Melanophoromas and Iridophoromas in Reptiles

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Summary

Chromatophoromas are tumours of pigment-producing cells of the skin and are rarely reported in reptiles. These tumours are subclassified on the basis of the type of pigment. The present study characterizes chromatophoromas arising in 26 reptiles, including six snakes, 19 lizards and a tortoise. These include the first reports of melanophoromas in a yellow anaconda (Eunectes notaeus), pigmy rattlesnake (Sistrurus spp.), southern water snake (Nerodia fasciata), veiled chameleon (Chamaeleo calyptratus) and leopard gecko (Eublepharis macularius); the first reports of benign iridophoromas in a savannah monitor (Varanus exanthematicus), veiled chameleon and bearded dragon (Pogona vitticeps); and the first description of a malignant iridophoroma in a bearded dragon. Additionally, in three bearded dragons a ‘mucinous’ type of melanophoroma is described for the first time. Chromatophoromas generally arose from the skin of the body and head and ranged in size from 0.2 to 2.0 cm in diameter. In six cases the animals were humanely destroyed immediately after diagnosis. Three further animals were humanely destroyed following recurrence of their tumour. Six of these nine reptiles had visceral metastases. Grossly, melanophoromas (n = 20) were grey or black, while iridophoromas (n = 6) were white in colour. Microscopically, most of the tumours were composed of spindle cells with varying pigmentation and 0–2 mitoses per 10 high power fields. Six of the 20 melanophoromas were classified as malignant due to the presence of intravascular tumour cells, visceral metastases, high pleomorphism and/or mitotic figures. Five of the six iridophoromas were classified as benign and the one malignant tumour was defined by the presence of intravascular tumour cells and visceral metastases. Immunohistochemically, melan A and S100 were coexpressed by all of the chromatophoromas.

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Introduction

Three types of pigment cells (chromatophores) occur within the integument of reptiles.

Tumours of pigment cells are generally referred to as chromatophoromas and are classified according to their specific chromatophores as: (1) melanophoromas or melanomas (melanin-producing cells), (2) xanthophoromas (carotenoid or pteridine-producing cells) or (3) iridophoromas (crystalline purine-producing cells containing reflecting granules of guanine, adenine, hypoxanthine or uric acid) (Bagnara, 1966; Bagnara and Hadley, 1973; Rohrlich, 1974; Bagnara et al., 1979; Gopalakrishnakone, 1986; Morrison, 1995).

In general, melanophoromas are considered rare tumours of reptiles (Korabiowska et al., 1997, 1998). In a study of 1,941 reptiles, Sinn (2004) found one melanophoroma in a snake, while Ippen and Schröder (1977) found no chromatophoromas in 5,000 reptile post-mortem examinations. Most reports of chromatophoromas have involved snakes such as colubrids, particularly the San Francisco garter snake (Thamnophis sirtalis tetrataenia), and in animals of the families Crotalidae, Boidae and Viperidae (Schlumberger and Lucke, 1948; Frye et al., 1975; Ryan et al., 1981; Ramsay and Fowler, 1992; Gregory et al., 1997; Garner et al., 2004; Suedmeyer et al., 2007). Metastases have been reported in several cases of melanophoromas (Ball, 1946; Elkan, 1974; Garner et al., 2004). Few cases of melanophoromas are described in lizards (Mikaelian et al., 2000; Johnson, 2003; Garner et al., 2004;
Irizarry-Rovira et al., 2006; Simpson, 2008). In tortoises there is one report of a malignant melanophoroma of the carapace in a Hermann’s tortoise (Testudo hermanni) (Heckers et al., 2011). A metastatic iridophoroma is described in a pine snake (Pituophis melanoleucus; Jacobson et al., 1989) and one malignant iridophoroma is reported in a boa constrictor (Boa constrictor; AFIP, 2000). One publication reports a xanthophoroma in a canebrake rattlesnake (Crotalus horridus; 2000). One publication reports a xanthophoroma in a boa constrictor (Jacobson et al., 2006). In bearded dragons the chromatophoromas were located in the skin of the thorax (Eublepharis macularius; Gregory et al., 1997).

The diagnosis of amelanotic melanomas in mammals is often based on immunohistochemical detection of melan A and S100 protein (Glage, 2001; Ramos-Vara et al., 2002). Melan A is expressed in melanocytes and in melanoma cells (Glage, 2001), while S100 protein is expressed in numerous cell types including glial cells and ependymal cells of the central nervous system, glial cells of peripheral nerves (Schwann cells), melanocytes and melanocytic tumour cells (Orchard and Wilson, 1994). Immunohistochemical characterization of reptilian chromatophoromas has rarely been reported. Irizarry-Rovira et al. (2006) were the first to report detection of melan A in reptiles in a melanophoroma from a green iguana (Iguana iguana). Reptilian melanophoromas do not always show expression of S100 (Gregory et al., 1997; Korabiowska et al., 1997; Kusewitt et al., 1997).

Although several case reports of chromatophoromas in reptiles are available, comparative pathological studies of a larger number of cases do not exist. Therefore, the aim of the present study was to characterize and compare the clinical findings and the gross, microscopic and immunohistochemical features of chromatophoromas in 26 reptiles.

Materials and Methods

Animals

Twenty-six chromatophoromas were selected for analysis from an archive of 179 reptilian tumours collected over 9 years. The chromatophoromas arose in a number of different species of varying age (Table 1). In each case the owners of the animals had observed skin masses that had been present for a period of several weeks.

In bearded dragons the chromatophoromas were located in the skin of the shoulder/thorax (n = 4), the head (n = 3), tail (n = 2), leg (n = 1) and in the oral cavity (n = 2). The origin was not documented in three cases. In other reptiles (snakes, chameleons and a savannah monitor) the chromatophoromas were located in the skin of the thorax (n = 6), abdomen (n = 3) and head (n = 1) or in the oral cavity (n = 1).

Most chromatophoromas were excised surgically (n = 23), fixed in 4% buffered formalin and submitted for histopathological investigation. Six animals were humanely destroyed at various times (1–635 days) after histological diagnosis and submitted for necropsy examination. These included a yellow anaconda (Eunectes notaeus), a bearded dragon (Pogona vitticeps), a veiled chameleon (Chamaeleo calyptratus), a leopard gecko (Eublepharis macularius), a Hermann’s tortoise and a pigmy rattlesnake (Sistrurus spp.). Three other reptiles were submitted for necropsy examination without previous histological investigation. These included a garter snake with small pigmented dermal tumours near to the eye and on the dorsum, a bearded dragon with a large non-excisable grey mass with some dark regions on the ventral abdomen and a bearded dragon with a non-excisable, ulcerated white mass ventral to the base of the tail (Table 2). During necropsy examination the animals were inspected in detail and representative samples were fixed in formalin for further examination.

Histopathology

The histological examinations were performed by two veterinary pathologists according to a standard protocol. Samples were embedded in paraffin wax and stained with haematoxylin and eosin (HE). Periodic acid–Schiff (PAS) staining was also performed for the mucinous tumours. If necessary, sections of heavily pigmented melanophoromas were bleached in H₂O₂ 10%. Polarized light was used to visualize birefringence, which is characteristic of iridophores (Jacobson et al., 1989; Irizarry-Rovira et al., 2006) and this examination was used to differentiate between melanophoromas and iridophoromas.

Mitotic figures and nucleoli were counted in 10 high power fields (×40 microscope objective) in randomly selected areas and the mean numbers were calculated. The degree of pigmentation was graded according to a modification of the system described by Smedley et al. (2011) as amelanotic (lack of pigment granules or only single cells containing very

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Chromatophoromas</th>
<th>Melanophoromas</th>
<th>Iridophoromas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lizards</td>
<td>105</td>
<td>20.0%</td>
<td>13.7%</td>
</tr>
<tr>
<td>Bearded dragons</td>
<td>51</td>
<td>29.4%</td>
<td>21.6%</td>
</tr>
<tr>
<td>Snakes</td>
<td>61</td>
<td>9.8%</td>
<td>9.8%</td>
</tr>
<tr>
<td>Tortoises</td>
<td>13</td>
<td>7.6%</td>
<td>7.6%</td>
</tr>
<tr>
<td>Turtles</td>
<td>Total number: 179</td>
<td>14.5%</td>
<td>11.2%</td>
</tr>
</tbody>
</table>
few granules), mild (some or most cells with mild pigmentation), moderate (most cells are moderately pigmented), marked (most cells are intensely pigmented) or dispersed.

**Immunohistochemistry**

The expression of melan A and S100 protein was detected by the streptavidin–biotin immunoperoxidase technique. Intensified pigmented melanophoromas were bleached in order to evaluate the immunohistochemistry (IHC). To avoid reduction of labelling intensity through the bleaching process, mildly pigmented melanophoromas were examined with and without bleaching. As a control for the melan A and S100 markers the expression of these molecules in three fibrosarcomas, a myxoma, a myxosarcoma and a squamous cell carcinoma from different reptile species was investigated.

Citrate buffer (pH 6.0) pretreatment (30 min, 96°C) was required for the detection of both antigens. An initial blocking step with 20% goat serum in Tris-buffered saline (TBS; 50 mM, pH 7.6) for 20 min at room temperature was performed. All antisera were diluted in TBS. Mouse monoclonal anti-human melan A (1 in 50; DAKO GmbH, Hamburg, Germany) was incubated with the sections for 24 h at room temperature. For this reaction the secondary antibody was rat anti-mouse IgG (1 in 250; Vector Laboratories, Burlingame, California, USA) incubated with the sections for 30 min at room temperature. Rabbit anti-S100 (1 in 400; DAKO GmbH) was incubated with the sections for 24 h at room temperature. The secondary antibody for this reaction was goat anti-rabbit IgG (1 in 800; DAKO Cytomation, Glostrup, Denmark) incubated with the sections for 30 min at room temperature. Avdin–biotin–peroxidase complex (horseradish peroxidase streptavidin; 1 in 250; Vector Laboratories) and AEC-substrate (Zytomed Systems, Berlin, Germany) were added subsequently to 'visualize' labelling. Slides were counterstained with Mayer’s haematoxylin (Bio Optica, Milan, Italy).

Negative controls were performed by substituting mouse (DAKO GmbH) or rabbit (DAKO GmbH) control serum for the primary antibodies. A canine melanoma served as a positive control, as did the labelling of chromatophores in normal skin adjacent to the tumours.

The labelling intensity was evaluated semiquantitatively and graded as follows: 0, negative; 1, weak (pale red); 2, moderate (red); 3, strong (deep red). The immunoreactive score (IRS) (Remmele and Stegner, 1987) was calculated according to the following formula, which was modified by Özgen et al. (1997):

$$IRS = \frac{1}{100} \sum \{ PP_n \times \max[\varphi \times (LI_n - 1), 1] \}_{n=2}$$

where $n$ is the index, $PP$ is the percentage of positive cells, $\varphi = 5$ is the weight and $LI$ is the labelling intensity. The assignment of labelling intensity and LI value is:

<table>
<thead>
<tr>
<th>Index number</th>
<th>LI</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>weak</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>moderate</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>strong</td>
<td>3</td>
</tr>
</tbody>
</table>

**Statistics**

Statistical analyses included the Shapiro–Wilk-test, Levene test, Fisher’s exact test (Median test) and Pearson and Spearman correlation tests. Results were
considered statistically significant where $P \leq 0.05$. All analyses were performed using SPSS software, version 16.0 (SSPS Inc., Chicago, Illinois, USA).

**Results**

Twenty of the 26 tumours were diagnosed as melanophoromas and six tumours with birefringent cytoplasmic granules were identified as iridophoromas. Six of the melanophoromas were classified as malignant due to the presence of intravascular neoplastic cells, visceral metastases, high pleomorphism and/or the presence of mitotic figures. Most iridophoromas were classified as benign ($n = 5$), with only one being considered malignant due to the presence of intravascular neoplastic cells and neoplastic cells in the viscera.

The age of the affected animals ranged between 1 and 17 years (median 5 years; Table 2).

When all 179 reptilian tumours were considered, chromatophoromas ($n = 26$) were the most frequent, followed by adenocarcinomas of different organs ($n = 23$), squamous cell carcinomas ($n = 18$), fibrosarcomas ($n = 18$), lymphomas ($n = 11$), papillomas ($n = 11$), osteochondrosarcomas ($n = 9$) and lipomas ($n = 9$). In lizards, chromatophoromas ($n = 19$) were the most frequent tumours followed by squamous cell carcinomas ($n = 14$), fibrosarcomas ($n = 10$), adenocarcinomas of different organs ($n = 9$), lymphomas ($n = 6$) and papillomas ($n = 5$). The most common tumours of snakes were adenocarcinomas of different organs ($n = 13$), fibrosarcomas ($n = 8$), chromatophoromas ($n = 6$), lymphomas ($n = 6$) and osteochondrosarcomas ($n = 5$). In the group of tortoises and turtles the most frequent tumours were papillomas ($n = 3$) and squamous cell carcinomas ($n = 3$).

**Gross Features of Melanophoromas**

Melanophoromas occurred in 11 bearded dragons and nine other reptiles. The melanophoromas ranged between 0.2 and 2.0 cm in diameter with the exception of one tumour that measured $4.0 \times 3.0 \times 2.5$ cm. The cut surface of the melanophoromas in bearded dragons after fixation was grey ($n = 4$), white ($n = 3$), black ($n = 2$) or mixed white and black ($n = 2$). In the other reptile species the cut surface was black ($n = 6$), grey ($n = 2$) or mixed white and black ($n = 1$) (Fig. 1). In general, the melanophoromas appeared significantly darker than the iridophoromas ($P = 0.004$). The consistency of the tumours was firm in 17 of 20 cases. In three bearded dragons a ‘mucinous’ type of melanophoroma was found. These had a gelatinous consistency and released jelly-like material into the formalin (Fig. 2). In six of the nine necropsy examinations visceral metastases were found (Table 3).
or moderate anisokaryosis. The number of nucleoli was 0–2. The mean number of mitoses per HPF was 0–2. Atypical mitotic figures were present in five cases. Neoplastic cells had a moderate amount of cytoplasm, but the degree of pigmentation was highly variable. In most cases amelanotic cells were interspersed with nests of markedly pigmented cells (Fig. 3; Table 3).

In one adult bearded dragon with a markedly pigmented melanophoroma of the face, marked cellular pleomorphism was present. The cells were spindle shaped, heavily pigmented and had hyperchromatic round to oval nuclei with <1 mitosis per HPF. Tumour cell emboli were found in lymphatic vessels, but no necropsy examination was performed in order to determine if visceral metastases were present.

In the three cases of mucinous tumours, all from bearded dragons, the defining feature was a large amount of basophilic, PAS-positive, mucinous interstitial matrix. The cells were arranged in interlacing bundles and loose whorls. The cells were mostly amelanotic with dispersed foci of moderate to marked pigmentation. The nuclei had a mildly dispersed chromatin pattern. The mean number of mitoses was 0–1 per HPF. The growth was invasive into the dermis and musculature in all cases, but tumour cell emboli in lymphatic or blood vessels and metastases were not found (Fig. 4).

Birefringence was not exhibited by any of the melanophoromas. Mild inflammatory infiltration was seen in seven tumours. Heterophils were the dominant inflammatory cells, but some lymphocytes, plasma cells and histiocytes were also present.

Metastatic melanophoromas had a significantly greater pleomorphism ($P = 0.047$) than those tumours without metastases. The number of nucleoli

<table>
<thead>
<tr>
<th>Species</th>
<th>Shape of cells</th>
<th>Pleomorphism</th>
<th>Mitotic figures/HPF</th>
<th>Degree of pigmentation</th>
<th>Metastases</th>
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<tr>
<td>Bearded dragon n = 11</td>
<td>Spindle shaped, n = 11</td>
<td>Mild, $n = 4$</td>
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<td>Amelanotic, $n = 2$</td>
<td>Yes</td>
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<tr>
<td></td>
<td></td>
<td>Moderate, $n = 6$</td>
<td>1, $n = 6$</td>
<td>Mild, $n = 3$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marked, $n = 1$</td>
<td>2, $n = 1$</td>
<td>Marked, $n = 1$</td>
<td></td>
</tr>
<tr>
<td>Veiled chameleon</td>
<td>Spindle shaped</td>
<td>Marked</td>
<td>3</td>
<td>Dispersed</td>
<td>Yes</td>
</tr>
<tr>
<td>Leopard gecko</td>
<td>Epithelioid</td>
<td>Marked</td>
<td>11</td>
<td>Dispersed</td>
<td>Yes</td>
</tr>
<tr>
<td>Garter snake 1</td>
<td>Spindle shaped, n = 2</td>
<td>Moderate, $n = 2$</td>
<td>0, $n = 1$</td>
<td>Dispersed, $n = 2$</td>
<td>No</td>
</tr>
<tr>
<td>Garter snake no. 2</td>
<td></td>
<td></td>
<td>1, $n = 1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow anaconda</td>
<td>Spindle shaped</td>
<td>Moderate</td>
<td>2</td>
<td>Dispersed</td>
<td>Yes</td>
</tr>
<tr>
<td>Pigmy rattlesnake</td>
<td>Spindle shaped</td>
<td>Mild</td>
<td>0</td>
<td>Marked</td>
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<tr>
<td>Boa constrictor</td>
<td>Spindle shaped</td>
<td>Moderate</td>
<td>0</td>
<td>Dispersed</td>
<td>No</td>
</tr>
<tr>
<td>Southern water snake</td>
<td>Spindle shaped</td>
<td>Mild</td>
<td>0</td>
<td>Marked</td>
<td>No</td>
</tr>
<tr>
<td>Hermann’s tortoise</td>
<td>Spindle shaped</td>
<td>Moderate</td>
<td>2</td>
<td>Dispersed</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 3. Dermal melanophoroma from a 5-year-old bearded dragon. The tumour consists of sheets of epithelioid to spindle-shaped cells with large hyperchromatic nuclei, a prominent nucleolus and a variable degree of pigmentation. HE. Bar, 10 μm.

Fig. 4. Mucinous type of dermal melanophoroma from a 7-year-old bearded dragon. Loosely-arranged tumour cells lie within large amounts of basophilic mucinous stroma. HE. Bar, 10 μm.
Histopathological Findings of Melanophoromas in other Reptile Species

The histomorphological findings in melanophoromas in other reptile species (n = 9) were not significantly different compared with the findings in the bearded dragons. The tumours were located in the dermis (8/9) and infiltrated the deeper dermis and musculature. One melanophoroma was located in the oral cavity (leopard gecko) and showed invasive growth into the interstitial tissue. Neoplastic infiltration of the epidermis was found in the yellow anaconda and the pigmy rattlesnake. Epidermal ulceration was present in the boa constrictor and the yellow anaconda. The morphology of the neoplastic cells was predominately spindle shaped (8/9 cases), but epithelioid in the leopard gecko (Table 3). Tumour cell emboli in lymphatic and/or blood vessels were found in the biopsy of the second recurrence in the yellow anaconda and in the Hermann’s tortoise. These tumours were characterized by spindle-shaped cells with hyperchromatic round to oval nuclei. Cells were mostly amelanotic with dispersed foci of moderate (yellow anaconda) or marked pigmentation (Hermann’s tortoise) and there were 1–2 mitoses per HPF. Five melanophoromas showed mild infiltration of heterophils. Additionally, in three cases some lymphocytes, plasma cells and histiocytes, and in the tumour of the yellow anaconda some multinucleated giant cells, were observed.

Visceral Metastases of Melanophoromas

Melanophoromas were present in the viscera in addition to the skin in five cases, including three lizards, one snake and one tortoise (Table 4) and these metastatic lesions affected 18 different tissues. Neoplastic foci occurred predominately in the kidney, liver, lung, intestine and abdominal fat bodies as well as in the stomach and heart. In four of five cases multiple organs were affected. In two cases the dermal tumours showed intravascular tumour cells and during necropsy examination, visceral metastases were seen. In three cases no tumour cell emboli were found in dermal neoplasms, but visceral metastases were located.

In general, the morphological features of the metastatic tumours corresponded to that described for the dermal neoplasms, but some differences are notable. In the veiled chameleon and the leopard gecko the primary tumours showed a higher number of mitotic figures and a higher degree of cellular atypia than the visceral metastases. The primary oral tumour of the leopard gecko was amelanotic, while the neoplastic cells in the lungs were moderately pigmented. In the yellow anaconda with recurring tumours the cellular differentiation changed from epithelioid (first dermal lesion) to spindle shaped (first recurrence in skin, second recurrence in skin and visceral metastases). At first, the nuclei showed a homogenous chromatin pattern that changed to a mildly dispersed and finally to a vacuolated pattern.

Clinical Outcome of Reptiles with Melanophoromas

In five cases there was no recurrence of the cutaneous tumour after surgical removal. The animals were monitored by the referring veterinarian over a period of 7 months (last cases) to 7 years. Recurrences were observed in three cases: one directly after surgery and, in the other two, within a period of 7 months after surgery. The animals were subsequently humanely destroyed. In the case of the yellow anaconda with a dermal melanophoroma two recurrences

| Table 4 | Characteristics of metastatic chromatophoromas |
| --- | --- | --- | --- |
| Species | Primary location | Metastasis | Gross findings in viscera |
| Bearded dragon | Skin, Melanophoroma | Lung, gut, liver, kidneys, fat bodies | Dark grey blotches, 0.2–0.4 cm |
| Bearded dragon | Skin, Iridophoroma | Skin, muscle, heart, lung, stomach, gut, liver, pancreas, kidneys, fat bodies, parietal serosa of the coelomic cavity | Firrm, 0.2–1.0 cm white spots and masses |
| Veiled chameleon 1 | Skin, Melanophoroma | Heart, lung, tongue, stomach, gut, liver, spleen, kidneys, bone, fat bodies, parietal serosa of the coelomic cavity, brain | Black spots 0.2–0.4 cm and diffuse black colour of the viscera |
| Leopard gecko | Oral cavity, Melanophoroma | Lung | One grey spot, <0.1 cm |
| Yellow anaconda | Skin, Melanophoroma | Heart, stomach, gut, pancreas, kidneys, uterus, ovaries, fat body | Firm, 1.0 × 1.0 × 0.4 cm mass in the heart, black spots 0.3–0.6 cm in other organs |
| Hermann’s tortoise | Carapace, Melanophoroma | Lung, liver, spleen, kidneys, ductus deferens, adrenal glands, thyroid gland | Mild to moderate diffuse swelling of liver and kidneys with black spots, 0.2 cm |

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occurred within a period of 21 months. After removal of the second recurrent tumour, the snake was humanely destroyed due to the poor prognosis. Eight reptiles were humanely destroyed directly after diagnosis because of poor prognosis and/or poor state of health. Another three reptiles died a few weeks to a few months after diagnosis, one with visceral metastases (Hermann’s tortoise) and two of secondary diseases (bearded dragon and pigmy rattlesnake). In five cases visceral metastases occurred; four of these patients were humanely destroyed and one died. In five cases no follow-up information was available.

Gross Findings in Iridophoromas

Iridophoroma occurred in four bearded dragons, one veiled chameleon and one savannah monitor. The iridophoromas appeared as single white intradermal nodules. The savannah monitor had one white mass (1.0 × 0.5 × 0.5 cm) on its neck (Fig. 5). Two bearded dragons had solitary pale nodules measuring 0.2 cm and 0.4 cm in diameter on the head and throat, respectively. One bearded dragon developed a solitary white mass measuring 3.0 × 2.0 × 2.0 cm on the thorax. Another bearded dragon had a 1.5–2.0 cm ulcerated mass on the ventral side of its tail and further masses in the viscera. In the veiled chameleon the iridophoromas appeared as multifocal white blotches of the skin (0.1–0.3 cm) near the yellow stripes of the thorax and abdomen.

Histopathological Findings in Iridophoromas

In HE-stained slides iridophores contained moderate to large amounts of fine to coarse golden-brown to olive-green pigment granules that were larger than the black pigment granules found in melanophores (Fig. 6). Diagnosis of iridophoromas \( n = 6 \) was based on the detection of numerous birefringent, strongly anisotropic granules within the neoplastic cells when examined under polarized light (Fig. 7). Further histomorphological findings in iridophoromas were not significantly different from the melanophoromas (Table 5). All iridophoromas showed infiltrative growth and inflammation was not found.

Visceral Metastases of Iridophoromas

The female bearded dragon with a tumour at the base of the tail had numerous iridophoromas in several organs (Table 4). The spindle-shaped cell morphology, invasive growth, marked pigmentation and mitotic index (1 per HPF) were similar in all sites. The lymphatic and blood vessels associated with the tumour of the tail contained numerous tumour cell emboli. Visceral metastases affected 11 different organs in this case.

Clinical Outcome of Reptiles with Iridophoromas

In three cases (bearded dragon, savannah monitor and veiled chameleon) surgical removal was curative.
and there was no recurrence of the tumours over a 2-year period. One bearded dragon was humanely destroyed directly after diagnosis because of the poor prognosis of an inoperable tumour and the animal’s poor state of health. In two cases no clinical follow-up information was available.

**Immunohistochemistry**

IHC was performed in 24 of the 26 cases. In four cases IHC was necessary for final diagnosis because the tumours were unpigmented. Treatment with H₂O₂ did not affect the IHC. Melan A labelling was not seen in any of the control tumours, while S100 protein was expressed in the fibrosarcomas, the myxoma and the myxosarcoma, but not in the squamous cell carcinoma.

In all chromatophoromas melan A and S100 protein were coexpressed in varying intensity (Figs. 8 and 9). The median expression intensity (IRS) of 18 melanophoromas was 2.3 (0.2–9.1) for melan A and 4.9 (1.9–9.5) for S100 protein. In the six iridophoromas it was 1.4 (0.2–2.6) for melan A and 4.3 (3.2–5.7) for S100 protein.

**Discussion**

Chromatophoromas appear to be relatively common tumours in reptiles. In bearded dragons melanophoromas represented 21.6% of all tumours in the study and represented 85% of the chromatophoromas found in lizards. To the authors’ knowledge there are no previous reports of melanophoromas in anacondas, pigmy rattlesnakes, southern water snakes, veiled chameleons, leopard geckos or savannah monitors.

In eight cases surgical removal of the tumour was curative. Recurrence was observed in three cases and visceral metastases occurred in six cases. In ten cases the animals were humanely destroyed and three other reptiles died a short time after diagnosis.

The microscopical appearance of the melanophoromas was similar in all species of reptiles (infiltrative growth, spindle-shaped cells, mild to moderate anisokaryosis, 1–2 nucleoli, few mitotic figures and variable pigmentation). These findings are consistent with previous reports (Garner et al., 2004; Irizarry-Rovira et al., 2006; Simpson 2008). Three melanophoromas with an atypical mucinous appearance were identified in bearded dragons. This type of melanocytic neoplasm has not been previously described in reptiles or mammals. The tumours were characterized by the presence of a proteoglycan-rich, PAS-positive mucinous interstitial matrix, which was released into the formalin during fixation. Myxosarcoma was considered as a differential diagnosis, but was excluded due to the presence of pigmentation, the cellular morphology and the expression of melan A. This type of melanophoroma has invasive growth and mild cellular atypia, but does not metastasize to the viscera.

Lymphatic and/or vascular invasion is generally regarded as the best indicator of malignancy (Garner et al., 2004); however, in cases with visceral metastases a significantly higher cellular pleomorphism and increased numbers of nucleoli and mitoses in dermal melanophoromas were also detected. These signs of malignancy were not as pronounced in the visceral sites as in the primary dermal tumours. Cellular atypia is a common feature of malignant tumours in the dog (Spangler and Kass, 2006), but in the present study some well-differentiated dermal chromatophoromas had widespread visceral metastasis, so this feature of malignancy may not necessarily apply to reptilian tumours. Similarly, mitotic activity is an important means of distinguishing between benign

Table 5

<table>
<thead>
<tr>
<th>Species</th>
<th>Shape of cells</th>
<th>Pleomorphism</th>
<th>Mitotic figures/HPF</th>
<th>Degree of pigmentation</th>
<th>Intravascular growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bearded dragon</td>
<td>Spindle shaped</td>
<td>Mild, n = 2</td>
<td>0, n = 2</td>
<td>Marked, n = 4</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate, n = 2</td>
<td>1, n = 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veiled chameleon</td>
<td>Spindle shaped</td>
<td>Moderate</td>
<td>0</td>
<td>Marked</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Mild</td>
<td>0</td>
<td>Marked</td>
<td>No</td>
</tr>
<tr>
<td>Savannah monitor</td>
<td>Spindle shaped</td>
<td>Mild</td>
<td>n = 2</td>
<td>Marked</td>
<td>No</td>
</tr>
</tbody>
</table>

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and malignant melanocytic tumours in mammals. In dogs, but not in cats, >3 mitoses per HPF are usually indicative of malignancy (Goldschmidt et al., 1998). In the present study a mitotic activity of ≥2 per HPF appeared to be associated with malignancy; however, melanophoromas with a mitotic activity of 1 per HPF also showed lymphatic invasion and visceral metastases. The location of melanophoroma in the skin (oral or digital) does not seem to be a prognostic factor in reptiles, as described in dogs (Spangler and Kass, 2006).

In mammals, cellular morphology is not of prognostic significance in malignant melanoma (Goldschmidt et al., 1998). In reptiles the cellular morphology was predominantly spindle shaped; however, epithelioid differentiation was seen in two of the five cases with multiple melanophoromas. Infiltrative growth was present in all solitary and multiple melanophoromas, so this feature does not appear to be a good criterion of malignancy in reptiles. Furthermore, the size of the tumour, the degree of pigmentation and the presence of tumour-associated inflammation were not predictive of the nature of the tumour. Infiltration of single cells or nests of neoplastic melanocytes in the upper epidermis, which is an indicator of malignancy in mammals (Goldschmidt et al., 1998), was not observed in tumours in the present study. In summary, lymphatic and/or vascular invasion and metastases are the best indicators of malignancy in reptiles, followed by a mitotic activity of ≥2 per HPF, greater cellular pleomorphism and increased numbers of nucleoli.

In reptiles, iridophoromas have been reported only in two species of snakes (Jacobson et al., 1989; AFIP, 2000). In the present study, iridophoromas were identified only in lizards, particularly in bearded dragons, with a frequency of 7.8% of total neoplasms. This is also the first report of benign iridophoromas in the savannah monitor, the veiled chameleon and the bearded dragon, as well as of a malignant iridophoroma in a bearded dragon. These tumours were characterized by a white colour after fixation. Microscopically, the amber colouration of the granules in the iridophores was noticeably different from the black melanin granules of melanophores. Furthermore, birefringence was clearly seen in these tumours confirming the diagnosis.

Similar criteria to those described above may be used to distinguish between benign and malignant iridophoromas. According to Gopalakrishnakone (1986) iridophores were normally present in small numbers in the internal viscera of a Chinese softshell turtle (Trionyx sinensis); however, we investigated the internal viscera of 10 bearded dragons by polarized light microscopy and iridophores were not found (unpublished data). This confirms the assumption that the numerous iridophoromas found in the viscera of one of the bearded dragons were visceral metastases.

Melan A is one of the most commonly used melanocytic differentiation markers for human and canine melanomas (Jungbluth, 2008; Smedley et al., 2011). Its specificity in canine melanoma is high (89–92% for melanomas; Ramos-Vara et al., 2000; Ramos-Vara and Miller, 2011). S100 protein is a widely used melanocytic marker with high sensitivity for human melanomas (97–100%) and with slightly lower sensitivity (76%) for canine melanomas (Ramos-Vara et al., 2000; Ohsie et al., 2008). Both, melan A and S100 protein were detected by IHC in all chromatophoromas in the present study. The
specificity of melan A and the sensitivity of S100 protein in melanophoromas and iridophoromas reported in other species were confirmed in the reptile chromatophoromas in this study. The expression pattern and intensity did not vary significantly between melanophoromas and iridophoromas. The detection of S100 protein in melanophoromas was in contrast to the results of other studies (Kusewitt et al., 1997, Irizarry-Rovira et al., 2006) where S100 protein was not detected. This is probably due to the fact that different antibodies and pretreatments were used. Kusewitt et al. (1997) used the same antibodies, but these authors examined a death adder (Acanthophis antarcticus), a species of snake not included in the present study.

Immunohistochemical studies of S100 protein and melan A expression in iridophoromas are not reported. The mild labelling of melan A in iridophoromas is probably caused by the similar origin of both cell lines; dermal chromatophores, including melanophores, xanthophores and iridophores, all derive from the neural crest (Bagnara et al., 1979).

In summary, chromatophoromas in reptiles, especially in bearded dragons, but also in other lizards, occurred more often than assumed by the low number of case reports in the literature and their potential malignancy should be considered clinically. Further studies are necessary to understand the biological behaviour of these tumours in more detail. Melan A is useful for differentiating reptilian amelanotic melanophoromas from other sarcomas.

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