The lysis buffer containing nucleic acids was transferred to a fresh eppendorf tube and stored for DNA isolation. The exoskeleton was stored in 70% ethyl alcohol at room temperature until used for preparing microscopic slides. DNA isolation was performed using a manufacturer's protocol for purification of total DNA from animal tissues. The only modification of the standard manufacturer's protocol concerned a 10 min incubation step in 70°C after adding AL buffer to the sample. DNA was eluted from the column using 0.1 ml of elution buffer AE.

The DNA barcode region (a 644-bp region near the 5` terminus of the COI gene) was amplified by PCR with degenerated primers: bcdF05 (5`-TTTTCTACHAAYCATAAAGATATTGC-3`) and bcdR04 (5`-TATAAACYTCDGGATGNCCAAAAAA-3`). PCRs were carried out in 25 µl reaction volumes containing reaction buffer (5 mM Tris-HCl pH 8.8, 25 mM KCl, 0.04% Nonidet P40), 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 1 µM each primer, 1.25 U *Taq* polymerase (*Allegro*, Novazym) and 5 µl of DNA template using a thermocycling profile of one cycle of 3 min at 96 °C followed by 35 steps of 10 sec at 95 °C, 30 sec at 50 °C, 1 min at 72 °C, with a final step of 5 min at 72 °C. After amplification, 5 µl of the PCR reaction was analyzed by electrophoresis on a 1.5% agarose gel. Samples containing visible bands were directly sequenced in both directions using 1–3 µl of the PCR reaction and 40 pmoles of primer. Sequencing was performed with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems). Contigs were aligned and manually assembled in ChromasPro v. 1.32 (Technelysium Pty Ltd.) and GeneDoc v. 2.7.000 (Nicholas & Nicholas

1997). Sequences were deposited in GenBank as species barcodes (GenBank Accession nos. EU271955–1958). Pairwise distance calculations between sequences were computed using K2P distance model (Kimura 1980) for all codon positions with MEGA version 3.1 (Kumar *et al.* 2004).

Microscopy. For morphological study mite specimens (exoskeletons) were mounted on slides in Faure medium (Evans 1992) and investigated under the light microscope Olympus BX50 with differential interference contrast (DIC) illumination. Drawings were made using a camera lucida drawing attachment. Photos were prepared by digital camera Olympus E330.

All measurements are given in micrometres. Dimension ranges of male paratypes are given in brackets following holotype data. Length of the gnathosoma was measured from the distal tips of palpi to the basal margin of the subcapitulum. The width of the gnathosoma was measured at the widest section of the subcapitulum. Idiosomal length was measured from the anterior margin of prodorsum to the posterior end of the body (females) or membranous tips of the opisthosomal lobes (males). Idiosomal width was measured at the level of setae *cp*. The nomenclature of chaetotaxy follows that of Gaud and Atyeo (1996). Scientific English and Latin names of birds as well the higher-level classification of birds are those of Sibley and Monroe (1990).

Glaucalges tytonis sp. nov.

Material examined. *Type material:* 1 male holotype, 3 male, 3 female paratypes from *Tyto alba* (Strigiformes, Tytonidae), near Osnabrueck (52.16 N 08.03 E), Germany, 12 November 1992, no other data; DNA barcode GenBank Acc. EU271955 for male holotype and 3 male paratypes, voucher AMUFM636; remaining paratypes vouchers AMUFM637–639.

Additional material: 17 males from same host (another bird individual), same data; DNA barcode GenBank Acc. EU271956 for 13 males, voucher AMUFM795; remaining individuals vouchers AMUFM791–794.

Comparative material: Glaucalges attenuatus, two samples: (1) 9 males from *Asio otus* (type host species), near Osnabrueck, Germany, 26 February 1993, no other data; DNA barcode GenBank Acc. EU271958 for 8 males, voucher AMUFM780, (2) 3 males, 2 females from same host, Poznan (52.25 N 16.52 E), Poland, 11 May 2005, coll. A. Badek; DNA barcode GenBank Acc. EU271957 for all individuals, voucher AMUFM211.

Description. *Male* (Figs 1, 2, 5A–B, 6, 7). Gnathosoma with rectangular subcapitulum and finger-like appendages on lateral margins, length of gnathosoma 50 (48–54), width 48 (46–50), length/width 1.0 (1.0–1.1). Idiosoma relatively stout, strongly narrowing terminally and shaped as inverted triangle, length 368 (320–375), width 243 (174–255), length/width 1.5 (1.5–1.8). Propodosoma and hysterosoma lengths 110 (96–115) and 258 (224–260) respectively, propodosoma/hysterosoma 0.4. Terminal margin of opisthosoma with elongated trapezoidal opisthosomal lobes fused medially. Remnants of interlobar cleft are connected with posterior part of ovate supranal concavity in a keyhole-like structure. Length of the cleft including supranal concavity 80 (62–87), width at the level of setae *h2* bases 11 (9–14). Terminal margins of lobes with tongue-like rounded membranes separated by roundly triangular incision reaching anteriorly level of insertion of setae *h3*. Lateral opisthosoma with narrow membranes extending from bases of legs IV to level anterior to bases of setae *f2*. Pronotal shield triangular in shape anteriorly to level of setae *si*, posteriorly to this level rectangular; length of the shield 105 (97–112), maximal width 90 (82–90). Shield uniformly dotted, occupying medial part of dorsal propodosoma. Scapular shields well developed, shaped as quadrangles. Setae *se* shaped as macrosetae, set on striated tegument between humeral and pronotal shields. Setae *si* minute, set on lateral margins of pronotal shield. Humeral shields large, rounded with medium-sized setae *c2* at anterior margins. Hysteronotal shield well developed and covers most of dorsal surface of hysterosoma; anterior margin concave. Maximal width of the shield 115 (109–122). The shield uniformly dotted with two longitudinal furrows extending from

the level posterior to cupules *im* to bases of setae *e2*. Dorsal setae *c1*, *d1*, *e1*, *h1* absent. Setae *ps1* longer than distance *d2–d2*. Lateral setae *d2*, *e2*, *f2* long; *d2* and *e2* extending beyond the level of tips of terminal membranes (Fig. 4A). Setae *h2* and *h3* shaped as macrosetae longer than idiosoma. All dorsal and lateral setae piliform. Openings of opisthonotal glands *gl* absent. Only cupules *im* visible, set posterior to bases of setae *d2*. Distances between setae: *c2–c2* 171 (136–186), *c2–d2* 62 (51–68), *d2–d2* 107 (100–108), *d2–e2* 90 (89–102), *e2–e2* 78 (71–77), *h3–h3* 37 (35–39), *ps1–ps1* 20 (18–19).

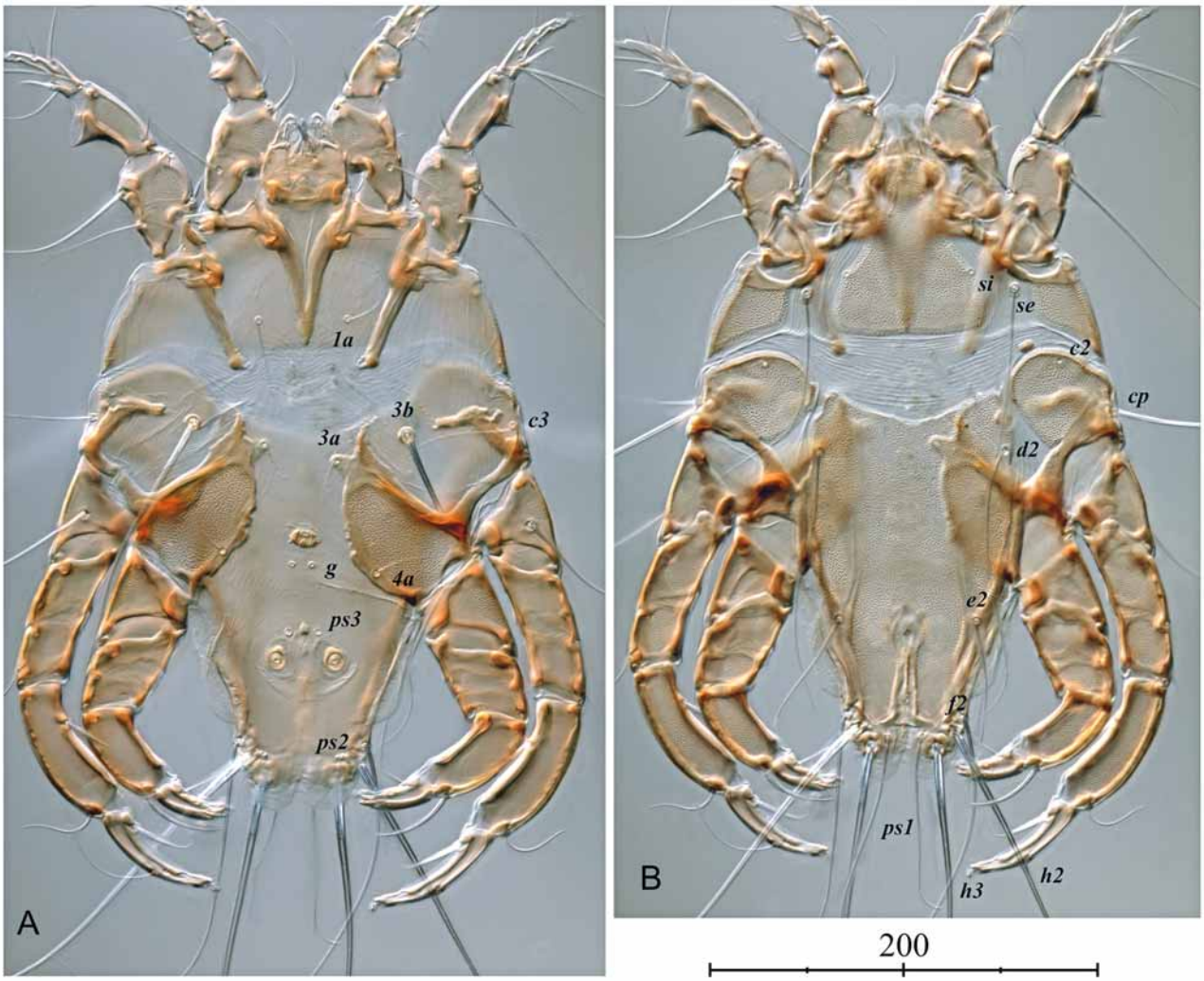

FIGURE 1. *Glaucalges tytonis* **sp. n.**, male. A – ventral view, B – dorsal view. Setal designations after Gaud and Atyeo (1996).

Epimerites I fused as Y with very short sternum (Fig. 5B). Coxal fields of ventral propodosoma and coxal fields III without sclerotized shields near epimerites. Coxal fields IV completely covered by well sclerotized shields with medial expansions encompassing bases of setae *3a*; in more weakly sclerotized individuals these expansions may be partly or completely reduced. Genito-anal region without cuticular shields with exception of minute square sclerite between setae *ps3*. Genital arch flat, length 11 (10–12), width 16 (14–15) with very short aedeagus. Genital acetabula set on epimerites IV laterally to genital organ. Setae *3b* shaped as long macrosetae reaching by tips bases of *f2*. Setae *ps2* at least as long as distance between them. Adanal discs slightly elliptical, diameter 13x12 (12x11–15x12); each disc with small antero-medial sclerite. Corollae of discs radially striated. Distances between setae: *3b–3b* 120 (64–111), *3b–3a* 41 (30–44), *3a–g* 68 (50–66), *g–ps3* 38 (35–42), *ps3–ps2* 70 (68–74).

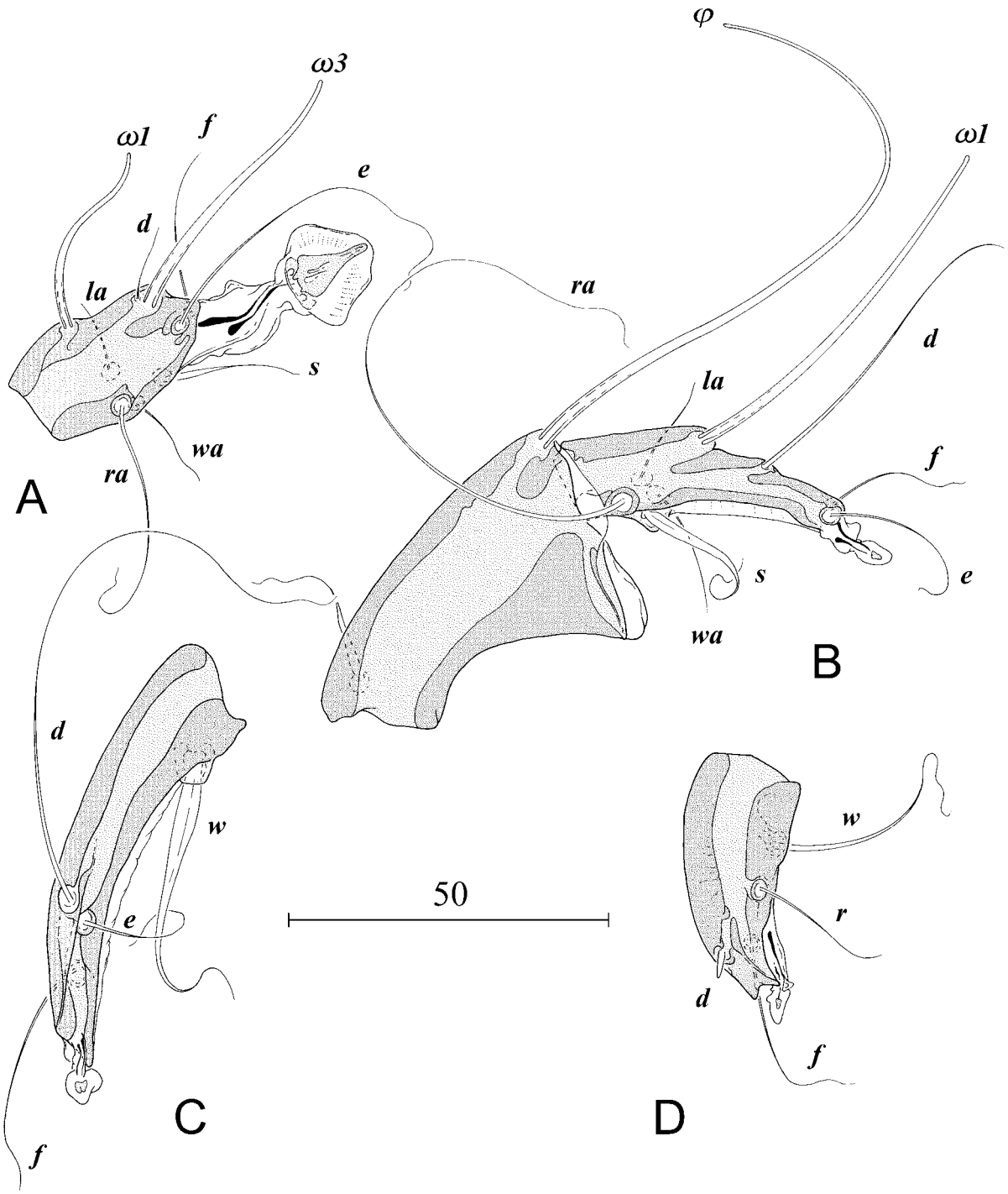

FIGURE 2. Distal podomeres of *Glaucalges tytonis* **sp. n.**, dorsal view, male. Designations of setae after Gaud and Atyeo (1996). A – tarsus I, B – tibia and tarsus II, C – tarsus III, D – tarsus IV.

Legs I–II of similar size, legs III longer than I–II, legs IV thicker than others. Tibiae I and II with ventral triangular apophyses. Femoragenua I and II with rounded dorsal retrograde apophyses. Tarsi II and III elongated with narrow ventral lamella. Tarsus IV short with two apical spines, tarsus III with single apical spine. Ambulacra I well developed, II–IV vestigial. Tips of leg IV tibiae reaching level of posterior margins of terminal membranes. Setae *ba* absent on tarsi I–II, *r, s* on tarsi III, and *r, e* on tarsi IV; setae *d* on tarsi IV stick-like, very short (Fig. 2A–D).

Female (Figs 3, 4, 5C). Gnathosoma shaped as in males, length 48–50, width 47–50, length/width 1.0–1.1. Idiosoma moderately elongated, parallel-sided, length 310–315, width 149–160, length/width 2.0–2.1.

Propodosoma and hysterosoma lengths 92–105 and 210–218 respectively, propodosoma/hysterosoma 0.4–0.5. Rounded or concave terminal margin with small terminal protuberance with copulatory opening on its tip; in some individuals copulatory opening situated more dorsally in which case protuberance is absent. Pronotum shaped and sclerotized as in male, pronotal shield length 92–96, width 85–92. Humeral shields absent. Hysteronotal shield shaped as strongly elongated trapezium, anterior margin longer than posterior one, both margins concave. Shield extends posteriorly slightly beyond the level of setae *e2*; length of the shield 149–153, width at anterior margin 78–83. Two elongated lacunae lying medially to bases of setae *d2*. All dorsal shields uniformly dotted. Setae of pronotum as in males. Setae of dorsal hysterosoma short and with exception of *d2* inserted on striated tegument outside the hysteronotal shield; setae *d2* set on lateral margins of the shield. Setae *e1* nearly twice as long as *d2* (Fig. 5C). Distances between setae: *c2–c2* 125–135, *c2–d2* 64–68, *d2–d2* 70–74, *d2–e2* 91–92, *e2–e2* 67–76, *h3–h3* 44–57.

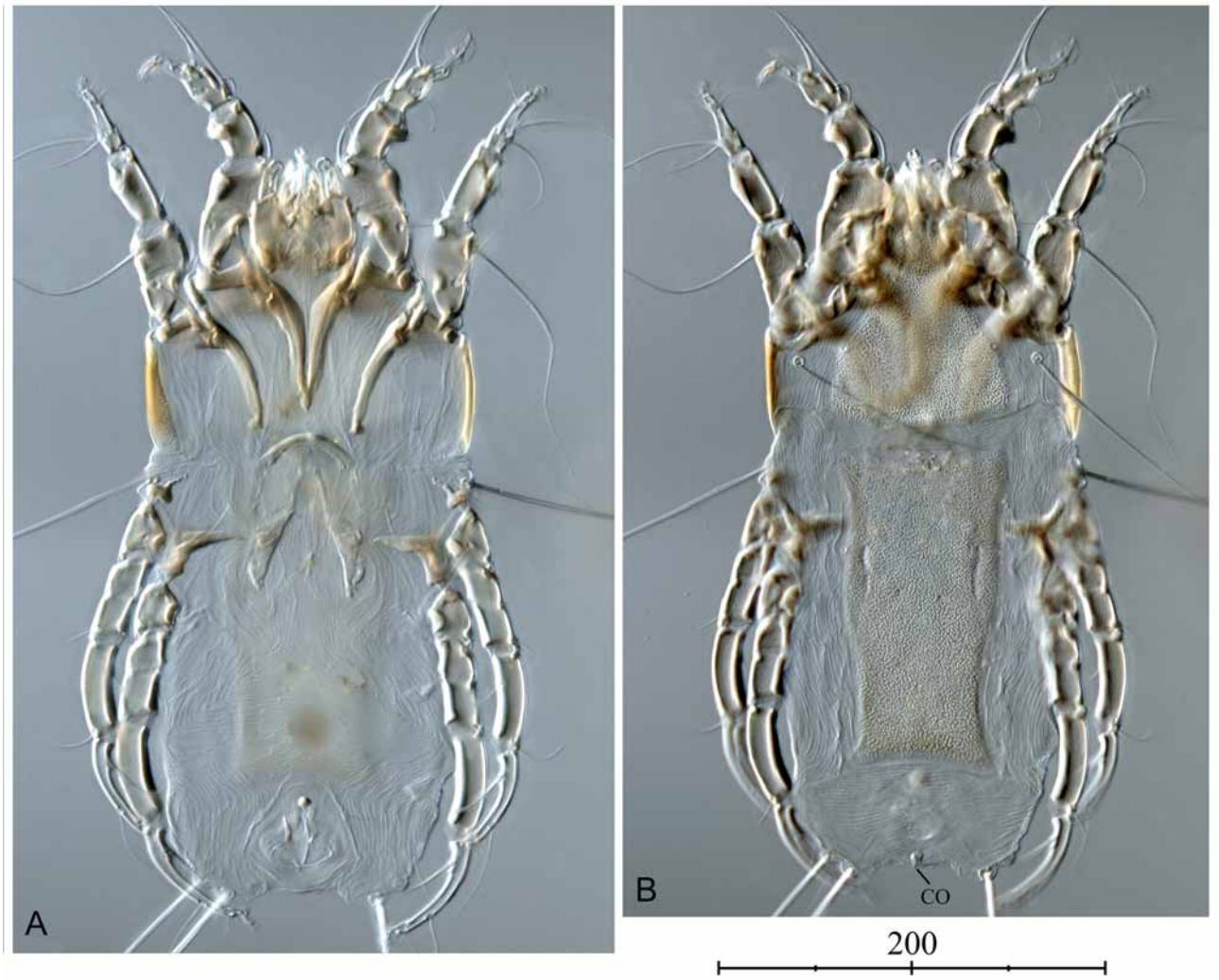

FIGURE 3. *Glaucalges tytonis* **sp. n.**, female. A – ventral view, B – dorsal view. CO – copulatory opening.

Ventral propodosoma shaped and sclerotized as in male. Epigynum well developed, arched, width 42–43, height 18–22 (max. 30 in additional material) (Figs 3A, 4). Branches of epigynum encompassing bases of setae *3a*. Latigenital sclerites extending posteriorly beyond the level of genital setae *g*. Setae *g* inserted posterior to the level of genital acetabula and posterior to the level of setae *3b*. Setae *3b* anterior to genital acetabula. Setae *ps2* inserted latero-terminal to *ps3*, *ps3* subequal to *ps2* in length.

Ambulacra of legs IV reaching terminal end of the body, legs III do not reach body terminus. Both posterior tarsi attenuate with narrow ventral lamella.

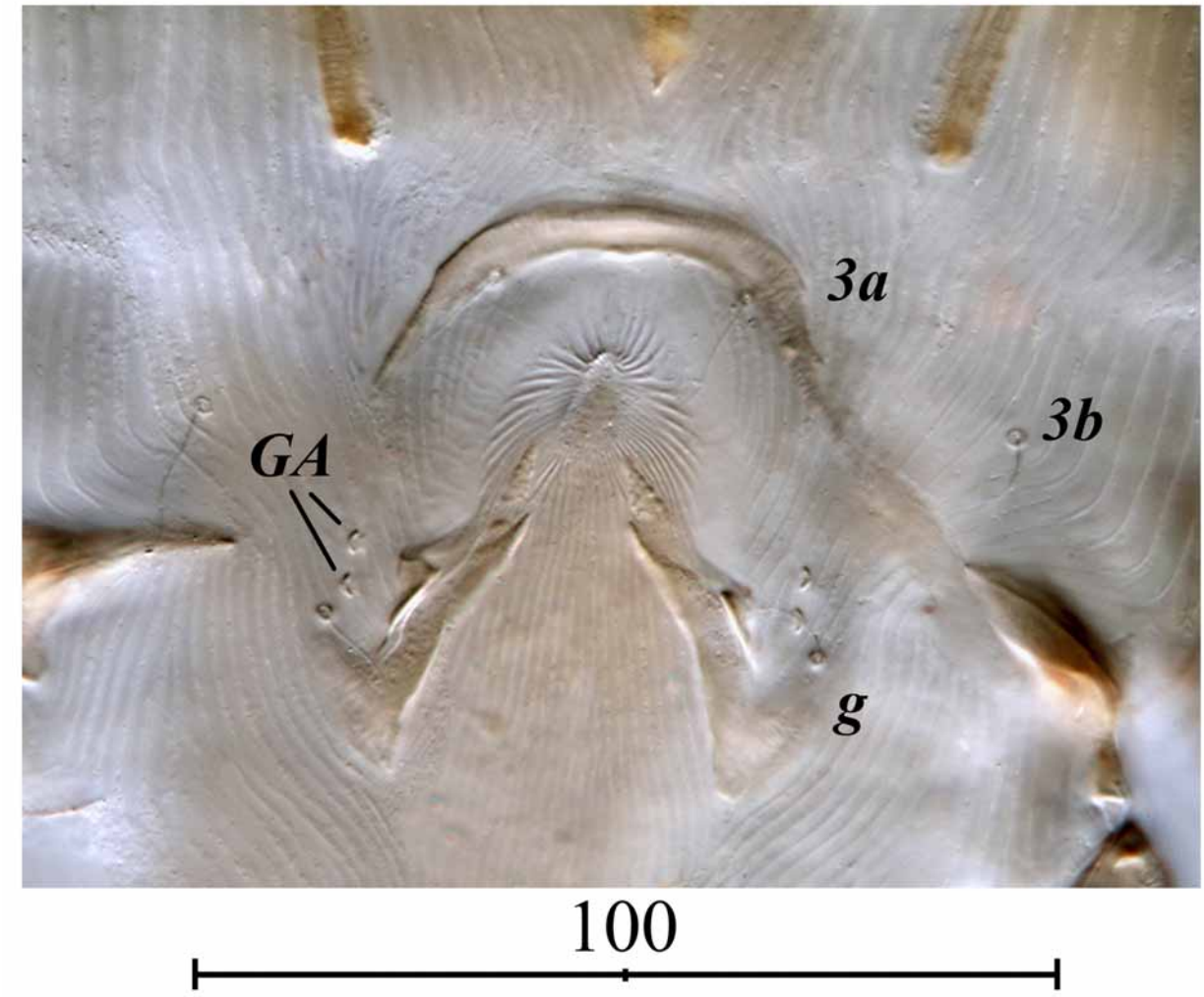

FIGURE 4. Epigynum and oviporus in female *Glaucalges tytonis* **sp. n.** GA—genital acetabula.

Differential diagnosis. *Morphology.* The new species is extremely similar to *Glaucalges attenuatus* (Buchholz, 1869) described from the long-eared owl *Asio otus* (Strigiformes, Strigidae). Males of both species differ by length of dorsal setae *d2* and *e2*: in *G. tytonis* **sp. n.** these setae are much longer than in *G. attenuatus* (Figs 5A, 6). Females are almost indistinguishable. The only difference is in the relative lengths of setae *d2* and *e2*: in females of *G. tytonis* **sp. n.** setae *e2* are clearly almost twice as long as setae *d2*; in *G. attenuatus* both setae are subequal (Fig. 5C). Both sexes of *G. tytonis* **sp. n.** have Y-shaped epimerites I with short sternum, while G. *attenuatus* has these sclerites V-shaped (Fig. 5B). Males of *G. tytonis* **sp. n.** are larger on average than *G. attenuatus*: 360x220 versus 330x200 (Fig. 7). However the shape of epimerites and the body size may sometimes fail as distinguishing characters.

DNA barcode. We amplified and sequenced a 644 bp fragment of the cytochrome oxidase subunit I (COI) gene (DNA barcode region chosen by the Consortium for the Barcode of Life, http://barcoding.si.edu) for one male holotype and 3 male paratypes (Acc. EU271955), remaining paratypes (Acc. EU271956) as well as for specimens of *G. attenuatus* (Acc. EU271957, EU271958). Intraspecific K2P divergence in *G. attenuatus* was 0.47%. No intraspecific variability in *G. tytonis* **sp. n.** was detected. Comparison of the sequences from *Glaucalges tytonis* **sp. n.** and *G. attenuatus* showed that proportion of different nucleotides ranged from 14.65% to 14.85% (mean 14.75%). Because most of the nucleotide substitutions were synonymous, they resulted only in one amino acid change (substitution of valine with isoleucine). This observed genetic distance in the nucleotide sequence of the DNA barcode is substantial, with the differentiation between species comparable to the majority of currently recognized species (Hebert *et al.* 2003).

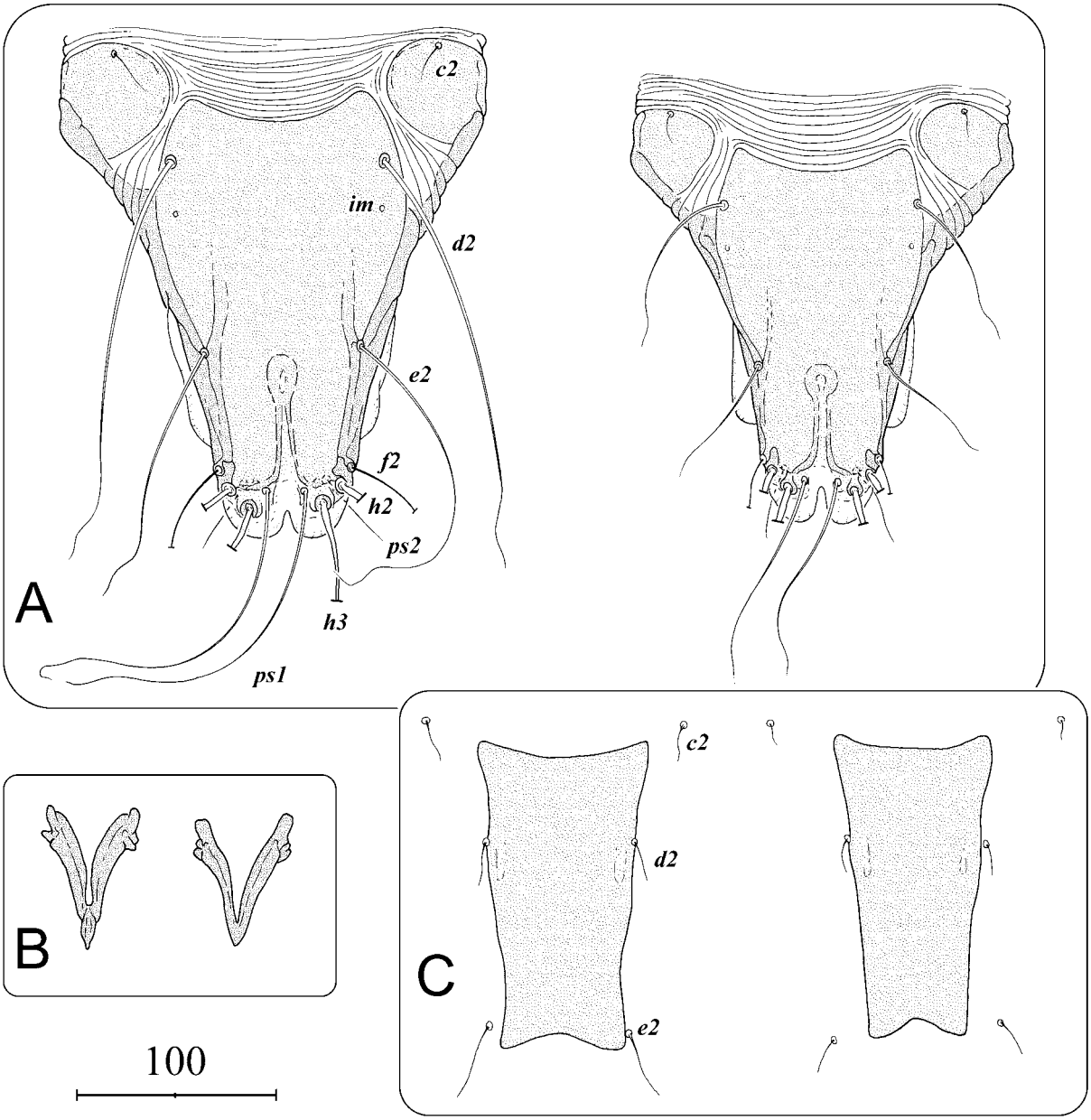

FIGURE 5. Morphological differences between *Glaucalges tytonis* **sp. n.** (left) and G. *attenuatus* (right). A – male hysteronotum, B – epimerites I of male, C – female hysteronotal shield with associated setae.

Etymology. The specific epithet is derived from the generic name of the host *Tyto alba*.

Remarks. Although DNA extraction from a single individual of feather mite is technically possible we suggest in case of small-sized mites such as these *Glaucalges* species (less than 0.5 mm) to publish DNA barcode sequence isolated from a sample of several individuals (holotype and paratypes = "type sample") along with information about possible genetic variability.

Results of the present study seem to confirm our previous observations that multihost feather mite species are often in fact a group of cryptic species. Each of these species is associated with limited host range of closely related bird species or even with a single host species (Badek & Dabert 2006, Dabert *et al.* 2005). If this evolutionary scenario holds true, it is likely that the *Glaucalges* species recorded on other strigid owls may also represent different species of mites. In our opinion, only thorough morphological analysis accompanied by DNA barcoding will significantly accelerate studies that could resolve this and many similar taxonomic problems.

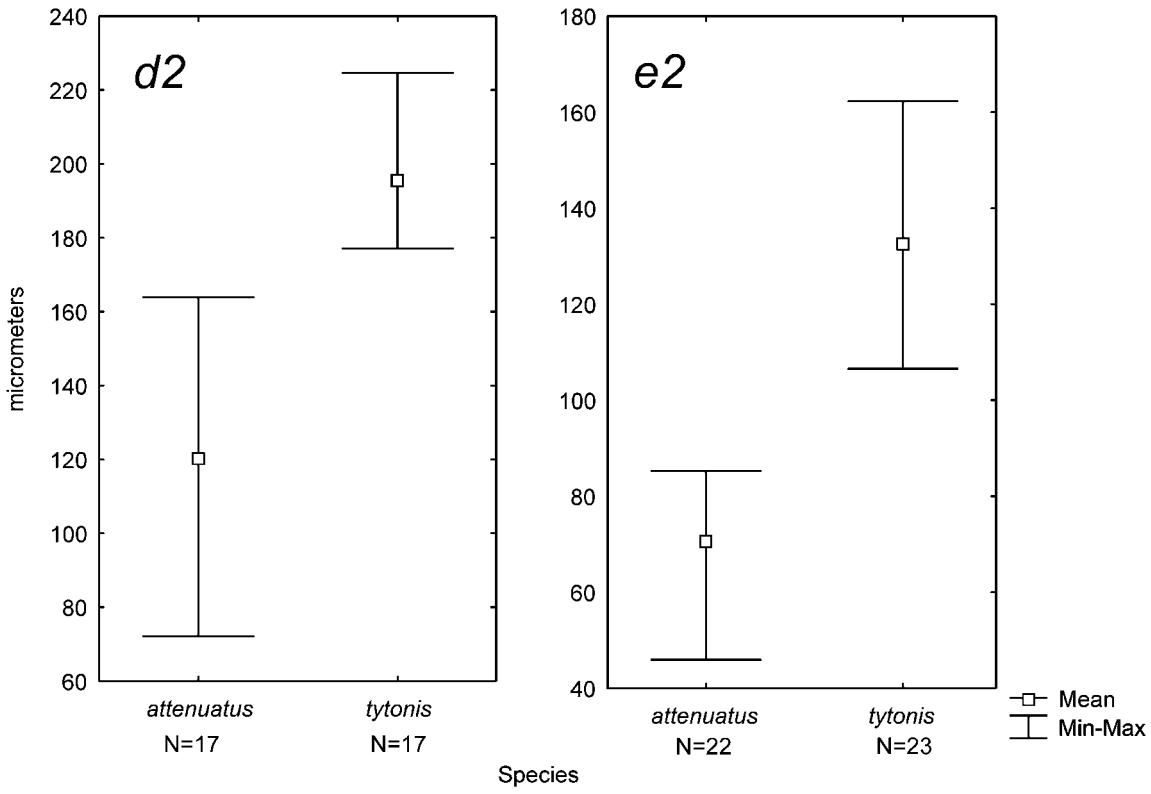

FIGURE 6. Length of setae *d2* and *e2* in males of *Glaucalges tytonis* **sp. n.** and *G. attenuatus*.

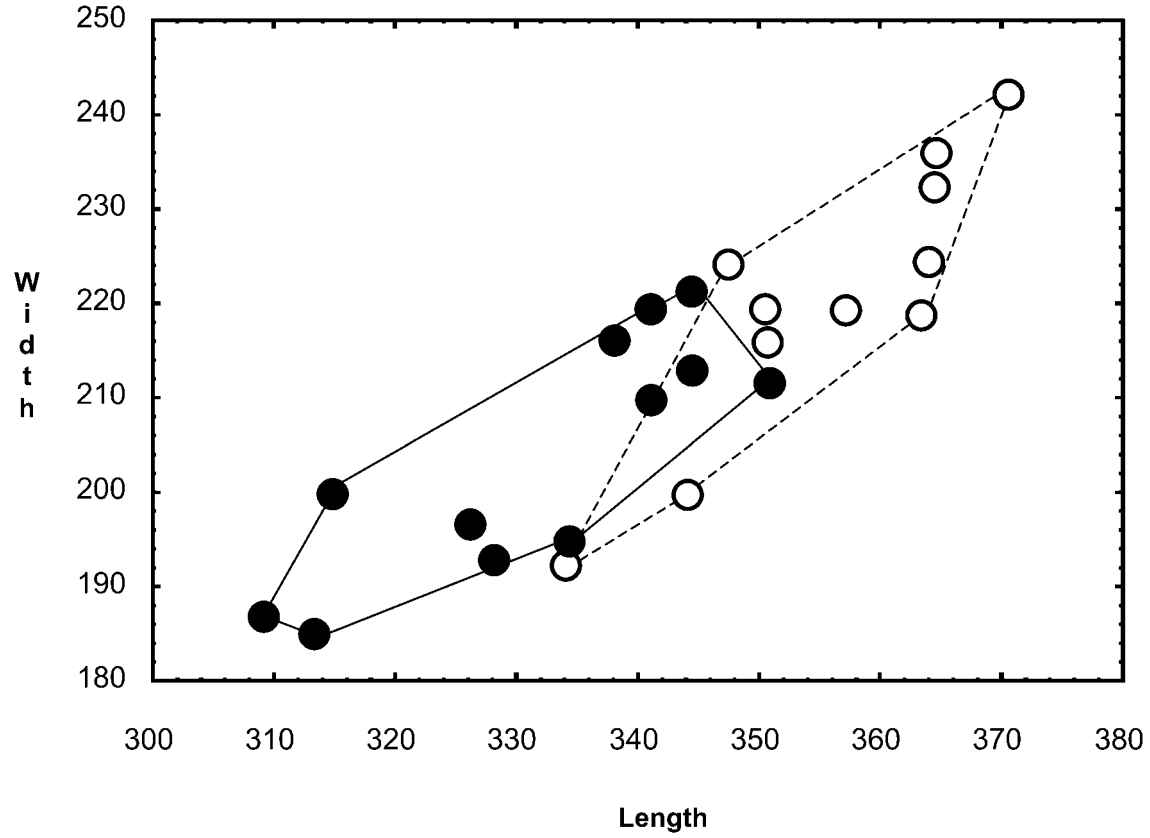

FIGURE 7. Idiosoma dimensions in *Glaucalges tytonis* **sp. n.** (white circles, N=12) and *G. attenuatus* (black circles, N=12).

TABLE 1. Host range of known *Glaucalges* species.

Mite species	Hosts	Locality	References
G. attenuatus (Buchholz)	Strigiformes Strigidae		
	Aegolius funereus (L.)	Germany	Eichler (1938)
	*Asio flammeus (*Pontoppidan)	Germany	Buchholz (1869)
	Asio otus (L.)	Morocco	Gaud (1980)
		Germany	Buchholz (1869)
	Athene noctua (Scopoli)	Rhodesia, Zaire, Zululand	Gaud (1980)
		Morocco	Gaud (1958)
	Bubo africanus (Temminck)	Rwanda, Zaire, Zululand	Gaud (1980)
	Bubo bubo (L.)	Zaire	Gaud (1980)
		Finland	Lönnfors (1937)
		Switzerland	Mumcuoglu and Müller (1974)
	Bubo lacteus (Temminck)	Rwanda, Zaire	Gaud (1980)
	Bubo leucostictus Hartlaub	Zaire	Gaud (1980)
	Bubo poensis Fraser	Morocco	Gaud (1958)
		Zaire	Gaud (1980)
		Cameroon	Gaud and Mouchet (1959), Gaud and Till (1961), Gaud (1980)
	Bubo shelleyi (Sharpe et Ussher)	Zaire	Gaud (1980)
	Bubo virginianus (Gmelin)	USA	Atyeo and Philips (1984)
		Cameroon	Gaud (1980)
	Scotopelia peli (Bonaparte)	Cameroon	Gaud and Till (1961), Gaud (1980)
	Strix aluco (L.)	no data	Philips (2007)
	Strix woodfordii (Smith A.)	Cameroon	Gaud (1980)
G. tytonis **sp. n.** [2]	Tytonidae		
	Tyto alba (Scopoli)	Poland, Germany	present study
		Morocco	Gaud and Petitot (1948), Gaud (1958)
		Senegal	Gaud and Mouchet (1959), Gaud and Till (1961), Gaud (1980)
		Cameroon, Transvaal	Gaud (1980)
		United Kingdom	Rothschild and Clay (1952)
		Cuba	Černý (1967)
		Japan	McClure and Ratanaworabhan (1973)[1]
		India	Philips (2007)
G. pteropus (Gaud et Mouchet)	Musophagiformes Musophagidae		
	Corythaeola cristata (Vieillot))	S Cameroon	Gaud and Mouchet (1959), Gaud and Atyeo (1981)

[1] Designed as *Protalges* sp. only.

[2] Provisional designations without checking the material described under name *Glaucalges attenuatus* (except present study).

Acknowledgements

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