

A new frog of the genus *Mantidactylus* from the rainforests of north-eastern Madagascar, and its karyological affinities

Franco Andreone¹, Gennaro Aprea², Miguel Vences³, Gaetano Odierna²

¹ Museo Regionale di Scienze Naturali, Sezione di Zoologia, Via G. Giolitti, 36, 10123 Torino, Italy
e-mail: f.andreone@libero.it

² Dipartimento di Biologia Evolutiva e Comparata, Università di Napoli "Federico II", Via Mezzocannone, 8, 80134 Napoli, Italy

e-mail: gaetano.odierna@unina.it

³ Institute of Biodiversity and Ecosystem Dynamics, Zoological Museum, Mauritskade 61, 1090 GT Amsterdam, The Netherlands

e-mail: vences@science.uva.nl

Abstract. We describe a new species of the genus *Mantidactylus* found during field inventories in the north-eastern rainforests of Madagascar. *Mantidactylus salegy* sp. n. reaches a snout-vent length of 45-50 mm, has evident dorsolateral ridges and whitish spots on the upper jaws (mainly in females). The vocal sacs in males are distinctly visible and paired subgular, without strongly inflatable areas recognizable between the blackish lateral skin flaps on the throat. This frog exhibits a mosaic of characters previously thought to be typical for either the subgenera *Gephyromantis* or *Phylacomantis*. Therefore, it is a crucial discovery towards better understanding of the relationships between the taxa included in these two subgenera, supporting previous molecular results which indicated their paraphyly. The call of *M. salegy* consists of a rapid series of 8-11 rattling notes emitted at 1000-3500 Hz. We also provide karyological data for *M. salegy*, and for several related species, *M. granulatus*, *M. leucomaculatus*, *M. moseri*, *M. redimitus*, *M. zavona*, and *M. pseudoasper* (attributed to the subgenus *Phylacomantis*) and *M. luteus* (subgenus *Gephyromantis*). In terms of general chromosome morphology and structure, NOR localisation and heterochromatin characteristics, *M. salegy* shows similarities to *M. redimitus*, *M. zavona* and *M. leucomaculatus*, all included within *Phylacomantis*. Our data confirm that NOR position is a phylogenetically informative character.

Introduction

The amphibian fauna of Madagascar is extraordinarily diverse (Blommers-Schlösser and Blanc, 1991): the number of nominal species has been assessed as 182 (by Glaw and Vences, 2000b), but many more undescribed taxa have already been identified: the total number of species may eventually stabilize between 250 and 300. The reasons for this high diversity are incompletely understood. Indications provided by molecular studies upon the

genus *Boophis* (Vences et al., 2002a) suggest that one key step in this process concerns the novelty (in terms of ecological adaptations) of colonisation of rainforest stream habitats.

Inventories in hitherto little-explored regions continue to yield new discoveries, some of which provide fresh insights into the evolutionary biology of the Malagasy anurans and which are crucial for the understanding of the phylogenetic relationships of their inclusive higher taxa (Andreone et al., in press).

In this respect, the bulk of material accumulated during recent survey work in Madagascar — spanning from morphology and bioacoustics to molecular biology and karyology — has turned out to be highly informative.

In some recent papers (Glaw and Vences, 2000a, 2001; Vences and Glaw, 2001; Vences et al., 1997, 2002) available material of two subgroups of the mantellid genus *Mantidactylus* (Dubois, 1992; Andreone, in press) was analysed and partially revised: *Gephyromantis* and *Phylacomantis*. These subgenera include (beside a third subgenus, *Laurentomantis*) the *Mantidactylus* representatives with direct development — or with a presumably similar specialized reproductive mode.

Glaw and Vences (1994) separated both subgenera taking into account characteristics such as different acoustics and aggregating behaviour: (a) mainly along brooks in *Phylacomantis*, indicating some association of breeding with free water; (b) independent from brooks in *Gephyromantis*, in which direct development was demonstrated for at least two species (*M. asper*, *M. eiselti*). Both subgenera are divided into two species groups: (1) *Phylacomantis* into the *M. pseudoasper* group (with free-swimming tadpoles), and the *M. granulatus* group (with still unknown reproductive mode, but probably without free-swimming tadpoles); (2) *Gephyromantis* into the *M. boulengeri* group (small species with diurnal and crepuscular calling behaviour), and the *M. asper* group (larger species with nocturnal calling). Recent discoveries, such as *Mantidactylus tandroka* and *M. schilfi* (Glaw and Vences 2000a, 2001) indicate that some characters previously used to support this classification are equivocal and should be reconsidered. A preliminary molecular phylogeny (Vences and Glaw, 2001) indicated monophyly of the *M. granulatus* group and possible paraphyly of the *M. asper* group.

A survey carried out in a rainforest area around the Anjanaharibe-Sud and Marojejy massifs resulted in the collection of many undescribed amphibians and reptiles, demonstrating that the north-eastern region of Madagascar holds an exceptionally rich and diverse herpetofauna. Noteworthy among these discoveries is one new species of *Mantidactylus* which shows a mosaic of morphological characters of the *M. asper* and *M. granulatus* groups (subgenera *Gephyromantis* and *Phylacomantis*). Herein we describe this new species and explore the chromosome complement in *Gephyromantis* and *Phylacomantis*, a novel data set which may be informative regarding the phylogenetic position among species of these two subgenera.

Materials and methods

Study sites and periods

The type locality is located in the Ambolokopatrika forest ridge, NE Madagascar. This rainforest is situated north-west of the Andapa Basin, between the Anjanaharibe-Sud and Marojejy massifs (Andreone et al., 2000). The vegetation belongs to the domains of East and Central (Humbert, 1955). The corridor appears as a mosaic of fairly intact primary forest, secondary full-grown or half-grown forest, and “savoka” (a degraded formation mainly constituted of herbaceous species).

The new *Mantidactylus* was found at Campsite 3, from 9 to 20 December 1997. The habitat around this campsite is transitional between lowland and mid-altitude rainforest. Beside the specimens from Ambolokopatrika, we also assign to the new species a specimen collected in 1996 next to Besariaka Forest (a classified forest lies at about 60 km southwards of Andapa, delimited at the North by the Anjanaharibe-Sud Massif, and southwards by the Tsararano Chain). Further specimens were observed and photographed (but not captured) at Anjanaharibe-Sud, western slope (Andreone et al., 2000).

According to Goodman and Lewis (1998) the Andapa region (within which the sites are included) has a humid, tropical climate. The mean temperature ranges from 18°C in July to 25°C in February. The annual precipitation is slightly more than 2,000 mm. On average it rains 271 days per year. The “dry” season lasts about two months (September and October), with 41.1 mm and 52.6 mm of rain distributed over 14.7 and 15.1 days respectively.

Capture, acronyms and morphometry

Frogs were located at night with the aid of electric torches, or oriented by the location of calling males. They were euthanised by means of immersion in Sandoz MS222, fixed in 10% formalin or in 90% ethanol, and preserved in a solution of 75% ethanol. Morphological measurements (see caption of table 1 for abbreviations) were taken with a calliper (at the nearest 0.1 mm). Webbing formula follows Blommers-Schlösser (1979). Vocalizations were recorded using a portable tape recorder (Sony TC-D3) with an external microphone, and analyzed with Cooledit software (Syntrillium Corp.). The following acronyms are used: MRSN, Museo Regionale di Scienze Naturali di Torino, Italy; PBZT, Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar.

Karyology

Frogs were injected in the field with 0.01 ml per g body weight of a 0.5 mg/ml colchicine solution. After this we waited for 2–4 hours: then the animals were euthanised. Intestines, spleens, lungs (and testes in the males) were removed and incubated for 30' in a 0.7% sodium citrate solution. In some cases also the left femur was cut to take the marrow. Specimens were kept in the reference collection as complementary vouchers.

Chromosomes were obtained by air drying and scraping (Olmo et al., 1986). Besides conventional staining (5% Giemsa at pH 7), the following techniques were applied: (1) AgNO₃-banding of the nucleolar organizer regions (NORs) (Howell and Black, 1980); (2) staining with the C+G DNA base specific fluorochrome chromomycin A₃ (CMA₃)/methyl green (Sahar and Latt, 1980), with a reduced exposure (a few seconds) to the non fluorescent dye, methyl green; (3) the A+T DNA base specific fluorochrome, Q-banding (Schmid, 1978); (4) C-banding as described by Sumner (1972), incubating the slides for 5 min at 45°C in Ba(OH)₂; (5) in situ digestion with Alu I endonucleases (Mezzanotte et al., 1983). Suitable results were also achieved by staining, either separately or sequentially, with CMA₃ and DAPI after hydrolysis in Ba(OH)₂. Metaphase chromosomes were stained with Giemsa. AgNO₃ and C-banding/Giemsa plates were viewed on a Zeiss® PHOM III phase contrast microscope, whereas the fluorochrome-stained metaphases (CMA₃ and DAPI) were viewed on a Leitz® epifluorescent microscope.

Of each taxon, at least four Giemsa-stained metaphases and two metaphases stained with the used banding methods were analysed. Measurements to determine relative chromosome length (RL, percentage ratio between the length of each chromosome to the total length of all the chromosomes) and centromeric index (CI, ratio between short arm and total length of each chromosome) were taken using the digitized images. In the text, DAPI-positive bands are sometimes referred to simply as “DAPI+”, CMA₃-positive bands as “CMA+”.

Table 1. Morphometric measurements (all in mm) of specimens of *Mantidactylus salegy* sp. n.: HT (holotype), PT (paratype), M (male), F (female), SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), ENL (eye-nostril length), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), FOL (forelimb length), FOTL (foot length), FOTL (foot length including tarsus), IMTL, IMTH (length and height of inner metatarsal tubercle), FGL, FGW (length and width of femoral gland), RHL (relative hindlimb length) gives the position reached by the tibio-tarsal articulation when the hindlimb is aligned along the body: 3, nostril; 6, beyond snout tip; 7, widely beyond snout tip.

Catalogue number	Rank	Sex	SVL	HW	HL	HL	TD	ED	END	NSD	NND	HAL	FORL	HIL	FOTL	FOL	IMTL	IMTW	FGL	FGW	RHL
MRSN A2038	HT	M	47.8	16.2	18.0	3.0	5.9	4.7	2.8	4.5	4.4	14.4	29.4	86.5	38.7	25.9	1.5	1.2	8.5	3.6	3
MRSN A2043	PT	M	45.8	15.5	16.5	2.5	5.2	5.0	2.2	4.4	13.6	29.8	84.4	37.9	25.3	2.1	1.3	8.0	3.5	6	
MRSN A2044	PT	M	47.0	15.5	17.2	3.0	5.3	5.1	2.5	4.2	14.1	29.9	88.3	38.2	26.7	2.3	1.4	7.4	2.9	7	
MRSN A2039	PT	F	49.3	16.3	17.8	2.8	6.1	5.1	2.7	4.2	14.8	29.8	90.8	39.5	26.0	1.7	0.9	-	-	6	
MRSN A2040	PT	F	44.6	15.0	16.8	2.6	6.0	4.5	2.6	4.4	13.2	28.4	86.4	38.0	24.7	1.9	0.8	-	-	7	
MRSN A2041	PT	F	47.0	15.5	16.9	2.5	5.4	4.8	2.3	4.6	14.9	30.3	89.5	39.8	26.9	1.8	1.0	-	-	7	
MRSN A2046	PT	F	50.1	16.8	19.0	2.6	5.6	5.0	2.7	4.5	15.3	33.0	97.1	43.0	28.5	1.9	1.0	-	-	7	
MRSN A2045	-	F	43.3	14.7	17.2	2.8	-	5.3	-	3.7	13.5	28.6	87.4	39.1	25.2	1.9	1.0	-	-	7	

Results

Description of Mantidactylus salegy sp. n. (figs 1-2)

Diagnosis. A species attributed to the genus *Mantidactylus* as featured by the following characters (a) presence of femoral glands, (b) absence of nuptial pads in males. Included in the *Mantidactylus granulatus* group within the subgenus *Phylacomantis*, upon the presence of interocular tubercles, partially connected lateral metatarsalia, and karyological data ($2n = 26$, $NF \geq 50$, NOR located on the short arm of the 6th chromosome pair). It can be distinguished from other species of the subgenus *Phylacomantis* by (a) relatively distinct pattern of inner and outer dorsolateral ridges (vs. absent or very faint, except for *M. tandroka*), (b) paired blackish subgular vocal sacs (vs. single or bilobate subgular, without distinct black edges in folded state in all *Phylacomantis* except for *M. granulatus* and *M. leucomaculatus*). It may be distinguished from representatives of the *M. asper* group (*Gephyromantis*) by combination of: (a) partially connected lateral metatarsalia (vs. separated), (b) large body size (male SVL > 45 mm vs. < 44 mm in all *Gephyromantis* species except for *M. plicifer*), and (c) presence of prominent interocular tubercles (vs. absence in *M. luteus*, *M. sculpturatus* and *M. plicifer*).

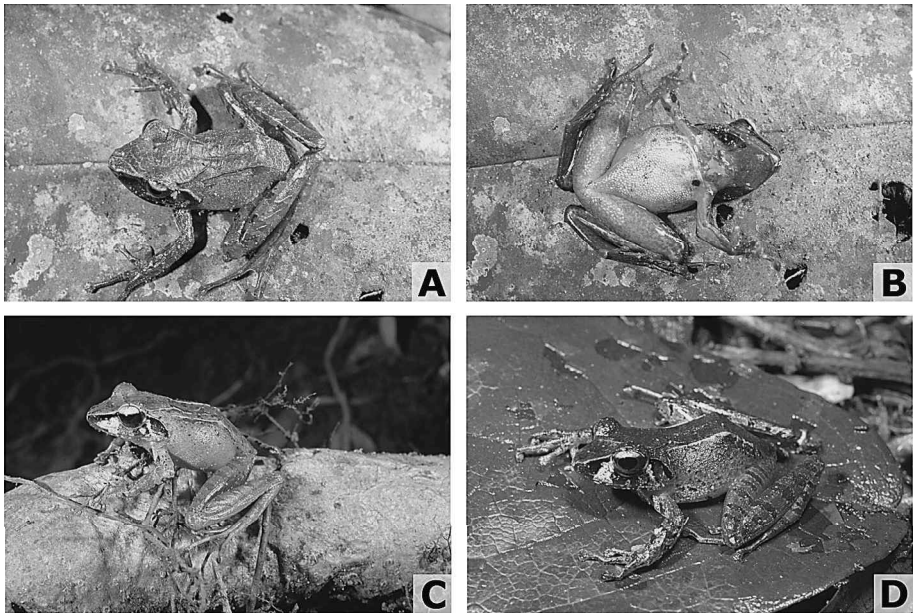


Figure 1. Specimens of *Mantidactylus salegy* in life: A) Holotype (male) from Ambolokopatrika. (MRSN A2038), dorsolateral view; B) ventral view; C) paratype (female) from Ambolokopatrika (MRSN A2039); D) female (not captured) from Analabe Valley, Anjanaharibe-Sud Massif, western slope.

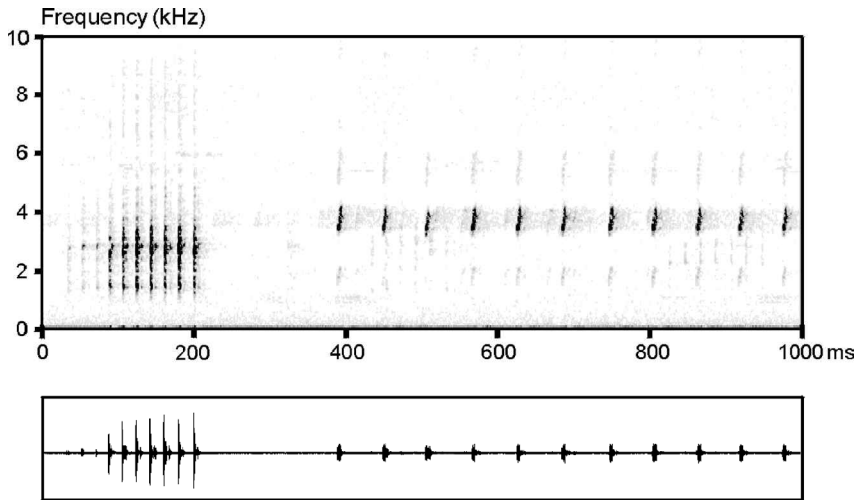


Figure 2. Sonogram (above) and time wave (below) of the advertisement calls in *Mantidactylus salegy* (left; one call composed of ten notes), and syntopic *M. luteus* (right, first 11 notes of a call) at Ambolokopatrika, NE Madagascar (recording temperature 19.5°C).

Holotype. MRSN A2038, adult male, collected in the Ambolokopatrika forest at Campsite 3 (named Antsinjorano), 14°32.6'S, 49°25.8'E, 950-1,250 m, Andapa Fivondronana, Antsiranana Faritany (Province) on 16.XII.1997, by F. Andreone, G. Aprea, and J.E. Randrianirina.

Paratypes. MRSN A2043-2044, two adult males (dissected for karyology), 13.XII.1997; MRSN A2039-2041, females, 9, 15, and 18.XII.1997; MRSN A2046, female, 13.XII.1997 (dissected for karyology); all five specimens collected at the type locality by F. Andreone, G. Aprea, and J.E. Randrianirina.

Further specimens. MRSN A2045, a presumably immature female (only immature oocytes recognizable after dissection) collected at Besariaka Forest, Andapa Fivondronana, Antsiranana Faritany, 14°50.8'S / 49°35.7'E, 965 m, in April 1996, by J.E. Randrianirina. Other specimens attributed to *M. salegy* were observed and photographed, but not collected (see fig. 1d) at Anjanaharibe-Sud, western slope (Analabe Valley), Befandriana Fivondronana, Mahajanga Faritany (Province), Campsite 1, 14°46'S, 49°27'E, about 1,000 m, on 10.II.1996, by F. Andreone, H. Randriamahazo, and J.E. Randrianirina.

Description of the holotype. Adult male in good state of preservation. SVL 47.8 mm; for other measurements see table 1. Body slender; head longer than wide, slightly wider than body; snout slightly pointed in dorsal view, rounded in lateral view; nostrils directed posterolaterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis distinct, straight; loreal region concave; tympanum distinct, rounded, its horizontal diameter 51% of eye diameter; supratympanic fold distinct, regularly curved; tongue

ovoid, distinctly bifid posteriorly; vomerine teeth distinct, in two elongate agglomerations, positioned posteromedially (= posterolaterally) to choanae; choanae rounded. Arms slender; subarticular tubercles single; paired outer and single inner metacarpal tubercles; fingers without webbing; relative length of fingers $1 < 2 < 4 < 3$; second finger distinctly shorter than fourth finger; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender. When hindlimb is aligned along body, the tibiotarsal articulation reaches the nostril; lateral metatarsalia partly connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing of foot 1(1), 2i(1.5), 2e(1), 3i(2.25), 3e(1), 4i(2.5), 4e(2.25), 5(1). Skin smooth on back; distinct but not very elevated inner dorsolateral folds. Very indistinct outer dorsolateral folds; faintly elevated rounded interocular tubercles. Supraocular tubercles are usually absent, excepting for the female MRSN A2040; rudiments of heel spines present; ventral skin smooth on throat and chest, granular on belly; distinct femoral glands, 8.5 mm in length and 3.6 mm in width; in internal view a well-delimited patch of 40 granules of relatively similar sizes (0.7-1.0 mm in diameter); no "humeral gland" visible. The vocal sacs are distinctly visible and are paired subgular, without strongly inflatable areas between the black lateral skin flaps on the throat. After about five years of preservation in ethanol, the holotype assumed a dorsal colouration shading from greyish to light brownish. A transversal darker brown band is visible in the shoulder region, as well as a faint brown interocular band. Dorsal folds and ridges are not distinguished in colouration from the rest of the dorsum, but in this male they appear anyway darkly contoured. The part of the head in front of the eyes is more greyish. The two interocular tubercles are blackish. The lateral part of the head in the canthal region is dark brown, as well as the supratympanic ridge until the shoulder. The tympanum is blackish, and a lighter spot is present just behind the eye and before the tympanum (here named loreal-tympanic spot). The lower border of the jaw is silvery whitish. Limbs are brownish, with dark brown crossbands: 4 on femur, 4 on tibia, 4-6 on tarsus and foot, 4 on lower arm and hand. On the flanks, the dorsal colour fades into the whitish ventral colour. The ventral side is uniformly cream-whitish on forelimbs and belly; the throat is pigmented with brown without a visible marbled pattern, laterally black (corresponding to the external vocal sacs); the hindlimbs have brown pigment which however is not very dense; hands and especially feet are dark brown. Femoral glands are protruding, and of a lighter colour. Colouration in life was relatively similar (fig. 1A-D). The chromatic patterns were much more contrasted, and the belly was more yellowish.

Variation. The two male paratypes (MRSN A2043-2044) are quite similar to the holotype in morphology and colouration (fig. 1C-D): in both specimens the canthal region is dark blackish and unspotted. In A2043 the loreal-tympanic spot is hardly visible, becoming almost invisible in A2044. In both the specimens the overall dorsal colouration is dark brownish, and dorsal plicae are bordered by black. Number of granules of femoral glands of these specimens in internal view was 36-38 (granule diameter 0.6-0.9 mm). The colour in the analysed females is quite different: they are lighter than males and in particular

the latero-posterior part of belly appears translucent yellowish. In most of them a light (whitish) irregular spot is present in the canthal region (here named canthal spot), while the loreal-tympanic spot is very contrasted and evident. Anyhow, MRSN A2046 has a dark unspotted canthal region, thus being quite similar to the observed males.

Etymology. The specific name “salegy” (pronounce: “sow-lai-ghee”) is a Malagasy term referring to a style of folk music and dance typical of northern Madagascar (especially of the Antsiranana Province). The name reminds the rhythmic calls of the species and its occurrence in the North; it is used as invariable noun standing in apposition to the generic name.

Natural history. The new species was observed at Ambolokopatrika in a patch of almost intact rainforest. The males were heard calling during the crepuscular and first night hours (from about 18.00 to 21.00). Later, their acoustic activity became less frequent. Although we do not have quantitative data, we noticed a sort of habitat segregation between *Mantidactylus salegy* and *M. luteus*. The latter usually called in rather open areas, in positions not particularly elevated from the ground (around 1-2 m). *Mantidactylus salegy* called almost always from a higher elevation from the ground (about 2-3 m), usually hidden within shrubs and other dense vegetation. Males were difficult to find also because their calling activity was easily interrupted. They were found quite far from the nearest forest stream (>10 m) and apparently aggregated in some areas, likely due to the presence of shrubs and similar selected vegetation. We do not have data about the type of reproduction in *M. salegy*: the fact that the observed specimens were found quite far from water makes it likely that its eggs undergo direct development. In the female MRSN A2046 (dissected for chromosome analysis), a single ovary remains attached to the body wall. It contains 14 oocytes of a diameter of about 2-2.5 mm. This low number supports the direct development hypothesis. The comparatively small size of the oocytes may indicate that they are not fully mature.

Advertisement calls. The call of *M. salegy* consists of characteristic rattling and sonorous notes repeated at irregular intervals. Each call is composed of a rapid series of notes. Each of these is generally represented by two components, which we here name “pulse groups”. The two pulse groups differ in intensity: the first group is more intense and generally shorter than the second one. Temporal characteristics are as follows: call duration 695-968 ms (808 ± 105 ms, $n = 7$), 8-11 notes per call (9 ± 1 , $n = 7$), note duration 34-53 ms (44 ± 9 ms, $n = 4$), duration of the first pulse group 10-26 ms (18 ± 9 ms, $n = 4$), duration of the second pulse group 23-28 ms (25 ± 3 ms, $n = 4$), duration of the inter-note intervals 42-50 ms (48 ± 4 , $n = 4$). In some calls, the two pulse groups of each note are separated from each other by intervals. In such cases it is difficult to consider them as component of one single note. Temporal characteristics, in these cases, are described as follows: duration of the first pulse group 28-38 ms (34 ± 6 ms, $n = 3$), duration of interval between first and second pulse group 14-20 ms (17 ± 3 , $n = 3$), duration of second

pulse group 20-23 ms (22 ± 2 ms), duration on interval between second and succeeding first pulse group 16-39 ms (24 ± 13 ms, $n = 3$). The first notes of each call consist of one pulse group and are shorter and much less intense than the succeeding notes; note duration is 21-33 ms (26 ± 5 ms, $n = 7$), inter-note interval duration is 48-74 ms (64 ± 10 ms, $n = 7$). Intervals between calls are irregular; three measured intervals lasted 2355-6588 ms. Frequencies ranged 1000-3500 Hz. The calls of *M. salegy* can be easily distinguished by those of syntopic *M. luteus*, which is shown in the same sonagram and time-wave of figure 2. In agreement with data from other populations (Glaw and Vences, 1994; Vences and Glaw, 2001), the calls of *M. luteus* at Ambolokopatrika were composed of 7-22 notes (12 ± 6 notes, $n = 5$). Call duration was 2764-6129 ms (3820 ± 1356 ms, $n = 5$). Note duration was 85-118 ms (99 ± 11 ms, $n = 12$), while duration of inter-note intervals was relatively variable: 146-401 ms (254 ± 100 ms, $n = 12$). This variability was mainly due to the motivational state of the calling specimen and accounted for a large variability in the note repetition rate of 2.4-4.1 notes per second. In some cases, the interval duration decreased within one call, corresponding to a progressive acceleration of the note repetition rate. Frequency bands were recognizable between 1600-2100 Hz, 3750-4500 Hz and 5800-6200 Hz. Dominant frequency was 3800-4200 Hz.

Morphological comparisons with other species. Among the wealth of species in *Mantidactylus*, *M. salegy* is phenetically most similar to the *M. luteus* complex (e.g., *M. luteus*, *M. plicifer*, *M. sculpturatus*) and to the recently described *Mantidactylus tandroka* (Glaw and Vences, 2001). In the *M. luteus* complex (subgenus *Gephyromantis*), both *M. sculpturatus* and *M. luteus* are smaller (male SVL 36-43 mm vs. 46-48 mm in *M. salegy*), and have smaller femoral glands (composed of up to 29 granules vs. 36-40 granules). *Mantidactylus plicifer* has a similar body size and similar femoral gland extension, but is characterized by much more elevated dorsolateral ridges and a different advertisement call. The species of the *M. luteus* complex furthermore lack the interocular tubercles which are visible in all the examined *M. salegy*. *Mantidactylus tandroka* (subgenus *Phylacomantis*) appears to share general morphology (distinct but not elevated dorsolateral folds; interocular tubercles). However, this species is smaller than *M. salegy* (male SVL 39-41 mm vs. 46-48 mm), and presumably has a single subgular vocal sac (no distinct black skin flaps laterally on throat vs. presence), small femoral glands (16 small granules vs. 36-40 large granules), and generally a marbled pattern on throat (vs. a more dispersed brownish pigmentation). The main similarity with *M. leucomaculatus* (which is also included in the subgenus *Phylacomantis*) concerns the presence of light spots (in most female specimens of both the species) in the canthal and loreal-tympanic regions. However, *M. leucomaculatus* is characterized by absence of interocular tubercles and of dorsolateral ridges, and by a comparatively smaller body size: in the sample examined by us (table 2), male SVL was 36.5 ± 2.7 mm ($n = 13$), and female SVL was 41.0 ± 3.1 mm ($n = 7$). It also has a shorter snout and different advertisement calls.

Table 2. Relative chromosome lengths (RL, upper values) and centromeric indices (IC, lower values) in the species studied. Data are given as mean values \pm standard deviations. Zero values correspond to acrocentric chromosomes.

SPECIES	CHROMOSOME PAIR												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Mantidactylus</i>	14.7 \pm 0.6	12.3 \pm 0.8	11.7 \pm 0.5	10.9 \pm 0.7	9.0 \pm 0.6	8.2 \pm 0.9	6.4 \pm 0.5	5.5 \pm 0.5	4.6 \pm 0.6	4.5 \pm 0.3	4.4 \pm 0.5	4.1 \pm 0.5	3.7 \pm 0.4
<i>salegy</i>	41.1 \pm 3.6	35.9 \pm 2.4	37.0 \pm 3.0	29.8 \pm 2.9	48.0 \pm 2.7	30.0 \pm 3.6	31.3 \pm 2.0	36.7 \pm 2.8	23.3 \pm 3.5	46.0 \pm 2.6	43.3 \pm 2.9	32.0 \pm 2.8	45.6 \pm 2.0
<i>Mantidactylus</i>	15.5 \pm 0.9	13.3 \pm 0.8	10.8 \pm 0.8	10.5 \pm 0.5	9.2 \pm 0.4	6.4 \pm 1.0	6.2 \pm 0.4	5.6 \pm 0.7	4.9 \pm 0.6	4.6 \pm 0.4	4.6 \pm 0.7	4.5 \pm 0.3	3.9 \pm 0.5
<i>redimitus</i>	44.2 \pm 4.0	34.9 \pm 2.1	38.0 \pm 3.8	32.5 \pm 2.5	47.1 \pm 2.9	26.1 \pm 3.7	42.3 \pm 2.5	33.7 \pm 2.8	46.3 \pm 2.8	0.0	49.0 \pm 2.7	31.0 \pm 2.9	47.5 \pm 3.0
<i>Mantidactylus</i>	14.7 \pm 0.6	11.9 \pm 0.8	11.3 \pm 0.4	10.4 \pm 0.7	9.6 \pm 0.8	6.2 \pm 1.1	6.1 \pm 0.7	6.0 \pm 0.5	5.3 \pm 0.4	5.2 \pm 0.6	4.8 \pm 0.6	4.6 \pm .5	4.2 \pm 0.3
<i>leucomaculatus</i>	42.6 \pm 2.6	33.3 \pm 2.8	34.7 \pm 2.8	34.3 \pm 3.0	40.8 \pm 2.0	29.8 \pm 3.6	35.9 \pm 2.7	40.5 \pm 2.5	42.4 \pm 2.4	39.4 \pm 2.2	30.4 \pm 2.6	0.0	46.0 \pm 2.4
<i>Mantidactylus</i>	15.3 \pm 1.0	13.9 \pm 0.6	12.7 \pm 0.6	11.6 \pm 0.8	10.0 \pm 0.4	6.0 \pm 2.6	5.8 \pm 2.9	5.8 \pm 2.7	5.4 \pm 2.6	5.0 \pm 2.9	4.6 \pm 2.7	4.1 \pm 2.8	3.9 \pm 2.5
<i>granulatus</i>	38.3 \pm 3.0	32.9 \pm 3.4	34.0 \pm 2.6	30.3 \pm 2.5	44.6 \pm 3.0	41.1 \pm 2.8	0.0	38.7 \pm 3.0	43.8 \pm 2.5	44.5 \pm 2.3	40.4 \pm 2.7	37.0 \pm 2.7	42.5 \pm 2.5
<i>Mantidactylus</i>	14.5 \pm 0.7	13.9 \pm 0.8	11.4 \pm 1.0	10.9 \pm 0.4	9.4 \pm 0.7	6.7 \pm 0.4	6.3 \pm 0.6	5.4 \pm 0.5	5.4 \pm 0.9	5.2 \pm 0.5	4.5 \pm 0.6	4.1 \pm 0.4	4.0 \pm 0.5
<i>zavona</i>	43.3 \pm 2.5	36.8 \pm 2.6	38.0 \pm 3.5	36.5 \pm 2.6	42.4 \pm 3.0	36.1 \pm 2.6	37.0 \pm 2.5	39.8 \pm 3.1	40.4 \pm 2.5	39.9 \pm 2.8	41.6 \pm 2.5	42.0 \pm 2.6	44.0 \pm 2.2
<i>Mantidactylus</i>	16.4 \pm 0.6	15.6 \pm 0.4	11.0 \pm 0.4	10.9 \pm 0.7	9.6 \pm 0.5	5.8 \pm 0.5	5.2 \pm 0.5	4.7 \pm 0.8	4.7 \pm 0.6	4.7 \pm 0.6	4.4 \pm 0.4	3.6 \pm 0.7	3.4 \pm 0.3
<i>moseri</i>	45.3 \pm 2.9	38.9 \pm 3.4	34.0 \pm 3.6	32.5 \pm 2.7	31.0 \pm 2.7	33.6 \pm 3.4	33.6 \pm 3.0	37.5 \pm 2.8	48.5 \pm 2.8	0.0	46.1 \pm 3.0	36.5 \pm 2.5	44.0 \pm 2.0
<i>Mantidactylus</i>	16.0 \pm 0.7	12.0 \pm 0.7	11.7 \pm 0.8	10.7 \pm 0.4	9.8 \pm 0.6	6.0 \pm 0.5	5.5 \pm 0.4	5.5 \pm 0.6	5.4 \pm 0.7	5.4 \pm 0.7	4.4 \pm 0.5	4.0 \pm 0.5	3.6 \pm 0.3
<i>pseudosper</i>	45.0 \pm 3.7	35.3 \pm 3.6	28.4 \pm 2.6	41.4 \pm 2.5	48.0 \pm 2.0	36.9 \pm 2.9	43.1 \pm 2.9	43.8 \pm 3.0	34.8 \pm 2.9	37. \pm 2.7	34.1 \pm 2.9	40.7 \pm 2.5	39.8 \pm 2.4
<i>Mantidactylus</i>	17.1 \pm 1.0	13.3 \pm 0.8	11.6 \pm 0.8	10.3 \pm 0.8	9.7 \pm 0.8	6.0 \pm 0.6	5.6 \pm 0.8	5.3 \pm 0.9	5.0 \pm 0.6	5.0 \pm 0.6	3.9 \pm 0.5	3.8 \pm 0.6	3.5 \pm 0.4
<i>lutens</i>	46.9 \pm 3.7	35.0 \pm 2.5	41.1 \pm 3.9	36.7 \pm 2.9	41.0 \pm 2.5	38.8 \pm 3.8	0.0	19.0 \pm 2.4	0.00	38.0 \pm 2.8	0.0	0.0	41.2 \pm 2.5

Karyology of Mantidactylus salegy and other scansorial Mantidactylus species

We obtained a sufficient number of metaphase plates to perform a karyological analysis from all the species examined, except *M. moseri* in which the low number of plates only allowed to detect the number and general morphology of chromosomes (fig. 3, table 2). Complementary results are provided from the Ag-NOR-, Q-, CMA, C-banding + CMA+, DAPI, and Alu I stainings (figs 4-5). These banding methods produced specific patterns in the examined species.

As summarized in table 3, the total number of chromosomes in all the examined species was constantly 26. The fundamental number varied anyhow from 44 in *M. luteus* up to 52 in *M. salegy*, *M. zavona*, and *M. pseudoasper*. In particular: *M. salegy*, *M. zavona* and *M. pseudoasper* had all the elements biarmed (NF = 52); *M. redimitus*, *M. leucomaculatus*, *M. granulatus* and *M. moseri*, had 12 biarmed pairs and one acrocentric pair (NF = 50). In *M. luteus*, 9 pairs were biarmed and 4 acrocentric (fig. 3 and table 3).

NORs were located as follows: (a) on the long arm, in pericentromeric position of the 6th pair (*M. salegy*, *M. redimitus*, *M. leucomaculatus*, *M. zavona*); (b) proximal to the centromer of the acrocentric 8th pair (*M. granulatus*), in interstitial position of the acrocentric 11th pair (*M. luteus*); (c) in peritelomeric position of the short arm of the 9th pair (*M. pseudoasper*) (fig. 3).

The Q and CMA₃ banding in *M. salegy*, *M. redimitus*, *M. leucomaculatus* and *M. zavona* show the presence of elements with Q+ bands (a pair in *M. zavona*, two in *M. salegy*, six in *M. redimitus* and eight in *M. leucomaculatus*), or elements with CMA+ bands (a pair in *M. redimitus*, *M. leucomaculatus* and *M. zavona*, and six in *M. salegy*) (fig. 4).

After C-banding (fig. 5), all the examined species, excepting for *M. granulatus* and *M. pseudoasper*, showed a variable number of elements with centromeric stain, while telomeric C-bands were absent only in *M. leucomaculatus* and *M. redimitus*. Furthermore, all the examined species had a nucleolus-associated heterochromatin. The centromeric, telomeric, and nucleolus-associated C-bands differed in all the species regarding their positivity/negativity to CMA₃ or DAPI, or for the sensibility/resistance at the action of the endonuclease Alu I.

Discussion

The herpetodiversity of northern Madagascar

In the last years the number of species in the genus *Mantidactylus* increased conspicuously. As it has been stressed in a recent paper (Vences et al., 2002), it is likely that the genus is paraphyletic, and it will be necessary, sooner or later, to elevate to generic rank some of its subgenera. Despite the incomplete taxonomic knowledge, it is clear that within Madagascar there are hotspots for specific diversity and endemism of different *Mantidactylus* groups. As it has been stressed by Lees et al. (1996, 1999), an important area is undoubtedly made

Table 3. Chromosomal characteristics of the examined *Manitadactylus* species: 2n = number of chromosome pairs; FN = fundamental number; NORs = location of NOR; q. = short arm; per = pericentromeric; tel. = telomeric; int. = interstitial A = absent; P = present; + = positive; - = negative. Q- and CMA₃ bands are referred only to presence/absence on the centromeric localisation and the number included in the brackets is referred to number of the intervening chromosomes. *Manitadactylus moseri* was not included for the negative staining results.

SPECIES	2n	FN	NOR	Q-	CMA ₃	C-BANDS														
						CENTROMERIC					TELOMERIC					NOR-ASSOCIATED				
						P/A	CMA	DAPI	Alu 1	P/A	P/A	CMA	DAPI	Alu 1	P/A	CMA	DAPI	Alu 1		
<i>Manitadactylus salegy</i>	26	52	6 th q. per.	P (2)	P (6)	P	+	-	S	P	P	-	-	S	P	+	+	+	R	
<i>Manitadactylus leucomaculatus</i>	26	50	6 th q. per.	P (8)	P (1)	P	+	+	R	A	-	-	-	S	P	+	+	+	R	
<i>Manitadactylus redimitus</i>	26	50	6 th q. per.	P (6)	P (1)	P	-	+	R	A	-	-	-	S	P	+	+	+	R	
<i>Manitadactylus zavona</i>	26	52	6 th q. per.	P (1)	P (1)	P	-	+	S	P	-	-	-	S	P	+	+	+	R	
<i>Manitadactylus granulatus</i>	26	50	8 th q. per.	A	A	A	-	-	S	P	+	+	+	R	P	-	-	-	S	
<i>Manitadactylus pseudoasper</i>	26	52	9 th q. tel.	A	A	A	-	-	S	P	+	+	+	S	P	+	+	+	S	
<i>Manitadactylus luteus</i>	26	44	11 th int.	A	A	P	-	+	R	P	P	+	-	R	P	+	+	+	R	

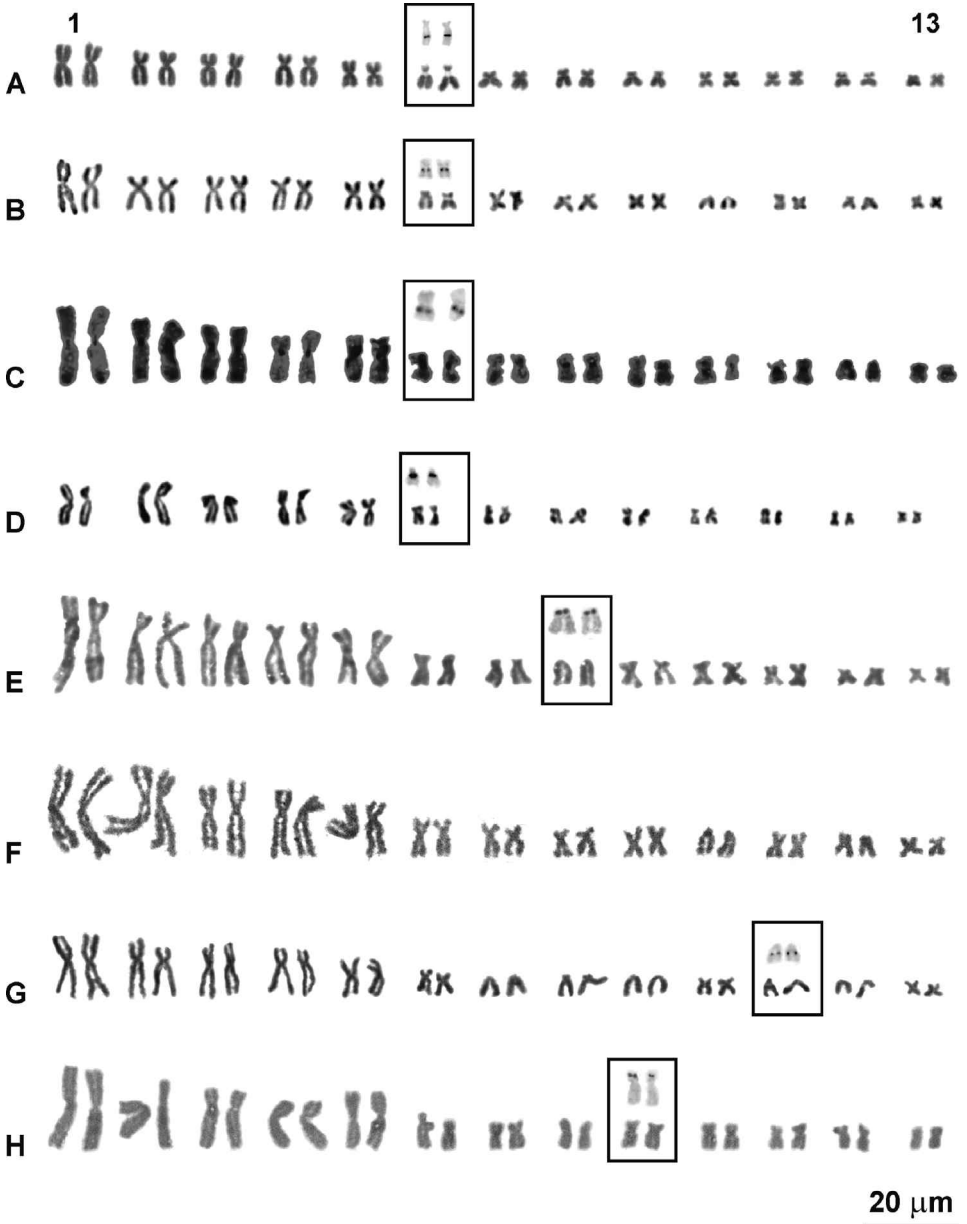


Figure 3. Giemsa stained karyotypes of *Mantidactylus salegyi* (A), *M. redimitus* (B), *M. leucomaculatus* (C), *M. zavona* (D), *M. granulatus* (E), *M. moseri* (F), *M. luteus* (G), and *M. pseudoasper* (H). The squares include the NOR bearing pair (after AgNOR staining). Chromosome pairs are given from first (1) to thirteenth (13) pairs.

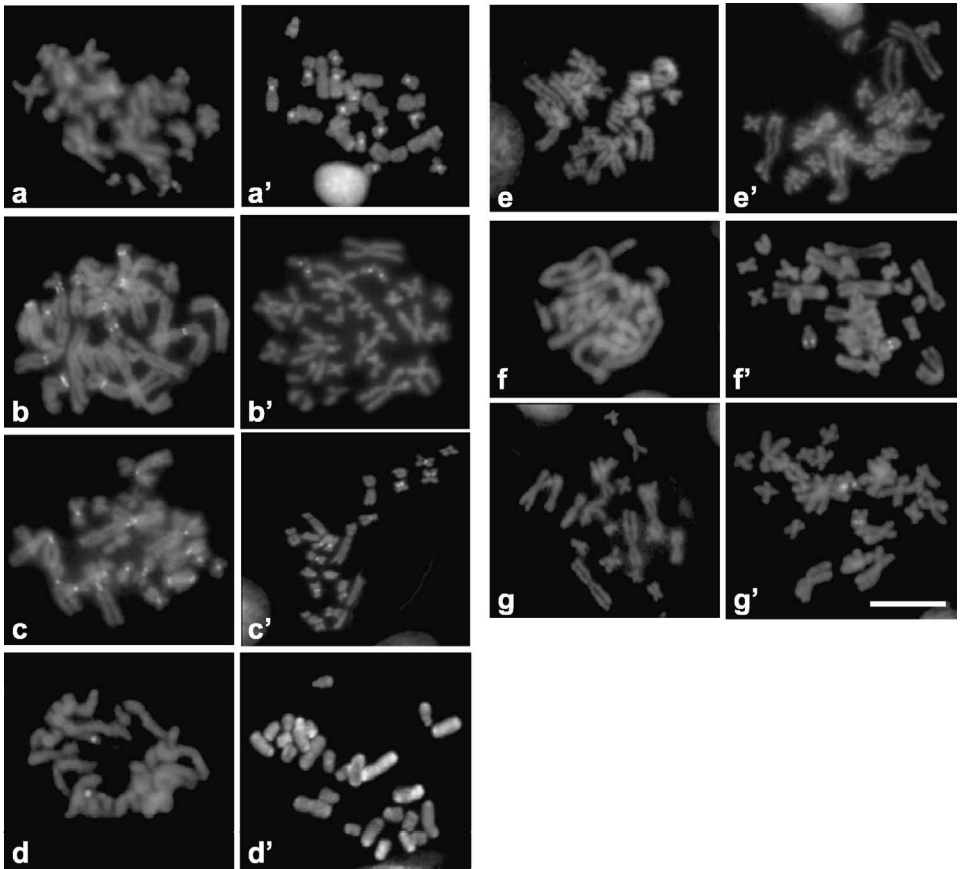
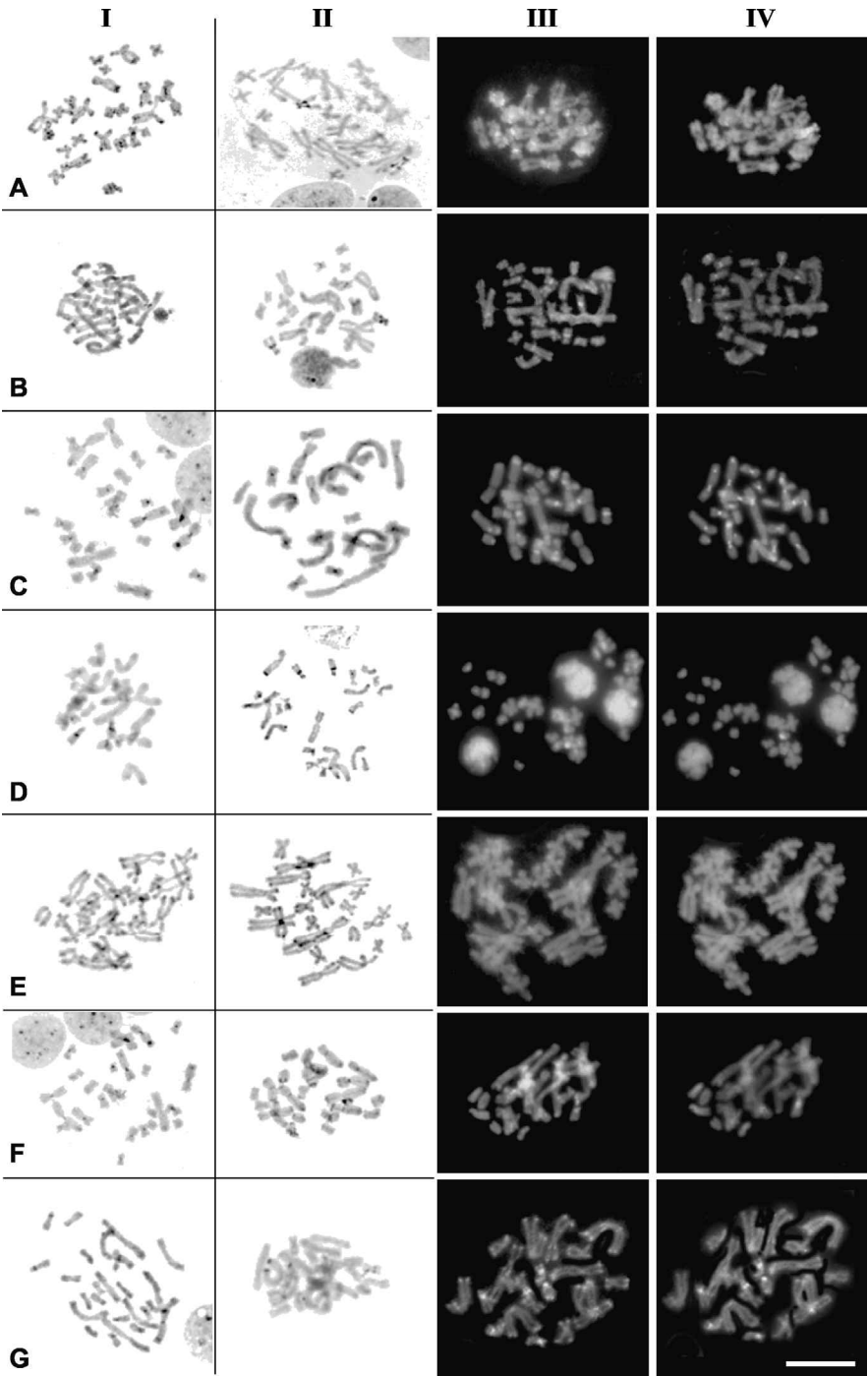


Figure 4. Metaphasic plates of *Mantidactylus salegy* (a, a'), *M. redimitus* (b, b'), *M. leucomaculatus* (c, c'), *M. zavona* (d, d'), *M. granulatus* (e, e'), *M. luteus* (f, f') and *M. pseudoasper* (g, g'). Staining with quinacrine (a, b, c, d, e, f, g), and CMA₃/MG (a', b', c', d', e', f', g'). The white line corresponds to 20 μ m.

up by the forests around Andasibe, in central-eastern Madagascar. This effect, however, is possibly caused by stochastic effects of the presence of both southern and northern faunal elements in the area.

In contrast, the biogeographic regions of the northern third of Madagascar are a hotspot of diversity and endemism of species of the direct-developing clade of *Mantidactylus*. Of the currently 29 species of the subgenera *Gephyromantis*, *Phylacomantis* and *Laurentomantis*, 16 have been recorded from northern Madagascar, a region that is characterized

Figure 5. C-banded (column I), Alu I digested (column II), C-banding + CMA₃ (column III), DAPI (column IV) metaphasic plates of *Mantidactylus salegy* (A), *M. redimitus* (B), *M. leucomaculatus* (C), *M. zavona* (D), *M. granulatus* (E), *M. luteus* (F), and *M. pseudoasper* (G). The white line corresponds to 20 μ m.



by the presence of several mountain massifs (i.e., Tsaratanana, Montagne d'Ambre, Marojejy, and Anjanaharibe-Sud).

So far, *M. salegy* has been found at Ambolokopatrika, Besariaka, and Anjanaharibe-Sud (western slope). All these areas are still non-protected, although they are included in the network of scheduled subregional areas (Anonymous, 2001). Ambolokopatrika and Besariaka are forest bridges between Marojejy and Anjanaharibe-Sud massifs and protected areas and between these and the large Masoala Peninsula. In this respect the conservation of *M. salegy* primarily depends on a careful management of the rainforest areas of NE Madagascar. However, *M. salegy* may also be present in other forests of the considered territory: further research will likely reveal its presence at other sites between Marojejy and Masoala.

Subgeneric attribution of a "mosaic" species

The characteristics of several recently discovered species of *Mantidactylus* indicate that external morphology is sometimes insufficient to establish phylogenetic affinities among these frogs. Some species, in fact, show a combination of phenetic characters formerly believed to be diagnostic for subgenera or species groups. This was the case, for example, in *M. brunae*, which is apparently intermediate between arboreal species of *Spinomantis* (e.g., *M. phantasticus*, *M. aglavei*, *M. peraccae*) and *M. elegans* (Andreone et al., 1998), and in *M. ambohitra*, which is intermediate between the *M. luteus* and *M. asper* complexes within *Gephyromantis* (Vences and Glaw, 2001). *Mantidactylus salegy*, as described herein, is a more extreme example, and highly supports the hypothesis of paraphyly of *Phylacomantis* and *Gephyromantis* which already had been indicated by the molecular data of Vences and Glaw (2001). By its general aspect it reminds a species of the *M. asper* group (subgenus *Gephyromantis*), such as *M. plicifer*, which is of similar size and also has large femoral glands. The blackish paired subgular vocal sac and the dorsolateral ridges are also a characteristic, though not unique, attribute of this group. In contrast, the partially connected metatarsalia and interocular tubercles are typical for several species in the *M. granulatus* group (subgenus *Phylacomantis*). A further relevant character in these groups is the presence of canthal and loreal-tympanic spots which seem to be largely restricted to females. Such markings are known, for instance, in *M. moseri*, *M. luteus* and *M. salegy*, and are extremely expressed in *M. leucomaculatus*. They may play a role in intersexual recognition in these species. Relying mainly on the karyological similarities, we here included *M. salegy* in the subgenus *Phylacomantis*, but a thorough revision and rearrangement of all these taxa is necessary.

Karyotype stability and NOR position as phylogenetically informative characters

A karyotype with 26 all banded elements (fundamental number = 52) is common (and possibly plesiomorphic) in many ranoid taxa (e.g., Kuramoto, 1990; King, 1991). This formula is almost invariably shown in most genera of Mantellidae (e.g., *Aglyptodactylus*,

Laliostoma, *Mantella*, and *Boophis*), with exceptional presence of single acrocentric elements in a few species of *Mantella* and *Boophis* (Blommers-Schlösser, 1978; Odierna et al., 2001). In contrast, in *Mantidactylus* many deviations from this “standard” pattern are known, from the karyotype of $2n = 24$ in the subgenera *Brygoomantis* and *Spinomantis*, to $2n = 30$ (*Ochthomantis*, see Andreone, in press), to the presence of acrocentric chromosomes in the direct-developing lineage (Blommers-Schlösser, 1978). It might be relevant that this variability of the karyotype is apparently restricted to a single monophyletic clade in *Mantidactylus*, which is characterized by brook breeding or further derived reproductive modes (Richards et al., 2000). It may be speculated that the subclades of this lineage have lost their karyotypic stability in the course of their evolutionary history. Our results would support this hypothesis by adding further examples of regular occurrence of acrocentric chromosomes in *Gephyromantis* and *Phylacomantis*, and by our observations of differences among specimens of *M. luteus*, as well as in *M. leucomaculatus* (G. Aprea and G. Odierna, unpublished).

We cannot exclude that this variability is symptom of already occurred or in act speciation, and that several additional species (still undetected using standard methods) can be recognised via karyological analysis. In the present study, as an example, the various banding staining colourations selectively differed among species with apparently similar karyotypes, and the resulting patterns turned out to be species-specific.

The localisation of the nucleolar organiser region (NOR) has been considered as useful taxonomic and phylogenetic marker (Schmid, 1978; Birstein, 1984; Mahony and Robinson, 1986; Odierna et al., 2001). Another interesting synapomorphy which is shared by *M. salegy*, *M. redimitus*, *M. leucomaculatus*, and *M. zavona*, is the presence of at least one pair of elements with Q+ and CMA+ bands, these bands being absent in *M. granulatus*, *M. pseudoasper*, and *M. luteus*. Comparing these states with the phylogenetic data of Vences and Glaw (2001), it is striking that the NOR position on the 6th pair and the presence of Q+ e CMA₃ bands, coincide with well supported lineage in the cladogram. The only exception is *M. granulatus*, which is part of that clade and which is the sister species of *M. zavona* based on the molecular results. Its differing NOR position must therefore be seen as autapomorphy, while the deviating positions in *M. pseudoasper* and *M. luteus* correspond well with their more distant phylogenetic position.

Our ongoing project is to screen karyotypes a large number of Malagasy genera and species, and in forthcoming papers we will provide new data on *Mantidactylus*, *Boophis* and other groups, such as cophyline microhylids. The value of parallel usage of chromosomal techniques and the detection of ecological data stresses once more the high importance of interdisciplinary work to get comprehensive results.

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Appendix

Further Mantidactylus species analysed for comparative karyology

Abbreviations and locations: A2 = Betaolana-Ambolokopatrika Forest, Andapa Fivondronana, Antsiranana Faritany (Province), Campsite 2 (Andranomadio), 14°32.4'S / 49°26.3'E; A3 = Ambolokopatrika Forest, Andapa Fivondronana, Antsiranana Faritany (Province), Campsite 3 (Antsinjorano), 970 m, 14°32.6'S / 49°25.8'E; L = Réserve Naturelle Intégrale de Lokobe, Nosy Be Island, Ambanja Fivondronana, Antsiranana Faritany (Province), 15 m, 13°25'S / 48°20'E; M1 = Masoala Peninsula, Antalaha Fivondronana, Antsiranana Faritany (Province), Campsite 1 (Ambatolelama), 450 m, 15°17.00'S / 50°01.3'E; M2 = Masoala Peninsula, Antalaha Fivondronana, Antsiranana Faritany (Province), Campsite 2 (Beanjada), 620 m, 15°17'S / 49°60'E; M3 = Masoala Peninsula, Antalaha Fivondronana, Antsiranana Faritany (Province), Campsite 3 (Andasin'i Governora), 650 m, 15°18.55'S / 50°0.22'E, 7.XII.1998; M5 = Masoala Peninsula, Antalaha Fivondronana, Antsiranana Faritany (Province), Campsite 5 (Menamalona), 780 m, 15°22.87'S / 49°59.27'E; TS = Antsahamanara, Manarikoba Forest, Marovato Fivondronana, Antsiranana Faritany (Province), 1100 m, 14°02.55'S / 48°46.79'E.

Mantidactylus (Gephyromantis) luteus — MRSN A2074, male, A3; *Mantidactylus (Phylacomantis) granulatus* — MRSN A2071, male, L; MRSN A2072, male, L; *Mantidactylus (Phylacomantis) leucomaculatus* — MRSN A2070, female, M5; MRSN A2078, male, M3; MRSN A2079, female, M3; *Mantidactylus (Phylacomantis) moseri* — MRSN A2073, female, M1; *Mantidactylus (Phylacomantis) pseudoasper* — PBZT 7632, male, M2; MRSN A2075, female, M3; MRSN A2076, male, L; MRSN A2077, female, A2; *Mantidactylus (Phylacomantis) redimitus* — MRSN A2067, male, A2; MRSN A2068, male, A2; MRSN A2069, male, A3; *Mantidactylus (Phylacomantis) zavona* — MRSN A2056, male, TS; MRSN A2054, male, TS.