Chapter 5
Bat Facial and Lip Projections: Unique Integumentary Morphology

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Abstract We examined the morphology of chin and lip facial projections of seven species of Phyllostomid and Noctilionid bats utilizing scanning electron microscopy and light microscopy of immunohistochemical and stained paraffin-embedded sections. Results showed that lip and chin facial projections were composed of glabrous skin except for some chin projections of *Noctilio leporinus* that were composed of haired skin. All projections contained a rich and diffuse concentration of nerves that were associated with dermal papillae and epidermal rete pegs that contained Merkel cell–neurite units and a diffuse network of epidermal free nerve endings. The existence of physically extended anatomical structures that contain a variety of receptor sensory units and their associated modalities must be important to be present throughout this diverse group of bats. These facial projections include a unique neural arrangement, but their specific functions remain unknown.

5.1 Introduction

Of the 18 families of bats recognized by Simmons (2005), the family Phyllostomidae (leaf-nosed bats) is by far the largest (55 genera out of 202, 160 species out of 1,116—Wilson and Reeder 2005). The Phyllostomid bats are well known for their nose leaf apparatus (nose leafs and lancet) and patterns of facial projections (aka warts, verrucae, facial projections, bumps, swellings, or excrescences located on lips and chin). The nose leaf apparatus of Phyllostomid bats is a morphological adaptation for enhancing and directing nasally emitted ultrasounds useful in acoustic orientation (Pye 1986). The patterns of facial projections, however, have not been investigated rigorously; their characterization has been minimal anatomically, and

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Fig. 5.1 Photograph of
A. jamaicensis face with
prominent chin and lip facial
projections. *Thin arrows*
distinguish lip projections,
large arrowheads distinguish
glabrous chin projections,
and *small arrowheads*
distinguish long sinus hairs
(vibrissae) from numerous
surrounding, shorter, terminal
hairs of fur
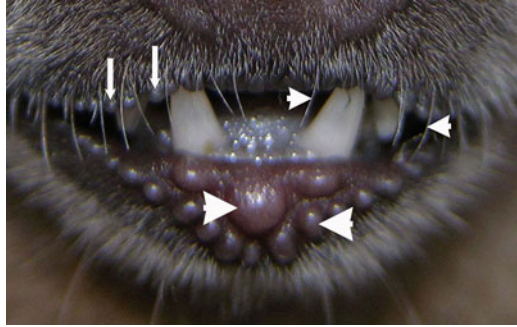
no function has been attributed to these structures. The existence of these facial
projections has been recognized by natural historians, for example, as identification
features (e.g., the original description of *Phyllostomus verrucosum* by Elliot 1905),
and they have been used as taxonomic characters in Phyllostomid taxonomy
(Wetterer et al. 2000). The purpose of this investigation was to characterize light
microscopic analyses of these unique structures for the first time.

The facial projections in question are paired chin pads or single chin pads with
or without clefts, chin papillae, chin flaps, and upper and lower lip papillae (Fig. 5.1)
at the vermillion border with haired skin. For representative figures of variations in
facial projections among Phyllostomids, see Wetterer et al. (2000). There is no
known sexual dimorphism associated with the chin and lip projections, and these
features are very similar within a genus but vary between genera (Wetterer et al.
2000). In the past, the facial projections had been characterized as glandular struc-
tures and pads of connective tissue and muscle without discreet morphological char-
acterization (Dalquest and Werner 1954; Harrison and Davies 1949; Quay 1970).
Facial projections, which are widely distributed (known to be present in the
Phyllostomidae, Noctilionidae, and Mormoopidae), are apparently conserved, albeit
variable, structures based on their presence in many species. These structures have
not yet been associated with any particular function; they may allow bats to better
exploit resources or amplify aspects of sociality. Interestingly, Silva-Toboada and
Pine (1969) noted internal labia papillae from specimens preserved in alcohol as
characteristic of the Stenodermines. These internal labial papillae were further char-
acterized as limited to the internal lip line or as covering most of the inside of cheeks
(Wetterer et al. 2000). Here, we show that the internal labial papillae are morpho-
logically continuous with the external lip projections, and together they form a uni-
structural anatomical entity.

Weiss (1990) investigated facial projections in routine histological, light micro-
scopic immunohistochemical, and scanning electron microscopic studies of three
Phyllostomid species. The unpublished results indicated the projections consisted
of glabrous skin with numerous nerve fibers and free nerve endings. Nerve fibers in
projections were found to terminate freely in the epidermis and at the dermal–
epidermal junction, many in association with epidermal Merkel cells juxtaposed at
this junction. Since nerve fibers and Merkel cells were prevalent in facial projections,

it was the conclusion of Weiss (1990) that these projections most likely provide an increased surface area for sensory reception. In this project, we attempt to characterize morphologically the nature of facial projections in six Phyllostomid bats and one Noctilionid bat with the ultimate goal being to attribute function to these conserved and widespread features. We report characterization of various facial projections by routine paraffin-embedded light microscopic histological, by light microscopic immunohistochemical, and by scanning electron microscopic methods. Our methods were similar to those of Weiss (1990), but whereas Weiss examined three Phyllostomid species, we examined six Phyllostomid species and one Noctilionid species. *Artibeus jamaicensis* was the only species in our study in common with the study by Weiss. Our working hypothesis was that the facial projections have a sensory function and that the projections provide increased surface area for concentrating epithelial-associated sensory structures and free nerve endings instead of typical epidermal content and epidermal derivatives.

5.2   Materials and Methods

Tissues from 21 bats were examined: *A. jamaicensis* ($n=7$), *Ardops nichollsi* ($n=2$), *Brachyphylla cavernarum* ($n=2$), *Carollia perspicillata* ($n=1$), *Noctilio leporinus* ($n=2$), *Phyllostomus discolor* ($n=3$), and *Sturnira lilium* ($n=4$). With the exceptions noted below, ongoing field studies in West Indian islands by Gary Kwiecinski provided field-caught bats that were euthanized, preserved, and transported according to approved University of Scranton IACUC protocols #8-03 and #1-08. The remaining tissue samples were obtained from specimens of captive colonies (*C. perspicillata* obtained from Rick Adams, University of Northern Colorado, and *P. discolor*, The University of Scranton).

Whole bats were fixed in neutral phosphate-buffered formalin. Excised tissues were fixed in either 4 % glutaraldehyde in 0.05 M cacodylate buffer with 1 mM $CaCl_2$ (Phillips 1985) or 0.05 M cacodylate-buffered 5 % glutaraldehyde/4 % paraformaldehyde (Karnovsky's fixative—Electron Microscopy Sciences, Hatfield, PA). After being held in fixative for 1–7 days at 4 °C, the bats or tissues were washed in running tap water and transferred to 70 % ethanol or 0.05 M cacodylate buffer for shipping to the laboratory if field collected. All specimens for microscopic studies were stored at 4 °C, in 70 % ethanol or in cacodylate buffer.

Tissue samples from the lips and chins of the bats obtained were subsequently processed for examination using routine paraffin histology, immunohistochemistry, and scanning electron microscopy techniques. Observations of whole or half lips and chins were made using a scanning electron microscope (SEM) from stored fixed tissues. Fixed tissues were dehydrated to 100 % ethanol for 24 h. Dehydrated tissue samples were critical point dried and sputter coated (SC7620 Mini Sputter Coater/ Glow Discharge System, Quorum Technologies, Kent, Great Britain) with gold under 100 mTorr for 120 s in an argon chamber prior to observation with the SEM. An ISI-ABT Scanning Electron Microscope (Model SX-40A, ISI, Brno, Czech

Republic) with a PGT Omega Digitizer (Model 0S16-I011, Omega Engineering, Inc., Stamford, CT) was used to obtain digital images. We utilized GW Electronics, Inc. Printerface for Windows (Oconomowoc, WI) to capture images.

For routine histological processing, selected areas of upper and lower lips and chins were removed from whole animal specimens, being careful not to damage the facial projections, and these removed areas were placed into fresh 70 % ethanol, while the remaining tissues were replaced into their respective solutions and stored at 4 °C until needed. Excised tissues were dehydrated to 100 % ethanol, cleared in chloroform, and infiltrated with melted paraffin (melting point 56 °C, Fisher Scientific, Pittsburgh, PA). Tissues in paraffin blocks were mounted on wooden blocks designed to fit a chuck/block holder for an AO 820 (American Optical Co., Buffalo, NY) rotary microtome fitted with a steel blade. Sections (6–8 μm) were mounted serially on glass slides subbed with VECTABOND (Vector Labs, Santa Cruz, CA), dried on a hot plate at 40–45 °C, and stained.

Histological stains employed included hematoxylin and eosin (H&E), Masson's trichrome, toluidine blue, eosin and methylene blue, cresyl violet, luxol fast blue, Fontana–Masson, orcein and methylene blue, periodic acid-Schiff, or aldehyde fuchsin (Bancroft and Stevens 1977; Presnell and Schreibman 1997), and Bielschowsky's silver stain for nerve fibers, Bielschowsky's silver stain for nerve fibers with a nuclear fast red counterstain for nuclei, and Bielschowsky's silver stain with a light-green counterstain for collagen (Luna 1960). Stained slides were preserved permanently by cover slipping with Permount (Sigma Chemical, St. Louis, MO) mounting medium. Examination of the slides included the use of an Olympus BH2 light microscope, and digital images were captured with a fitted SPOT 7.2 Color Mosaic camera and accompanying software (Spot RTKE, Diagnostic Instruments, Sterling Heights, MI).

Selected tissue samples stored in 70 % ethanol or 0.05 M cacodylate buffer were embedded in paraffin, sectioned, and analyzed with a modified immunoperoxidase method (Naish 1989) utilizing VECTASTAIN Elite ABC Kits (Vector Labs, Santa Cruz, CA). As described above for histological preparations, microtome cut sections were mounted on glass slides and allowed to dry. Dried slides with sections were deparaffinized, hydrated, and washed with a 0.1 % hydrogen peroxide/pH 7.4 phosphate-buffered saline (PBS) solution (Fisher Scientific, Pittsburgh, PA) for 30 min. The slides were then washed in PBS three times for 5 min each, followed by incubation for 30 min in blocking solution from the VECTASTAIN Elite ABC Kit following the manufacturer's instructions. The slides were subsequently washed in PBS three times for 15 min each and incubated in primary antibody solution made in rabbit [anti-neuron-specific enolase (a-NSE), at a dilution of 1:100 to 1:200 (Calbiochem, Santa Cruz, CA); anti-vasoactive intestinal peptide (a-VIP), at dilutions of 1:100 to 1:200; and anti-neurofilament 200 (a-NF), at dilutions of 1:50 to 1:1,000 (Sigma-Aldrich, St. Louis, MO)] for 2–7 days at room temperature. Slides were then washed in PBS three times for 15 min each, followed by incubation with the secondary antibody solution (anti-rabbit, VECTASTAIN Elite ABC Kit, following the manufacturer's instructions) for 1 h. Slides were then rinsed and incubated in the ABC peroxidase reagent solution (VECTASTAIN Elite ABC Kit) for 60 min

and washed in PBS three times for 15 min. A solution of diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) was added until a sufficient brown color was attained (approximately 4 min). The slides were then washed in PBS three times for 15 min, rinsed twice in distilled water, and mounted with VectaMount water-soluble mountant (Vector Labs, Santa Cruz, CA). Acting as negative controls, normal rabbit serum (pre-immune) was used at dilutions of 1:100 in place of immunized primary antisera on adjacent slides of serial sections. Slides were examined with an Olympus BH2 light microscope, and digital images were captured with a fitted SPOT 7.2 Color Mosaic camera and accompanying software (Spot RTKE, Diagnostic Instruments, Sterling Heights, MI).

5.3 Results

Lips and chins of all species, with the exception of *P. discolor*, were examined by scanning electron microscopy (SEM). The extent and shapes of facial projections and presence or absence of internal papillae were revealed by these analyses. Facial projections for all bats lacked hairs and were without any obvious external evidence of epidermal appendage, except for *N. leporinus*. The SEMs revealed the cornified nature of epithelial surfaces of glabrous projections by the presence of sloughing keratinocytes. Some projections had concavities that possessed a reticular network appearance (Fig. 5.2a, b), but these were not consistent from specimen to specimen or between species. Higher magnifications revealed various types of holes and portals in facial projection surfaces, but they were not consistent from one projection to the other or between individuals of the same species. All other bats including *A. jamaicensis*, *A. nichollsi*, *B. cavernarum*, *C. perspicillata*, *P. discolor*, and *S. lilium* had lip and chin projections that were glabrous. The surfaces of posterior chin projections of *N. leporinus* were not comparable to those of the Phyllostomid bats investigated in this study. For *N. leporinus*, the chin consisted, in part, of glabrous projections appearing as flaps and folds that were contiguous with the lower lip and, in part, by haired chin folds or projections, posterior to those connected to the lower lip. The posterior projections were covered with a sparse population of terminal hairs (predominant, contain hair and follicular sheath), and each projection contained vibrissae (elongated hair and follicular sheath surrounded by an encapsulated blood sinus). Low-magnification SEMs demonstrated clearly the continuous nature of external lip projections and internal labial papillae (Fig. 5.2c, d) for those species that possessed internal labial projections that were contiguous with external projections (all species examined except *B. cavernarum* and *N. leporinus*). The internal labial papillae were conical, triangular, or rectangular. There were no internal projections associated with lips or cheeks in *B. cavernarum*. Sparsely located, rectangular-shaped internal labial papillae of *N. leporinus* were found inside the lower cheek and were not associated with the lips.

Through routine paraffin-embedded histological analysis of cut sections, the glabrous-appearing facial projections for all species (except *N. leporinus* chin
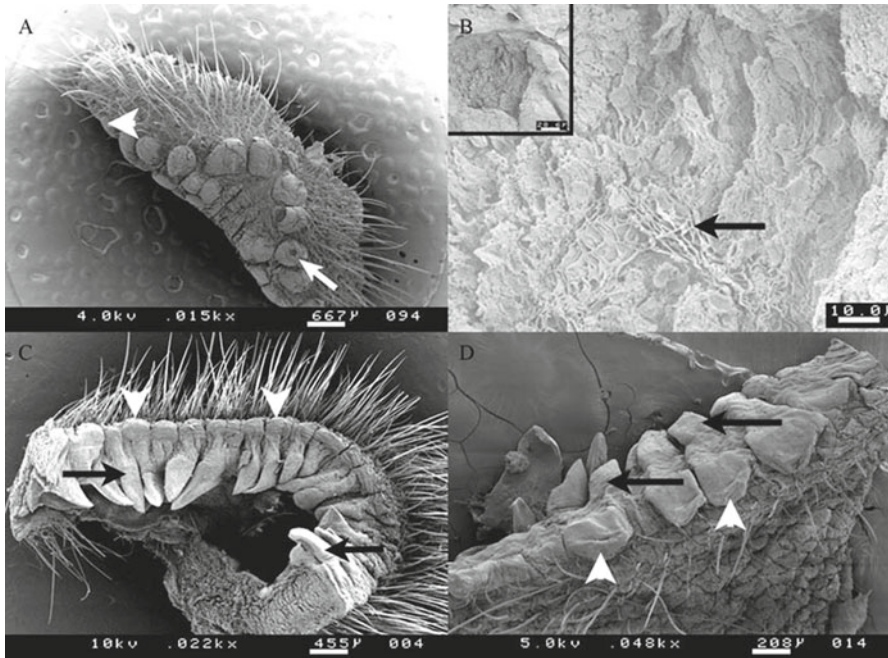
Fig. 5.2 Scanning electron micrographs of lips from *B. cavernarum* (**a**, ×15, and **b**, ×1,000 with inset ×350) and *A. jamaicensis* (**c**, ×22 and **d**, ×48). Note lip projection (*arrowhead*) and chin projection at *arrow* in (**a**) have a concavity; that concavity is magnified in inset of (**b**) and further magnified in (**b**) to reveal a reticular-like network (*arrow*) at the surface of concavity. Buccal internal papillae (*black arrows*) and lip projections (*white arrowheads*) and their continuities are shown in (**c**) and (**d**)

projections) were confirmed as being hairless skin with little epidermal variation between lip, chin, and internal papillae. The posterior portions of chins from *N. leporinus* contained a series of complex folds of haired skin, with each fold containing at least one vibrissa (Fig. 5.3). Since the posterior chin projection skin of *N. leporinus* was not different from the haired skin of all other bats examined, except there being fewer follicles and they contained vibrissae, the results that follow pertain to all projections from all bats examined except the posterior chin projections of *N. leporinus*. The glabrous lip projections of all bats examined were composed of a keratinized stratified squamous epidermis and a collagenous-rich and cellular dermis (Fig. 5.4a). The epidermal stratification clearly displayed a stratum basale resting on a PAS+ basal lamina. A stratum spinosum, a stratum granulosum, and a keratinized stratum corneum were consistently present. A sloughing stratum corneum was commonly found, especially on chin pads. The epidermal thickness of projections was greater than adjacent haired skin region by four to six cell layers or more. The junctions between the epidermis and dermis of facial projections were scalloped with well-developed epidermal rete pegs and dermal papillae (Fig. 5.4b). The epidermal portions of the projections also contained other cellular elements
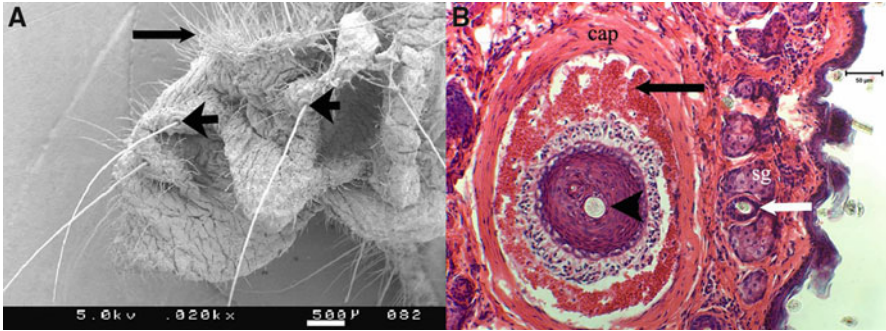
Fig. 5.3 (**a**) Scanning electron micrograph of chin projections from *Noctilio leporinus* (×20) distinguishing sinus hairs (*arrowheads*) from terminal hairs (*thin arrow*). (**b**) Histological section of skin contrasting a sinus hair and its follicle (*black arrowhead*) from a terminal hair (*white arrow*) with its follicle and associated sebaceous glands (sg). Note the much larger size and complexity of sinus hair follicle and its associated structures including the blood sinus (*black arrow*) and sinus capsule (cap)
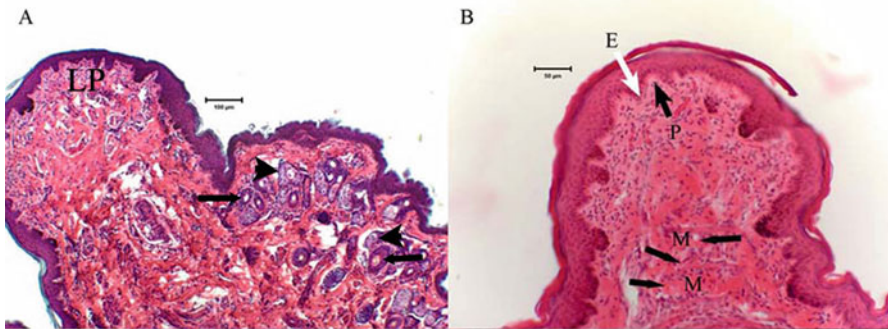

Fig. 5.4 Light photomicrographic representatives of lip and chin projections. (**a**) Low magnification (×143) of lip projection and haired skin junction from *A. jamaicensis*. Note lip projection on *left side* of micrograph lacks hair follicles (*thin arrows*) and sebaceous glands (*arrowheads*) present in haired skin on *right side* of micrograph, H&E stain. (**b**) Higher magnification (×286) image of chin projection from *A. jamaicensis* highlighting aglandular and glabrous nature of papillae, with an epidermal rete peg (E *arrow*) and dermal papillae (P *arrow*) labeled. *Smaller arrows* at M point to skeletal muscle elements, H&E stain

commonly found in mammalian epidermis, including melanocytes and their extracellular product melanin between keratinocytes, Langerhans cells (wandering immune cells), and Merkel cells (somatosensory-associated cells). In H&E- or toluidine blue-stained sections, Merkel cells were concentrated in the stratum basale; particularly dense populations were in the epidermal rete pegs along with melanocytes and pigment, while halo-appearing Langerhans cells could be found within epidermal layers above the stratum basale.

A constant, readily observed feature of all projections was the absence of epidermal appendages: no hair follicles, no sebaceous glands, and no sweat glands. Hair follicles

with sebaceous glands, but no sweat glands, were found in skin adjacent to projections (Fig. 5.4a) in all species examined. The dermis of projections (lip, chin, and internal papillae) consisted of a rich collagenous matrix, and an elastic fiber network was found in the reticular layer, but not the papillary layer. The dermis contained an unusually rich cellularity compared to adjacent haired skin areas, containing cellular profiles typical of dermal fibroblasts, but many more cells with a rounded or oblong profile, unlike typical flattened fibroblasts. An especially rich dermal network of nerve bundles were observed in routine H&E-stained sections, were confirmed by silver and immunohistochemical staining, and are discussed further below. The dermis of chin projections contained skeletal muscle fibers, with many oriented parallel or oblique to the skin surface (Fig. 5.4b). The collagenous matrix of the dermis contained the usual network of blood vessels and other typical connective tissue elements, including mast cells, lymphocytes, and polymorphonuclear leukocytes, but a paucity of adipocytes, except for the internal papillae of *N. leporinus*. Adipocytes were routine dermal constituents of *N. leporinus* internal labial papillae. Within the reticular dermis, there was an unusual appearance of some small arteries, similar to periarterial lymphatic sheaths (sheathed arteries) typical of the spleen (Fawcett 1994; Sasou and Sugai 1992). Meissner's corpuscles were not observed in the dermal papillae of projections nor were Pacinian corpuscles or any other encapsulated sensory organs found in the dermis of any species examined.

Analysis of the facial projections by various methods demonstrated a rich dermal nerve fiber content and many intra-epidermal nerve fibers originating from the dermis. The fibrous array of nerves was demonstrated further by immunohistochemical staining with antibodies specific for nerve cells. Epidermal and dermal nerve fibers and cell bodies (or cells with nuclei) were positively stained for immunoreactivity in facial and chin projections and were greater in number than those observed in surrounding haired skin or oral mucosa. In the dermal papillae of facial projections, many free nerve endings were found, and no corpuscle-like endings were located. In silver-stained and a-NSE preparations, many nerve endings appeared to abut the basal lamina, while others appeared to penetrate into the epidermis. The nerve fibers in the dermis appeared to be ensheathed by supporting cells (Fig. 5.6a), which also appeared to be stained by a-NSE (Fig. 5.5c).

Individual facial projections contained an array of nerve fibers (Fig. 5.6a), some of which terminated at the basal layer of the epidermis, while others were observed coursing through the epidermis (Fig. 5.6b). The array of intra-epidermal nerve fibers was a consistent finding in projections from all species examined. These fibers were not visible in routine H&E-stained sections but were demonstrated by silver and immunohistochemical staining. Silver staining showed some nerve fibers traversing the entire epidermis to the surface (Fig. 5.6b); this was confirmed by immunohistochemical staining with a-NSE that also demonstrated trans-epithelial nerve fibers terminating at the surface (Fig. 5.5b) as well as many nerve fibers terminating at various levels within the epidermis. Some intra-epidermal nerve fibers were observed to intertwine with varicosities, while other fibers terminated within the epidermis with knobbed or expanded endings (Fig. 5.5a, d). The intra-epidermal fibers with knobbed terminals were most prominent in strata nearer the surface rather than in basal strata.
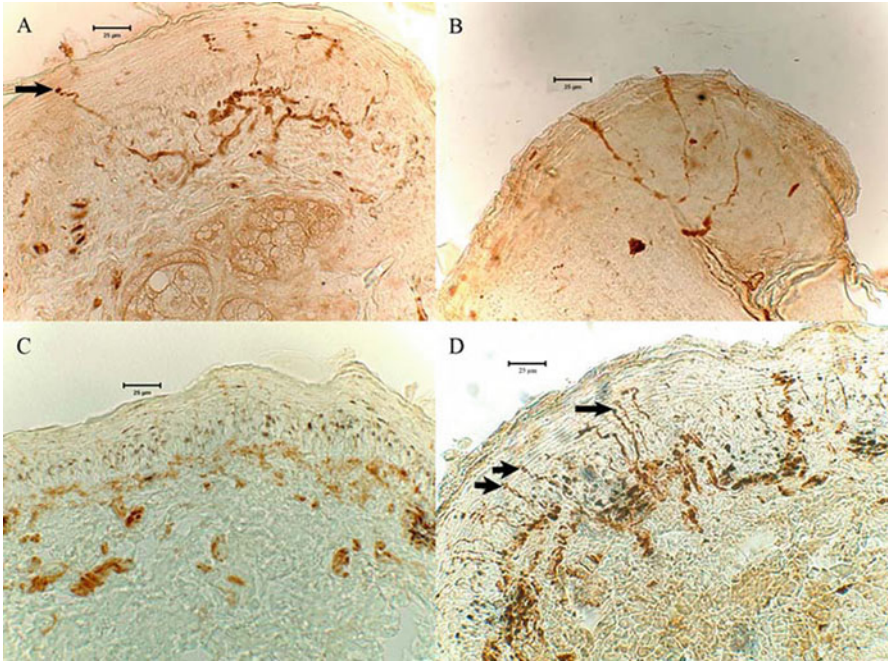
Fig. 5.5 Staining of neuronal elements by DAB (gold/brown color) localization of antineuron-specific enolase (a-NSE) as described in methods, ×572. (**a**) Section through facial projection of *Brachyphylla cavernarum* with a-NSE activity in intra-dermal and intra-epidermal nerve fibers. Note knobbed nerve fiber terminus (*arrow*). (**b**) Section from facial projection of *Sturnira lilium* showing trans-epidermal nerve fibers. (**c**) Section from facial projection of *Artibeus jamaicensis* showing abundant a-NSE activity at the epidermal–dermal junction. (**d**) Section from facial projections of *Artibeus jamaicensis* with epidermal and dermal a-NSE activity. Note knobbed nerve fiber termini (*arrows*) in epidermis and abundant a-NSE activity at epidermal–dermal junction associated with epidermal rete pegs
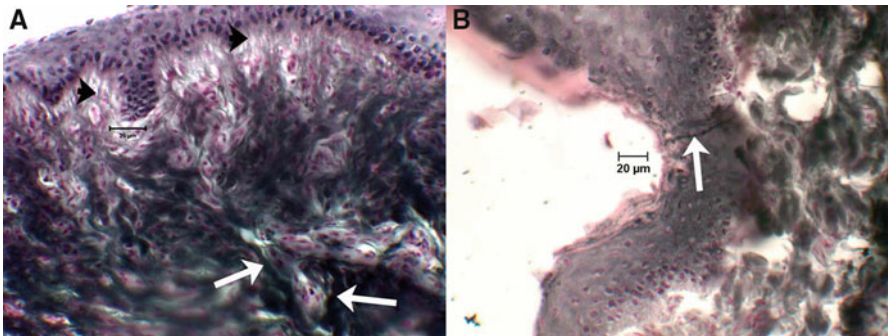

Fig. 5.6 (**a**) Bielschowsky's silver stain for nerve fibers highlights rich dermal innervation (*white arrows*, nerve bundles), particularly free nerve endings at the epidermal–dermal junction (*arrowheads*), ×243. (**b**) Trans-epidermal nerve fiber (*arrow*) from *Phyllostomus discolor*. Bielschowsky's silver stain, ×572

Merkel cells were also observed repeatedly in routine sections of facial projections and were stained positive with a-NSE but not with a-VIP. Merkel cells associated with external root sheaths of vibrissae and terminal hairs in haired skin were stained positive by a-VIP. Merkel cells of facial projections were distinctly concentrated in the stratum basale layer of the epidermis, primarily located in epidermal rete pegs. The failure to observe cytoplasmic staining in either H&E- or toluidine blue-stained sections does not validate the recognition of the Merkel cell. Subsequent positive immunohistochemical staining and demonstration by the Fontana–Masson method (Luna 1960) of neurosecretory granules inside the cell cytoplasm (Negri bodies) support our identification of these cells as Merkel cells. Because neurosecretory granules in Merkel cells when stained by the Fontana–Masson method are indistinguishable from melanin granules within melanocytes, it is difficult to discriminate between these two cell types. To resolve this problem, Merkel cells with associated Negri bodies were identified by a melanin bleaching treatment with 0.25 % potassium permanganate and 5 % oxalic acid prior to Fontana–Masson staining (Luna 1960) to eliminate confounding melanin.

5.4   Discussion

The results show that facial projections are unique structures consisting of cornified epidermis lacking epidermal derivatives/appendages but rich in nerve networks, compared to surrounding haired skin. The epidermis of the facial projections is arranged with epidermal rete pegs, forming a scalloped junction with the dermis, and rich in free nerve endings. Both dermis and epidermis contain numerous nervous elements without typical epithelial sensory structures but an abundance of free nerve endings. The dermis of facial projections was unusual in lacking glands and other epidermal derivatives and in containing many round and oblong cells in histological profiles. These dermal cells deserve further characterization by ultrastructural and cytochemical methods. The dermis of chin projections contained skeletal muscle fibers, and although we did not characterize these muscle fibers further, they may be important; for example, they may move, spread, elongate, or further elevate the projection away from the skin surface. Such skeletal muscle-powered movements may increase sensitivity or contact range of the projection. These muscle fibers need further characterization to define their role(s) in facial projection structure and function. We also noted an unusual appearance of some small dermal arteries, similar to sheathed arteries in the spleen (Fawcett 1994). These arteries, although noted, were not characterized further in this study. Although sheathed arteries are considered immunological structures (Fawcett 1994; Sasou and Sugai 1992), they are also in need of further attention in the facial projections of bats. For example, do these arteries play a role in hydrostatic control mechanisms associated with elevation/retraction of facial projections or are they associated with the integumentary immune system?

Mammalian cutaneous innervation involves a variety of morphologically distinct sensory receptors that include encapsulated mechanoreceptors, free nerve endings, and Merkel cells (Delmas et al. 2011; Iggo and Andres 1982; Oaklander and Siegel 2005). Many skin receptors are mechanoreceptors, but thermo-, chemo-, and noci-ceptors are also part of the sensory innervation formed by peripheral terminals of afferent nerve fibers in both haired and non-haired (glabrous) skin. Cutaneous sensory neurons also deploy a variety of specialized transducers with highly specific sensory functions and differing distributions and densities at various regions of the skin (Oaklander and Siegel 2005). The pattern of innervation within the glabrous facial projections of the Phyllostomid and Noctilionid bats examined in this study is organized around epidermal rete pegs, dermal papillae, and intra-epidermal free nerve endings. No encapsulated mechanoreceptors were found in any of the bats examined. Within these facial projections, intra-epidermal free nerve endings and clusters of epidermal rete pegs with Merkel cell concentrations appear to aggregate, forming a unique sensory organ.

Based upon SEM and histological analyses, the lip and chin facial projections of the bats examined were similar to glabrous skin without any epidermal appendages but instead contained an abundance of nerve fibers. Standard histological methods and immunoreactivity to anti-neuron-specific enolase (a-NSE) revealed that facial projection skin contained an abundance of nervous elements that were more numerous than adjacent haired skin. The pattern of innervation within the facial projections was organized around the dermal papillae, the epidermal rete pegs, and including free nerve endings that projected into the layers of the epidermis. These observations were similar to those of Weiss (1990). The neural elements were not associated with epidermal appendages, since they were absent in the glabrous facial projections. Although there were some skeletal muscle fibers in projections, there was not a rich nerve plexus associated with them. Based on the profusion of afferent terminations at their tips in the epidermis and at the epidermal–dermal junction in association with Merkel cells in epidermal rete pegs and the lack of other epidermal appendages or effectors, it is most likely that facial projections have a sensory function.

In routine paraffin-embedded tissue, we stained sections with Bielschowsky's silver stain, a technique relatively specific for nerve fibers. But this technique might also stain reticular fibers, which could be misleading. Our staining, however, appears to be neural for several reasons. Reticular fibers are shorter than nerve fibers (Fawcett 1994) and are stained purple instead of being black as is typical for nerve fibers stained by Bielschowsky's silver stain (Luna 1960). Furthermore, the extra-cellular fibrous matrix of the papillary dermis is normally devoid of reticular fibers except those of the basal lamina. We further confirmed the specificity of Bielschowsky's silver stain for nerves by observing a similar pattern of nerve networks by positive immunoreactivity to a-NSE.

It is difficult to differentiate Langerhans cells and Merkel cells in isolation due to their similar appearance in the epidermis when stained with H&E or toluidine blue. Although both cell types appear with a cytoplasmic halo (perinuclear negative image), they are site specific (Lacour et al. 1991). Merkel cells were found in the

stratum basale of the epidermis, while the Langerhans cells could be located in all strata of the epidermis (Fawcett 1994; Satthaporn and Eremin 2001). Although some of the cells we call Merkel could be Langerhans cells in the stratum basale, the numbers of Merkel cells we observed are consistent with clusters of Merkel cells at the bases of epidermal rete pegs in lips, oral mucosa, and digital skin observed in other mammals (Halata and Munger 1983; Munger and Halata 1983).

The innervation pattern of the facial projections examined in the bats surveyed seems to be typical for glabrous skin, in that somato-neural arrangements around epidermal rete pegs and dermal papillae have been noted previously (Halata and Munger 1983; Montagna et al. 1975). Also, free nerve endings penetrating the epidermis and clusters of epidermal rete pegs as observed in this study have been observed in the noses of other mammals (Montagna et al. 1975). In tree shrews (*Tupaia glis*), moles (*Scapanus townsendii*), and opossums (*Didelphis virginiana*), expanded nerve endings and myelinated fibers form nerve nets that penetrate the epidermis up to the stratum corneum. Although reminiscent of Eimer's organs (Catana 1995) of star-nosed moles (*Condylura cristata*), the epidermal neurites of bats and other mammals tend to be longer and are densely concentrated in the center of epidermal columns that form epidermal rete pegs, and they are not as distinct or morphologically organized as Eimer's organs. Interestingly, at the central base of Eimer's organs in the star-nosed mole is a single Merkel cell–neurite complex. In the bats we examined, the Merkel cell–neurite complexes seem to be structured sensory end organs that resemble Eimer's organs. Eimer's organs consist of stacks of epithelial cells, intra-epidermal neurites, a single Merkel cell–neurite complex, and a single lamellated corpuscle (Catana 1995). The bat epidermal rete pegs contain all the elements of an Eimer's organ except the bats lack a lamellated end organ, the bat epidermal rete pegs contain numerous Merkel cells instead of one, and the bat free nerve endings are not encased within keratinocytes. We suggest that bat epidermal rete pegs with Merkel cells and free nerve endings are a variation of the Eimer's organ that is structured to increase cutaneous sensibility. Such epidermal rete pegs would be advantageous to nocturnal, routing animals whose movements are limited by vision; they would make it more aware of disturbances and obstacles that might be injurious as well as aid in food selection and transport.

Merkel cells have been extensively characterized by means of light and transmission electron microscopy in the integument of many vertebrates (e.g., Catana 1995; Merkel 1875; Tazaki and Sakada 1989; Toyoshima and Shimamura 1988). Clusters of Merkel cells have been observed in epidermal rete pegs in primate eyelids, lips, oral mucosa, and digits (Halata and Munger 1980b, 1983; Munger and Halata 1983, 1984). Merkel cells have also been found in the external root sheaths of regular and sinus hairs (Halata and Munger 1980a, b). Anti-NSE is an accepted marker of Merkel cells and has been utilized in a variety of animals to identify this cell type (Gu et al. 1981; Smoller 2002). In the present study, Merkel cells were most abundant in the bases of epidermal rete pegs in facial projections, while few, or none, were located in haired skin adjacent to facial projections as evinced by intense immunoreactivity to a-NSE. Only weak, if any, immunoreactivity by a-NSE was observed in the external root sheaths of hair follicles. The Merkel cells of external

root sheaths were clearly demonstrated by immunoreactivity to a-VIP, while Merkel cells of epidermal rete pegs were weakly, if at all, immunoreactive to a-VIP. Localization of neuropeptides in mammalian Merkel cells seems to be highly variable, and their locations are also variable (Hitchcock et al. 2004; Lucarz and Brand 2007; Tachibana and Nawa 2005). For example, calcitonin gene-related peptide has been immunolocated in Merkel cells of human, pig, dog, and cat (Alvarez et al. 1988; Garcia-Caballero et al. 1989; Cheng Chew and Leung 1993), while met-enkephalin immunoreactivity has been observed in mice but not human, pig, dog, and cat (Cheng Chew and Leung 1991, 1992; Hartschuh et al. 1979, 1983). It is possible that the varied immunoreactive staining with the antibodies selected in this bat study reflects functional divergence in different groups of Merkel cells.

It is known that Merkel cells can be visualized immunohistochemically with a variety of cytokeratin and neuroendocrine markers. Controversial results have been obtained with neurofilament protein 200 kDa, which was detected in only a small percentage of Merkel cells or not at all (Moll et al. 1995; Narisawa et al. 1994; Sauret and Didierjean 1984). The cytoplasm of Merkel cells contains cytoskeletal intermediate filaments, and the low molecular weight cytokeratin CK20 is known as a specific marker for cutaneous Merkel cells (Moll et al. 1995). Our negative results of no immunoreactivity with bat tissues when reacted with a-NF, the anti-200 kDa neurofilament protein, could be because a-NF is not a consistent marker of Merkel cells or rabbit-derived antibodies for a-NF do not cross-react with bat tissues. Future identification of Merkel cells and their distribution might be better served with other Merkel cell markers such as anti-cytokeratin CK-20 and/or the neuronal cytoplasmic enzyme, protein gene product 9.5 (PGP9.5).

The Merkel cell is a neuroendocrine cell that also functions as a slowly adapting mechanoreceptor and is often observed in association with nerve endings, collectively known as the Merkel cell–neurite complex (Hitchcock et al. 2004). This complex is made of clusters of 50–70 cells in contact with the terminals of a single myelinated Aß axon (Delmas et al. 2011). They function as slow-adapting low-threshold mechanoreceptors, respond to indentation depth of skin, and have the highest spatial resolutions of the cutaneous mechanoreceptors. As such, they are purported to transmit a precise spatial image of tactile stimuli and are responsible for form and texture perception (Delmas et al. 2011; Iggo and Muir 1969; Maricich et al. 2009). Such structures would seem to fit the conditions that specialized diets of nocturnal, volant animals would require. The Phyllostomid bats as a group exploit and consume a variety of food items: plant fruits, flowers, and leaves; insects; and a variety of vertebrates including fish, frogs, and mammals (even other bats). As individuals, these bats are unusual in that their food choices are opportunistically flexible as food resources change. To be able to evaluate the safety and nutritional value of a food item quickly is essential for survival. They must evaluate, grasp, remove, and transport a prey item in a short period of time and be able to secure their investment until relocation for ingestion to avoid predation themselves. Feeding behavior would be facilitated by the precise spatial and textural characterizations that clusters of Merkel cells seem able to provide. Observing feeding behavior by high-speed cinematography may facilitate understanding the function(s) of these facial projections.

The absence of corpuscles (e.g., Meissner's) and the presence of high concentrations of free nerve endings in the facial projections of these bats are unusual. This pattern may be unique in these bats. Sensory corpuscles have been described in connective tissues of the tongue, urethra, rectum, and anus of some bat species (Quay 1970), and the sensory innervation in the bat facial projections differs from the innervation that has been typically found in other mammalian glabrous skin. The general principle that morphologically distinct receptors occupy typical and regular locations in skin has been noted in a variety of mammalian species other than bats. Meissner's corpuscles are coiled mechanoreceptors and the predominant sensory corpuscle found within dermal papillae of glabrous skin, for example, in human and primate fingers, ridged skin of rodents, primate palatal mucosa and tongue, and glabrous skin of other mammals (Delmas et al. 2011; Halata and Munger 1983). Meissner's corpuscles were also the most numerous sensory structure of the monkey lip vermilion border (Halata and Munger 1983). In light of these commonly observed innervation patterns, the absence of Meissner's corpuscles and the presence of free nerve endings in close adjacent areas and in close association with clusters of Merkel cells in epidermal rete pegs are noteworthy.

Why are there so many free nerve endings and an absence of sensory corpuscles in these bat facial projections? The meandering properties of highly arborized nerves and the small size and inaccessibility of sensory nerve endings have hampered investigations of morphological and functional studies. Several assays of cellular responses to mechanical stimulation have been developed in recent years and have begun to uncover the molecular basis of mechanotransduction (Delmas et al. 2011). The best-known mechanoreceptors in mammals are located in the skin. Cutaneous somatosensory receptors detect a wide range of mechanical stimuli, including light brush of the skin, texture, vibration, touch, and noxious pressure (Delmas et al. 2011). This variety of stimuli is matched by a diverse array of specialized or encapsulated sensory nerve endings that respond to cutaneous motion and deformation in specific fashions. Some cutaneous nerve endings are classified as low-threshold mechanoreceptors because they respond preferentially to innocuous mechanical forces, while others are considered high-threshold receptors because they are excited by injury (Delmas et al. 2011). In general, for some specialized or encapsulated nerve endings, there is a strict correlation between morphology and functional characteristics, and the complex morphology of corpuscles can be regarded as facilitating their specific mechanoreceptive function (Iggo and Andres 1982). In mammals, free nerve endings represent afferent terminals of both small, myelinated A delta fibers and unmyelinated C fibers that typically terminate in the subepidermal skin. The perception of painful stimuli is initiated by high-threshold C fibers that can be mechanosensitive or polymodal in nature (Perl 1996). Some C fibers are low-threshold mechanoreceptors that respond preferentially to innocuous tactile mechanical forces and signal a pleasant sensation in associative social body contact in humans (Loken et al. 2009; Valbo et al. 1999). It has been proposed that inflammation or trauma may change the sensation conveyed by C fiber low-threshold mechanoreceptors from pleasant touch to pain (Seal et al. 2009). Such information indicates that the functional implications of a high concentration of free nerve endings in bat facial projections do not delimit morphological constraints on their functional potentials. Without morphological characteristics that covary with the

diversity of modality transductions exhibited by free nerve endings, discrete functions of free nerve endings in bat facial projections need to be determined by other means such as neurophysiological recordings.

Cutaneous sensory units with unmyelinated fibers exhibit great variation in their sensitivity to different stimuli. Sensory units can be highly responsive (low threshold) to either gentle mechanical stimuli or innocuous thermal stimuli or have a high threshold requiring a strong stimulation for activation, such as noxious heat and irritant chemicals, for example, dilute HCl or acetic acid (Delmas et al. 2011; Bessou and Perl 1969). The epidermal free nerve endings, which would fall into the category of C fibers, may be composed of different types of unmyelinated axons projecting to different locations and may be associated with different modalities. That free nerve endings offer a potentially wide range of modalities within a given skin region creates a potential site for selection of specific receptors that would be useful to the life-history constraints on an animal.

The function of the nerve associations in the facial projections of these Phyllostomid bats appears to be sensory, but the modalities cannot be determined from morphological studies. Without transmission electron microscopic, biochemical, physiological, and behavioral studies, it is impossible to discern if the nerve endings in facial projections are homoplastic or heteroplastic populations. Heterogeneous populations of neurons and receptor modalities would be advantageous for cutaneous sensation in the faces of nearly all Phyllostomid and Noctilionid bats, which are species of bats that occupy a diversity of biomes and exploit variable resources. The elevated concentrations of neuron sensory endings in facial projections allow for heterogeneous populations of neurons that would potentially be important in chemoreception and thermoreception as well as in mechanoreception. The fact that facial thermoreceptors have been identified in the common vampire bat (*Desmodus rotundus*) that function as an aid in detecting warm body prey and surface vasculature (Kürten and Schmidt 1981) is testimony to the importance of receptor diversification and plasticity in this group of bats and to the importance of behavioral and physiological correlation. We hope that these initial characterizations of bat facial projections provide a foundation for future research that will examine the interplay between form, function, and selective adaptations in neuronal plasticity.

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