Madagascan Poison Frogs (*Mantella*) and Their Skin Alkaloids

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ABSTRACT

Mantella is demonstrably convergent with aposematic Neotropical poison frogs of the family Dendrobatidae in presence of a variety of similar skin alkaloids, and variously convergent in a few other traits. *Mantella cowanii* is the best example of color pattern convergence to a *Dendrobates*. Inasmuch as resemblances are primarily to derived features of phylogenetically advanced dendrobatids, the old notion of relationship, although intriguing, is not supported by current understanding. But the phylogenetic placement of *Mantella* is nonetheless unclear and even its relationship to other "mantellines" requires corroboration.

Alkaloid profiles in skin extracts of 9 of 11 recognized species of *Mantella* are reported. Levels of alkaloids ranged from relatively low (*M. betsileo*, *M. expectata*, *M. laevigata*) to moderate (*M. aurantiaca*, *M. crocea*, *M. pulchra*) to high (*M. baroni* (*madagascariensis*, auctorum), *M. cowanii*, *M. viridis*). However, there was variation in levels for different populations of the same species. The major alkaloids in most species and populations were of the pumiliotoxin class, consisting mainly of pumiliotoxins and allopumiliotoxins, although homopumiliotoxins and other pumiliotoxin-class alkaloids also occurred. A decahydroquinoline and related "dimeric" alkaloids were prominent in certain populations of *M. betsileo*, *M. pulchra*, and *M. laevigata*, but were absent in other populations and in other species. "Izidine" alkaloids, namely disubstituted pyrrolizidines, indolizidines, and quinolizidines, were prominent in all populations of *M. baroni*, but were usually relatively minor or absent in other species.

Although the monophyly of *Mantella* is supported by presence of lipophilic skin alkaloids, these compounds may be sequestered from arthropod prey and, if so, are unlikely to be phylogenetically informative within the genus. Interspecific and interpopulational differences in alkaloid profiles may be a reflection of dietary differences correlated with habitat diversity. *Mantella* habitats include upland swamp forest (*M. aurantiaca*, *M. crocea*), boggy areas within forest (*M. pulchra*), upland riverine forest (*M. baroni*), both disturbed and relatively undisturbed lowland forest (*M. betsileo*), and semiarid streambeds (*M. expectata*, *M. viridis*). Although most species are terrestrial, *M. laevigata* is semiarboreal in forest and in bamboo groves.

Excluding published conjectures based on frogs without provenance (from commercial dealers), assumptions of extraordinary intraspecific or intrapopulational variability in *Mantella* color patterns have proven unfounded (unlike the situation in *Dendrobates*), and most species are readily identified in life by coloration alone. The name *Mantella baroni* Boulenger, 1888, is resurrected from synonymy for a widespread upland species currently known as *M. madagascariensis* (Grandidier, 1872)—a nomen dubium not certainly applicable to any known species (but conceivably based on *M. pulchra* Parker, 1925). Use of the name *M. betsileo* (Grandidier, 1872) for a widespread lowland species is accepted with reservation because the type locality falls outside the known distribution and habitat.

INTRODUCTION

All amphibians have cutaneous granular ("poison") glands, that in most species secrete diverse pharmacologically active compounds—including lipophilic alkaloids, which have a peculiar taxonomic distribution in amphibian skin (Daly et al., 1978, 1987, 1993: 276).

The first amphibian lipophilic alkaloids to be chemically elucidated, the samandarines from the European fire salamander (Schöpf, 1961), remain known only from the Old World genus *Salamandra* (for comment on a contrary report, see Daly et al., 1993: 198). Different lipophilic alkaloids were subsequently found to characterize a monophyletic group of several genera of poison frogs within the Neotropical family Dendrobatidae (summary in Myers et al., 1995: 17), with over 200 lipophilic "dendrobatid alkaloids" having now been reported (Daly et al., 1987, 1993: 276).

Prior to 1984, most lipophilic frog alkaloids were known in nature only from dendrobatids (e.g., Daly et al., 1978) until the discovery of some in the South American bufonid genus *Melanophryniscus* (Daly et al., 1984; Garraffo et al., 1993a), in Australian myobatrachids of the genus *Pseudophryne* (Daly et al., 1984, 1990), and in presumptive ranids, rhacophorids, or mantellids of the Madagascan genus *Mantella* (Daly et al., 1984; Garraffo et al., 1993b). Lipophilic alkaloids have not been detected in skin extracts from some 70 other genera of amphib-

ians. A few structurally distinct classes of alkaloids—batrachotoxins, epibatidines, gephyrotoxins, and histrionicotoxins—are still not known to occur naturally in amphibians other than dendrobatids (but see comment under *Mantella pulchra* on histrionicotoxins in a pet-trade specimen). Homobatrachotoxin, however, was recently identified in skin and feathers of a toxic bird from Papua New Guinea (Dumbacher et al., 1992).

Although they represent four families, anurans with lipophilic skin alkaloids share a number of attributes. All are small (1–5 cm SVL), primarily terrestrial frogs that lay terrestrial eggs. The great majority are brilliantly colored (and often boldly patterned) and all except the Australian *Pseudophryne* are diurnal. Many are toothless.

Lipophilic skin alkaloids appear to function defensively and the ability to synthesize and/or uptake and sequester them seems to have evolved at least five times in the Amphibia. The samandarine alkaloids are currently thought to be synthesized by the salamanders (comment in Daly et al., 1993: 199), and the possibility that dendrobatid frogs also synthesize some of their diverse alkaloids cannot be disproved at this time.

Nonetheless, evidence is accumulating that many "dendrobatid alkaloids" are sequestered from some of the small arthropods on which they feed. This discovery originated in the observation that skin extracts of captive-raised dendrobatids contained no alkaloids (Daly et al., 1980, 1992). It was then demonstrated that alkaloids fed to captive-raised frogs were effectively sequestered into skin (Daly et al., 1994a). The ability to sequester alkaloids is absent in the dendrobatid *Colostethus*, which lacks lipophilic alkaloids in the wild. Finally, feeding leaf-litter insects to dendrobatids being raised in terraria led to establishment of significant levels of many, but not all, of the alkaloids present in wild-caught frogs from the leaf-litter locality (Daly et al., 1994b). What arthropods might be the source of alkaloids not detected in dendrobatids that were fed leaf-litter insects is unknown. Indeed, it is still possible that such alkaloids (pumiliotoxins, histrionicotoxins, decahydroquinolines) may be synthesized by the anuran in the wild, but not when raised in captivity.

Sequestering of lipophilic alkaloids provided in the frogs' diet has recently been demonstrated for *Mantella*, which resembles dendrobatids in that frogs reared in captivity on Collembola, *Drosophila*, and *Acheta* do not contain detectable alkaloids (Daly et al., in manuscript). Although it is not known whether the other non-dendrobatid genera (*Melanophryniscus* and *Pseudophryne*) with skin alkaloids have similar sequestering systems, it is now conceivable that many, if not, all frog alkaloids are "second-hand" chemicals that are not manufactured by the frogs themselves. This helps explain some, albeit not all, otherwise puzzling instances of inter- and intraspecific variation in skin toxins, and gives a clearer perspective on the taxonomic usefulness of toxins. Myers et al. (1995), in a preliminary assessment of the systematic implications, pointed out that the underlying genetic mechanisms for alkaloid sequestering provide useful synapomorphies, but that differences in alkaloid profiles of related species may for the most part reflect dietary differences.

Profiles of lipophilic skin alkaloids differ remarkably in different species and populations of frogs containing them (Daly et al., 1984, 1987, 1990; Garraffo et al., 1993a, 1993b; Myers et al., 1995). If sequestering systems and diet are the prime determinants, then alkaloid profiles ought to show strong correlations with habitats and kinds of prey. With this in mind, the occurrence of skin alkaloids in nine species of *Mantella* are presented here in conjunction with general observations on habitats. Specifics of the diet of *Mantella* spp. and of possible interspecific differences in the genetic base of their sequestering systems remain open questions.

SUBFAMILY MANTELLINAE LAURENT, 1946

The correct familial placement of the Mantellinae is uncertain, as discussed below under *Mantella*. The content of the group varies somewhat according to author, but always includes at least the genera *Mantella* and *Mantidactylus* (s.l.). Noble (1931, and *in* Noble and Parker, 1926: 4n) proposed a close relationship between these frogs on the basis of certain anatomical similarities and a Madagascan distribution. Laurent (1946) erected a subfamily to include the Madagascan gen-
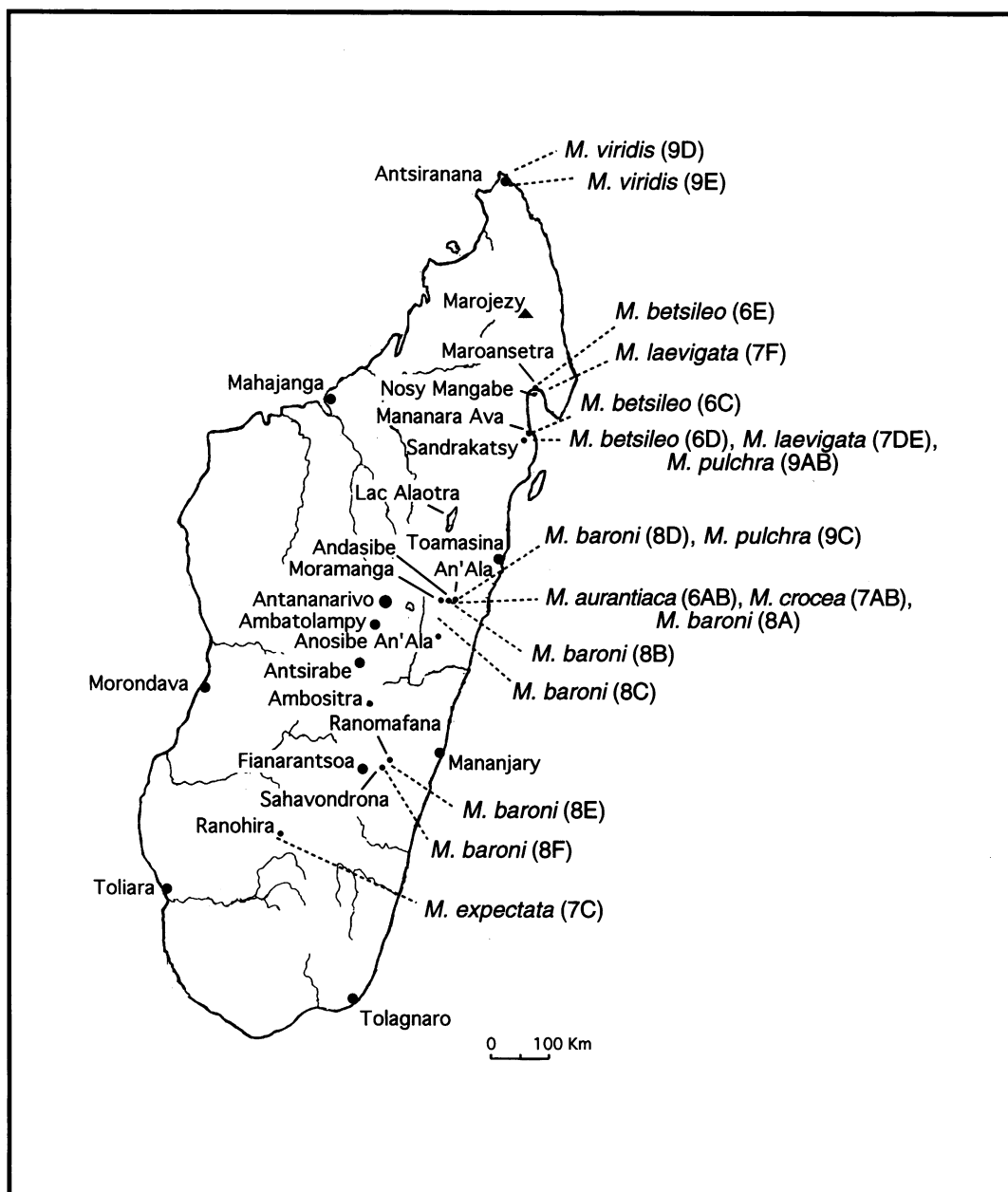
Fig. 1. Madagascar, showing collecting localities for *Mantella* spp. discussed in this paper. The number/letter combinations within parentheses match the population designations in figures 6–9 (gas chromatograms) and in appendix 2.

era *Mantella, Mantidactylus, Gephyromantis, Trachymantis* (= *Laurentomantis*), and the Sri Lankan *Pseudophilautus*.

Except to designate *Mantella* as type genus of the Mantellinae, Laurent's work (1943a,

1943b, 1946) makes no mention of the genus and one assumes that he accepted at face value Noble's proposal that it is related to *Mantidactylus*. Since then, a few authors (notably Liem, 1970, and Guibé, 1978) have not rec-

ognized the subfamily but neither have they been concerned with the relationships of *Mantella* itself. Otherwise, except for doubts concerning the inclusion of *Pseudophilautus* (see Frost, 1985: 439), no one seems to have explicitly questioned the naturalness of the Mantellinae, which has been uncritically accepted as a unit for various phylogenetic analyses (see below). Half a century after Noble first proposed the relationship, Blommers-Schlösser (1979: 65) asserted that:

> The genus *Mantella* is very closely related to the genus *Mantidactylus* (especially to the *wittei*, *depressiceps* and *pulcher* groups, see Table IV for comparison). The cytogenetic data agree with those of these three species groups (cf. Blommers-Schlösser, 1978). The tadpole resembles those of the *wittei* and *depressiceps* groups (cf. Arnoult, 1966). The lateral metatarsalia are entirely connected. The omosternum is widely forked posteriorly and the sternum is slightly forked anteriorly in *Mantella cowanii* (cf. Guibé, 1978) and the femoral glands are diffuse.

In habitus and general color pattern, some *Mantella* are also reminiscent of the several bicolored species of the *Mantidactylus albofrenatus* species group (see p. 160 and color photographs 111–113 in Glaw and Vences, 1994). In fact, it is not clear whether *Mantidactylus* (s.l.) possesses any synapomorphies that would exclude the monophyletic *Mantella*.

Nonetheless, the significance of any phenotypic similarity remains to be elaborated. Convincing synapomorphies still have not been presented to link *Mantella* with any other genus and the monophyly of the Mantellinae must simply be questioned as a matter of course.

The only explicit mantelline synapomorphy proposed by Blommers-Schlösser (1993: 210, 212, fig. 2) is a loosely defined behavioral trait:

> Abbreviated mating contact . . . no real amplexus; the male covers the head and shoulders of the female with his thighs (femoral glands) in arboreal species or embraces the female very loosely, inguinally or axillary, in ground dwelling species, which lasts very short, from seconds to a few minutes. This behavior is known only from the Mantellinae.

But, as noted by Blommers-Schlösser (1993: 210), femoral glands occur in at least a half-dozen [petropedetine and ranine] genera other than mantellines, and one might suspect that the glands correlate with similar mating behavior at least in some cases. Femoral glands, shown as a derived character in two places on her cladogram (her fig. 2, with character 28 as part of nodes D and J), were considered to be a primitive feature in the Mantellinae (Blommers-Schlösser, 1993: 212), although the supposedly correlated mating behavior was proposed as a synapomorphy for the subfamily.

Femoral glands in *Mantella* have not been figured or described to our knowledge. Blommers-Schlösser (1979: 65) said that the glands are diffuse and Blommers-Schlösser and Blanc (1991: 263) indicated that femoral glands are a male character in *Mantella*. Glaw and Vences (1994: 70) indicated that the glands are indistinct or absent in male *Mantella* and absent in females. Cursory examination of some of our voucher material (appendix 1) suggests that the purported glands are coexistent with the patch of granular skin on the underside of the thigh; the granular patch occurs in both males and females, but sexual dimorphism seems evident only in *M. baroni* and *M. betsileo*. In female *baroni*, the granular thigh patch is pale brownish, whereas it is paler and has a more sharply defined perimeter in males. In female *betsileo*, the patch is black and consists of large angular "granules," whereas it is pale brownish and smoother in the males. In these species, male and female specimens could be separated by the naked eye on the basis of differences in thigh granulation. There were no pigmentation differences in a few *M. crocea*, although granulation is perhaps stronger in the female, and there were no obvious sexual differences in *M. pulchra*, *M. expectata*, *M. laevigata*, and *M. viridis* (unskinned males were not available for *M. aurantiaca* or *M. cowanii*). However, in addition to species differences, there is notable individual variation in the granular thigh area at least in *M. expectata*. Some male and female *expectata* have the ventral thigh patch appearing "normally" granular, whereas others have deep parallel grooving in a superficially smoother patch. Strongly granular skin underneath the thigh is commonplace among frogs, but this area seems exceptionally variable in *Mantella*.

Femoral "glands" in *Mantella*, when they occur (e.g., in *betsileo* and *baroni* as described
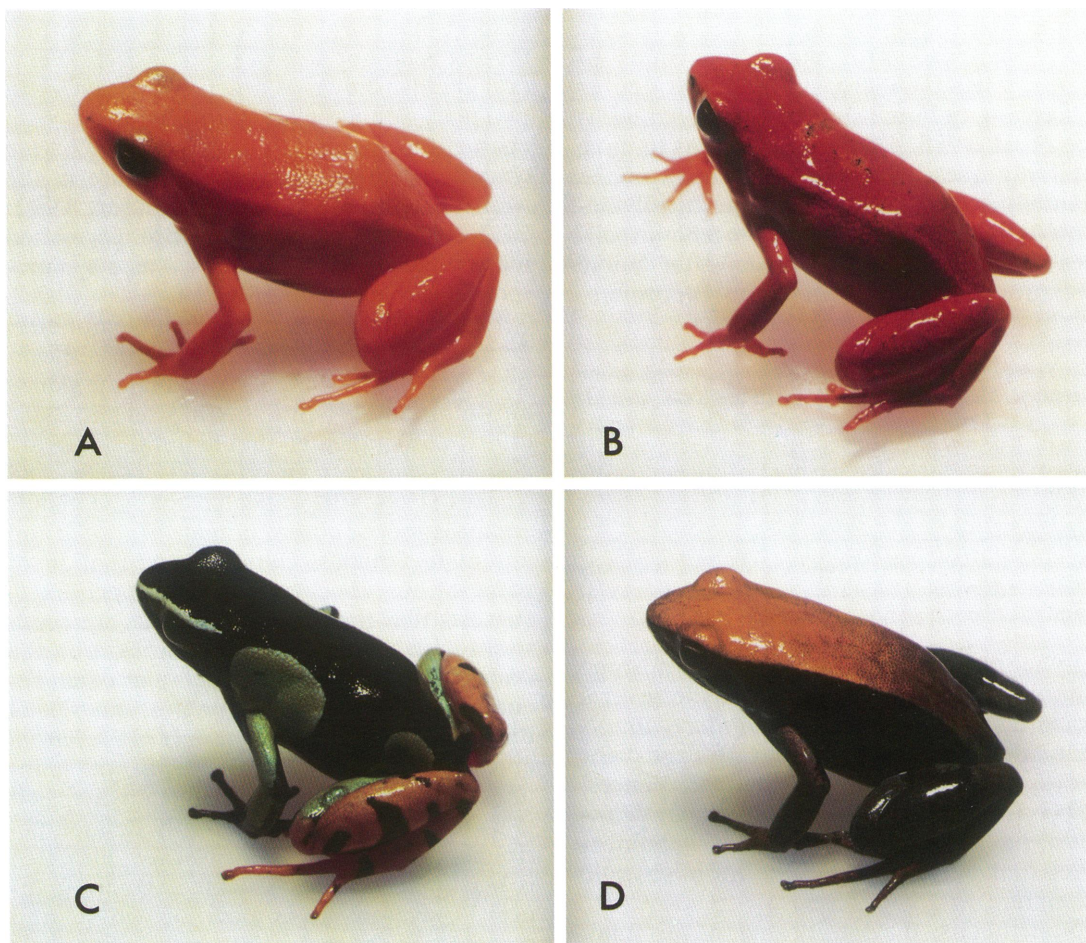
Fig. 2. **A, B.** *Mantella aurantiaca* from the Andasibe region; orange and red variants from different demes (AMNH 136892, ♀ 23 mm SVL, and 136921, ♀ 22 mm SVL, respectively). **C.** *Mantella baroni* (*M. madagascariensis*, auctorum), from An'Ala (AMNH 140556, ♂ 24 mm SVL). **D.** *Mantella betsileo* from Ambavala (AMNH 140573, ♀ 23 mm SVL).

above), bear little superficial resemblance to the often strikingly well-defined glands in *Mantidactylus* (s.l.), some of which have a central pore,[4] and the homology might therefore be questioned. However, Blommers-Schlösser (1979: 35, 44, 65) indicated a similarity in stating that some *Mantidactylus* have the femoral glands "diffused" or not always

visible. Glaw and Vences (1994: 70) tabulated presence-absence and distribution by sex of the glands in the subgenera or species groups of *Mantidactylus,* and histological comparisons with *Mantella* would be useful. The possibility of seasonal variation should also be investigated.

Alkaloids were lacking in three species of *Mantidactylus* (Garraffo et al., 1993b: 1017), although present in the nine species of *Mantella* examined. The possibility that *Mantidactylus* (s.l.) is paraphyletic with respect to *Mantella* is mentioned above, even though no one has made a convincing case for relating these genera. We neither assume nor

[4] Blommers-Schlösser's (1979: 66) schematic drawings of femoral gland variation in *Mantidactylus* (s.l.) are variously misleading in either not explicitly distinguishing the glands from the area of coarsely granular skin on the underside of the thigh or in implying the absence or restriction of such granular skin.
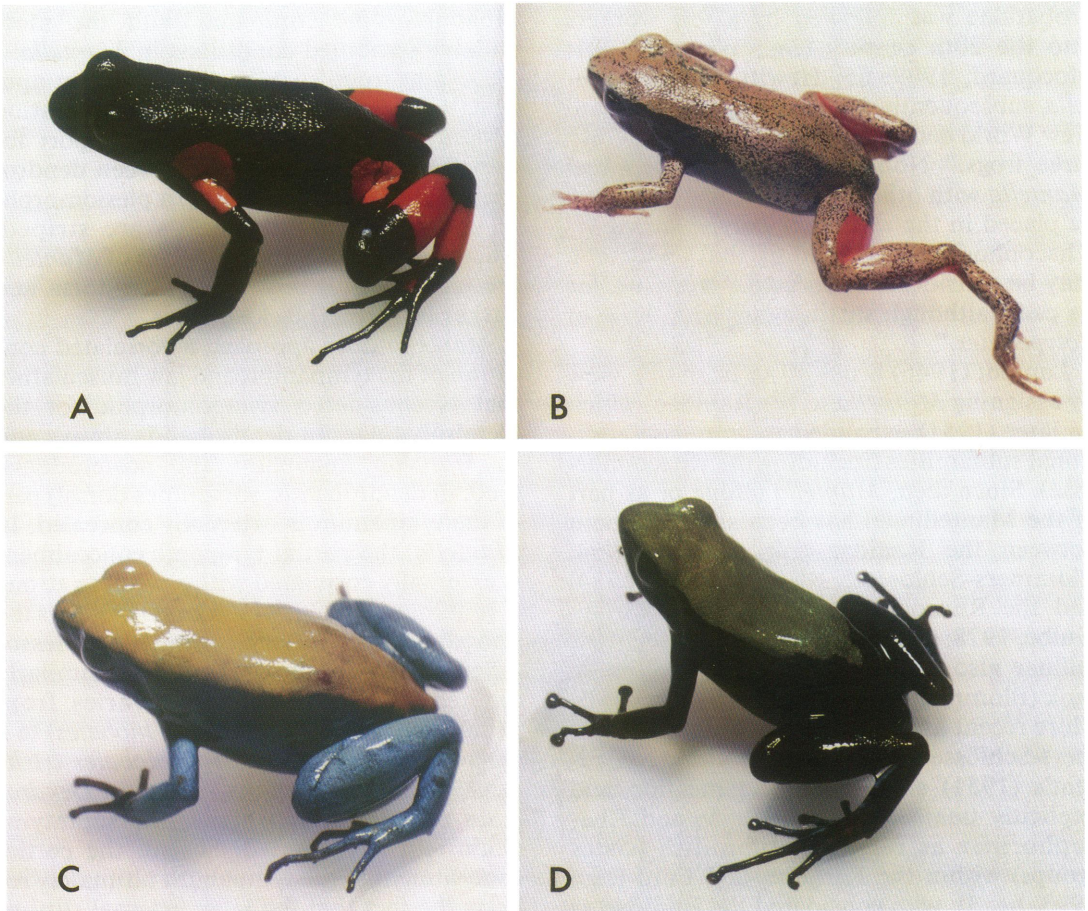
Fig. 3. **A.** *Mantella cowanii* from unknown locality (AMNH 140550, ♀ 26 mm SVL). **B.** *Mantella crocea* from 14–18 km N Andasibe (AMNH 136897, adult ♂, 17.5 mm SVL). **C.** *M. expectata* from Massif Isalo (AMNH 136938, ♀ 26 mm SVL). **D.** *Mantella laevigata* from Ambavala (AMNH 140564, ♀ 24 mm SVL).

reject the monophyly of the Mantellinae in this paper, which focuses on *Mantella* without further reference to *Mantidactylus* (s.l.) or other putative mantelline genera. *Mantella* probably is monophyletic as defined by Guibé (1978: 81–82) and Blommers-Schlösser (1979: 61), and we here suggest that the genetic mechanism leading to presence of diverse lipophilic alkaloids in the skin (whether from sequestering or biosynthesis) is an additional synapomorphy for the genus. But the aposematic frogs of the Neotropical Dendrobatidae are similarly defined (e.g., Myers et al., 1995) and so the questions of relatedness or convergence are revisited below.

Genus *Mantella* Boulenger, 1882

The three oldest species names assigned to this genus were first placed in *Dendrobates* (*betsileo* and *madagascariensis* of Grandidier, 1872, and *ebenaui* Boettger, 1880). Boulenger (1882: 141) erected the genus *Mantella* in the family Dendrobatidae only for these three nominal species, basing his concept primarily on available specimens of *M. betsileo* (which, therefore, was appropriately designated as type species by Liem, 1970: 100). Boulenger (1882: 471–472) named *Mantella cowanii* as an addendum to his new genus. Although the placement within the Den-

drobatidae was followed by a few workers into the 20th century (e.g., Werner, 1901; Mocquard, 1909: 65; Hewitt, 1911), *Mantella* subsequently became aligned not with New World dendrobatids but with Old World "tree frogs." Noble (1931: 524–526) allied *Mantella* with other Madagascan genera that he placed in the redefined Polypedatidae (= Rhacophoridae), concluding that "*Mantella* may be considered a terrestrial tree frog, for its pads, although small, agree with those of *Polypedates*."

Laurent (1946) broke away from this view by assigning *Mantella* to the Ranidae, which he later (1951) expanded to comprise additional subfamilies (including the Rhacophorinae). Since then, *Mantella* (alone or as part of the Mantellinae) has been shuffled about between the Ranidae (e.g., Guibé, 1964; Blommers-Schlösser, 1979; Dubois, 1984) and the Rhacophoridae (e.g., Liem, 1970; Guibé, 1978; Channing, 1989), and the Mantellinae also have been elevated to familial rank (Blommers-Schlösser and Blanc, 1991). More recent analyses include those of Blommers-Schlösser (1993), who returned to Laurent's (1951) subfamilial classification and explicitly treated the Mantellinae and Rhacophorinae as related subfamilies (sister groups) within the Ranidae, and Ford (e.g., 1993: fig. 3), who considered the Ranidae as being nonmonophyletic and regarded the mantellines as being nested within the family Rhacophoridae. To summarize, the mantellines have been on a kind of taxonomic seesaw but have not fallen off definitively on one side or the other.

One of the few things implicit in taxonomic discourse of the last six decades is that mantellines are not closely related to dendrobatids, even though general resemblances between *Mantella* and *Dendrobates* still receive comment. Cited anatomical differences include external and internal structure of the digits (supradigital scutes in dendrobatids, and intercalary cartilages and Y-shaped terminal phalanges in *Mantella*), and thigh musculature (distal tendon of the m. semitendinous pierces that of the m. gracilis complex prior to insertion in dendrobatids but inserts deeper than the m. gracilis in *Mantella*). The aforesaid dendrobatid character states are

synapomorphic compared with the more widely distributed conditions in *Mantella*.

Despite some striking similarities in aposematic colorations (see below), coloration does not provide any obvious support for hypothesizing relationship between dendrobatids and *Mantella*. The most plesiomorphic aposematic dendrobatids are striped, whereas the least colorful species of *Mantella* are bicolor, with brown to green dorsa and darker brown or blackish sides.

Mantella does approach a correlated condition of the tympanum and jaw musculature that is considered synapomorphic for the Dendrobatidae. In dendrobatids always and in *Mantella* frequently, the posterodorsal (and, in dendrobatids, sometimes dorsal) part of the tympanum is externally concealed. In *Mantella*, the partial tympanic concealment is externally correlated with a weak to strong supratympanic fold, which largely follows the underlying anterior edge of the m. depressor mandibulae. In dendrobatids, a similarly aligned supratympanic fold varies from primitively present (e.g., in *Aromobates*) to a vague swelling (e.g., in *Epipedobates trivittatus*) to completely absent (e.g., in *Dendrobates pumilio*). But whether the supratympanic fold is present or absent, the partial concealment of the tympanum ultimately reflects the fact that the large external slip[5] of the m. depressor mandibulae partially overlaps the tympanum in dendrobatids (Myers et al., 1991: fig. 5), and, similarly overlaps or else skirts the edge of the tympanic ring in *Mantella*. The usual condition in *Mantella* (i.e., in the nine species in appendix 1) is for

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[5] The m. depressor mandibulae is similarly configured in dendrobatids and *Mantella*. A large superficial slip originates broadly from the dorsal fascia, completely concealing a smaller, deeper slip originating from the long otic ramus of the squamosal bone. In *Mantella*, a third slip, not concealed ventrally, originates from the posterior and posteroventral margin of the tympanic ring. In dendrobatids, the third slip is more variable and often poorly defined, sometimes virtually absent or not separable from the slip that originates mainly from the otic ramus (e.g., Myers et al., 1984: 8). Such divisions of the m. depressor mandibulae occur in certain other anurans, but the overlapping of the tympanum by the largest slip may be less common.
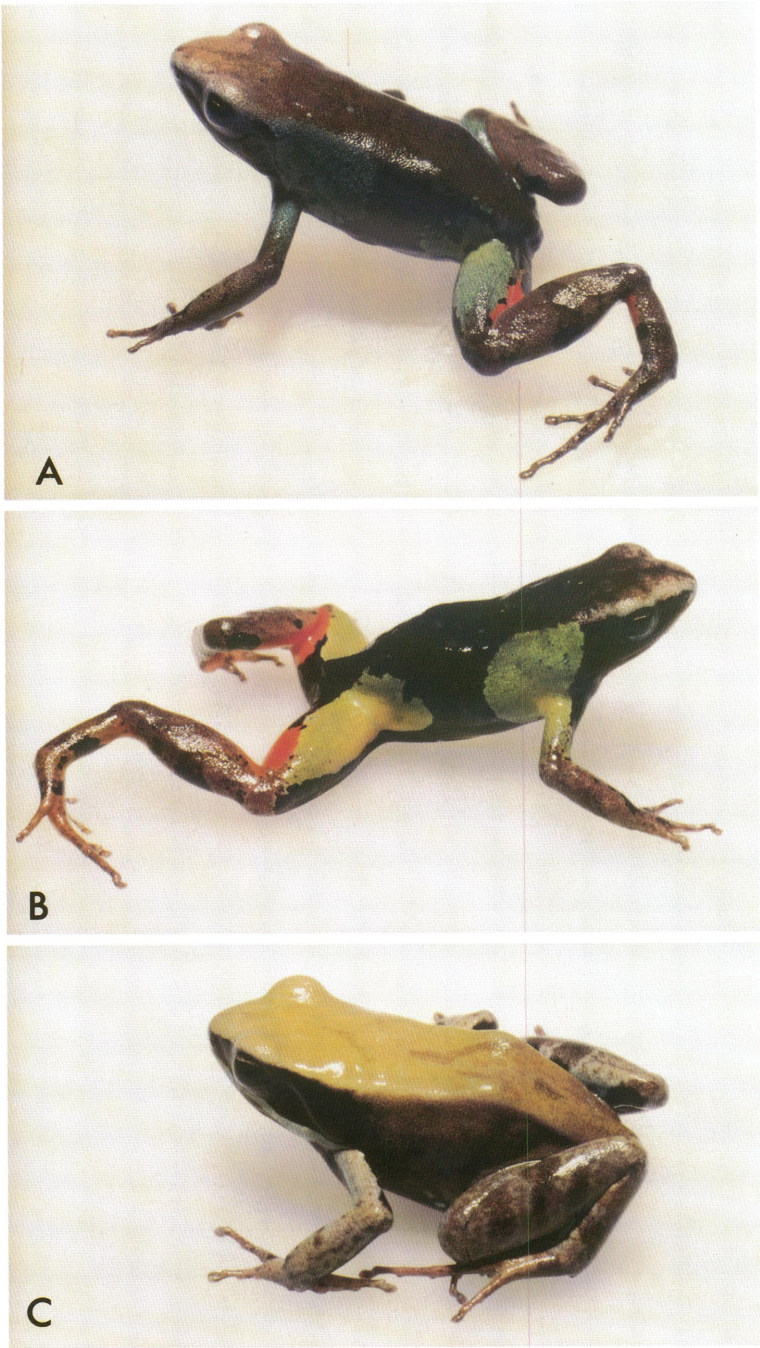
Fig. 4. **A, B.** *Mantella pulchra* from An'Ala (AMNH 136907, ♂ 21 mm SVL, 136908, ♀ 22 mm SVL). **C.** *Mantella viridis* from Montagne des Français (AMNH 140580, ♀ 26 mm SVL).

Fig. 5. Comparisons in dorsal and ventral views of *Mantella baroni* (AMNH 140556) on left side, and *Mantella pulchra* (AMNH 140552) on right. Both from 2 km SW An'Ala. These specimens (♂ left, ♀ right) are equivalent in size (24 mm SVL), but, taking sexual dimorphism into account, *M. baroni* is the larger species.

the depressor muscle to slightly overlap the posterodorsal part of the tympanic ring, but there is no overlap at all in several skinned carcasses of *M. pulchra*, in which any external concealment is due simply to muscle bulk raising the skin away from the tympanum.

Another, even more striking similarity between *Mantella* and the "advanced" (aposematic) dendrobatids is of course the subject of this paper—the shared presence of lipophilic alkaloids. This trait, however, also is shared with Australian *Pseudophryne* and South American *Melanophryniscus*, as first reported by Daly et al. in 1984. Maxson and Myers (1985: 54) noted that dendrobatid antisera showed no cross reactivity with *Mantella* albumins or *Pseudophryne* antisera, which was taken to imply that "any phylogenetic association could be no more recent than some 100–120 million years ago."

Clearly, much remains to be learned about the comparative anatomy and phylogenetic relationships of major anuran groups. Nonetheless, present evidence suggests that aposematic dendrobatids and *Mantella* are monophyletic groups that are derived relative to other dendrobatids and other purported mantellines. All the similarities therefore may be postulated as striking examples of convergence beyond ranoid (or hyloid and ranoid) synapomorphies. More than 60 years ago, G. K. Noble penned the following note to himself:

> It is queer that loss of teeth often runs to iridescent colors. Note *Dendrobates* and *Mantella*. (From *Genera Salientia* [n.d.], a bound volume of typed literature excerpts and hand-written notes, in the AMNH Dept. Herpetology library.)

We can now suggest a few pieces to Noble's puzzle (but in recognition of widespread toothlessness, in dull toads for example, we should reverse his statement—iridescent colors often run to loss of teeth). The bright hues are warning colors advertising the existence of noxious skin alkaloids. Loss of teeth in these cases probably reflects specialization on very small prey, some of which may provide the alkaloids sequestered and accumulated by the frogs. It is an ecological niche entered more than once.

In color patterns, as well as in accumula-

tion of skin alkaloids, *Mantella* is convergent with aposematic dendrobatids in a general way. Thus, the unicolored *Mantella aurantiaca* (fig. 2A) is comparable to a few *Phyllobates* (although the ancestral patterns are very different). Bright flash marks in concealed parts of the limbs of some *Mantella* have their counterparts not only in the dendrobatid genus *Epipedobates* but in a variety of other frogs as well. As long recognized, some *Mantella* are convergent mainly with *Dendrobates* because the iridescent coloring is displayed as rounded markings and even limb bracelets. *Mantella cowanii* (fig. 3A), which resembles some populations of *Dendrobates histrionicus*, is the best example of a *Dendrobates*-like pattern. Species of *Dendrobates*, however, are highly variable in color pattern both within and among populations. Such variation has not yet been clearly demonstrated for any *Mantella* (see below).

Some species of *Dendrobates* (e.g., Panamanian populations of *D. pumilio*) do not uniformly occupy available habitat but occur as dense populations of well-separated demes. It is an intriguing possibility that at least *Mantella baroni* may exhibit similar population structure (see observation by John Cadle on page 18). If so, one might also expect similar territorial behavior (which might also correlate with lack of pronounced sexual dimorphism in size).

The number of recognized species of *Mantella* has about tripled in the last decade (since Frost, 1985), in part due to discovery of new species concurrent with increased fieldwork in Madagascar, and in part due to resurrection of valid species names from synonymy. Much of the taxonomic confusion has involved belief that at least one species is extraordinarily variable (like some *Dendrobates*)—a concept for which the name *Mantella* "*madagascariensis*," has been most recently used, even though Guibé (1964) set this name aside as being unattributable.

Glaw and Vences (1992a, 1994) seem to have renewed the belief that extreme variability in color pattern is expected in single populations of *Mantella*, first (1992a: 166) in their announcement of a "variable colour morph," and then by resurrection (in quotation marks) of the unused name *Mantella*
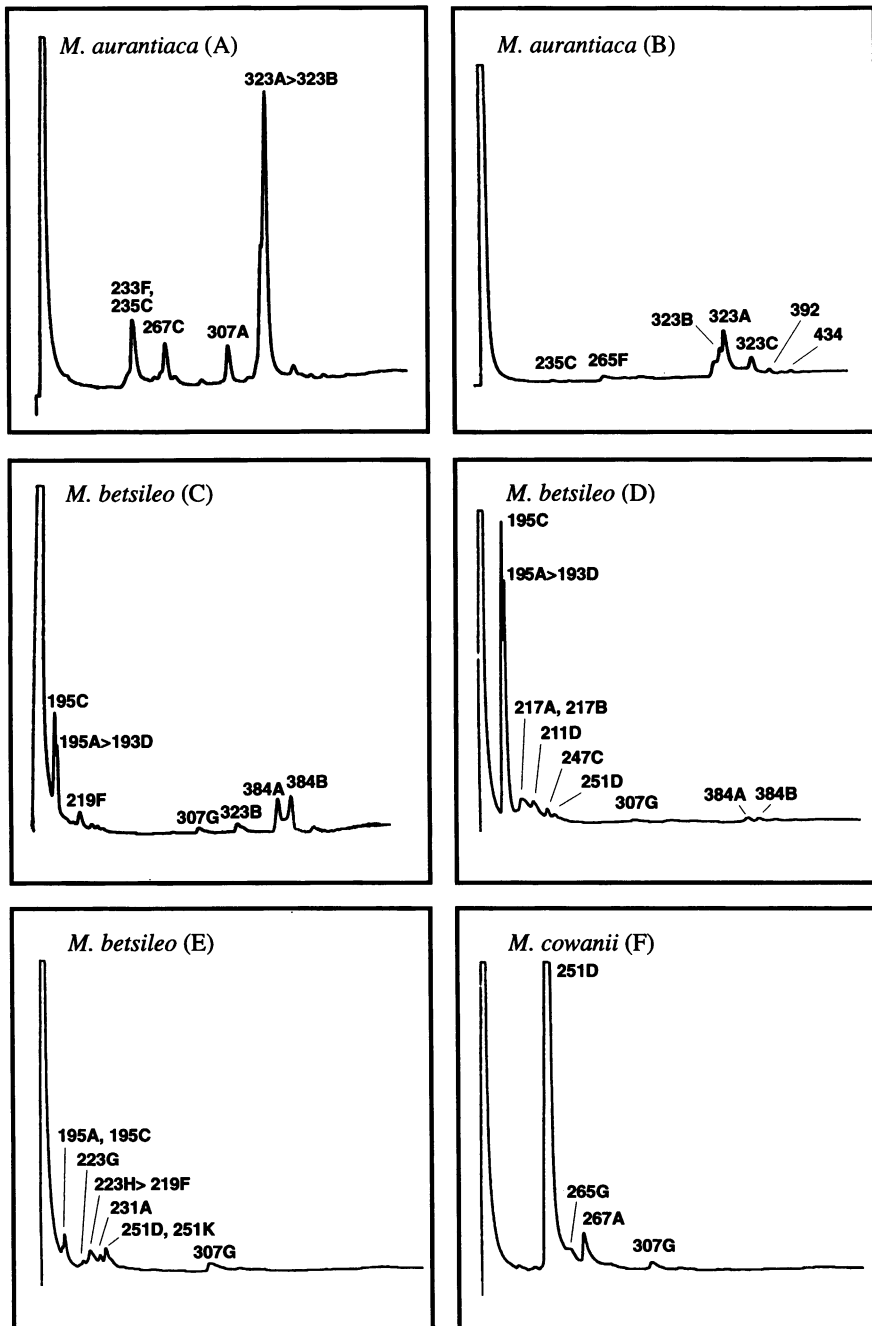
Fig. 6. Gas chromatographic traces showing alkaloid profiles from *Mantella* spp. **A.** *M. aurantiaca* (10 skins, approx. 14–18 km N Andasibe, upland swamp forest, Nov. 1989). **B.** *M. aurantiaca* (5 skins, same region and habitat as preceding, Jan. 1993). **C.** *M. betsileo* (11 skins, Antanambaobe, inland clove tree forest, Dec. 1990). **D.** *M. betsileo* (8 skins, Ambavala, remnant forest around a bamboo grove, Jan. 1994). **E.** *M. betsileo* (12 skins, Farakaraina, disturbed coastal forest, Dec. 1993). **F.** *M. cowanii* (3 skins, from dealer, Dec. 1993).

Boldface designations of alkaloids match those in appendix 2. The OV-1 column is programmed to 280°C at 10°C per minute from an initial temperature of 150°C; other conditions are as given in Daly

loppei (from Roux, 1935) for some highly diverse specimens (1994: 185, black and white figs. 335–337 and color photos 58–60). But most, if not all, of these seem to be frogs from the animal trade and there is no telling how many populations or demes or species might be represented—nor can the possibility of captive-produced hybrids be ruled out a priori.

Commercial and other exportation of *Mantella* (see Glaw and Vences, 1994: 31–32[6]), especially to Europe, has beneficially stimulated much of the recent work on these frogs but also is introducing a new kind of confusion into the literature. Localities, if available at all, are sometimes based on second- or third-hand information that is automatically suspect until verified in the field, and we especially urge authors to indicate *clearly* any locality data provided by dealers (who understandably might be unwilling to share their sources of income).

The number of species of *Mantella* will increase with investigation of possibly unnamed species (e.g., see mention under *M. betsileo* and *M. laevigata*) and discovery of others. But, excluding *Mantella loppei* (see above), the 11 named species of *Mantella* that seem clearly recognizable at this time are as follow:

Mantella aurantiaca Mocquard, 1900
Mantella baroni Boulenger, 1888 (*M. madagascariensis*, auctorum)
Mantella bernhardi Vences et al., 1994
Mantella betsileo (Grandidier, 1872)
Mantella cowanii Boulenger, 1882
Mantella crocea Pintak and Böhme, 1990
Mantella expectata Busse and Böhme, 1992
Mantella haraldmeieri Busse, 1981
Mantella laevigata Methuen and Hewitt, 1913
Mantella pulchra Parker, 1925
Mantella viridis Pintak and Böhme, 1988

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[6] We agree with these authors that controlled exportation and especially habitat management are probably better conservation solutions for *Mantella* than attempted "protection" through CITES listing, which often has the unintentional effect of making research so difficult as to be impractical.

All these have been investigated for skin alkaloids except *Mantella bernhardi* and *M. haraldmeieri*, which are both from southeastern Madagascar. The nine species sampled are shown in figures 2–5 and the localities sampled are mapped in figure 1.

The following accounts summarize field observations[7] and results of alkaloid analyses for each of the nine species. Gas chromatographic profiles are shown in figures 6–9. The occurrence of individual alkaloids of various classes is presented in appendix 2 and the structures of representative alkaloids are shown in figures 10–12.

Mantella aurantiaca Mocquard, 1900
Figure 2A, B

This easily recognized unicolored frog appears to occupy a very small range in the uplands of east-central Madagascar, where its distribution correlates with wet montane forests (Glaw and Vences, 1992a, 1994; Zimmerman et al., 1990). Specimens used for alkaloid analysis were collected with the help of local people in upland swamp-forest habitats in the region north of Andasibe. The widely disjunct swamp forest localities (about 1000 m elev.) were separated mainly by cultivated areas comprising open fields and forest of pine or eucalyptus. The swamp forests themselves contained varying amounts of standing water, grassy hillocks and a variety of trees, including *Pandanus*. Frogs were active throughout such habitat. The general area is located in the region of the type locality, "une forêt entre Beforona et Moramanga" (Mocquard, 1900: 111).

Demes from different swamp forests differed somewhat in coloration, varying from yellow through orange to red, any given color being nearly uniform dorsally and ventrally (including palms and soles), with a red calf spot in the concealed part of the shank that

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[7] Fieldwork was accomplished by the first two authors and local guides, with help at times from the third author and A. Rabemanantsoa.

←

et al. (1993). Emergent temperatures can differ somewhat with different columns and variations in flow rates. Some typical emergent temperatures for some common mantelline alkaloids are: **217B** 166°, **251D** 172°, **267C** 190°, **307A** 216°, **323A** 230°.

Fig. 7. Gas chromatographic traces showing alkaloid profiles from *Mantella* spp.; conditions as in figure 6. **A.** *M. crocea* (10 skins, approx. 14–18 km N Andasibe, upland swamp forest, Nov. 1989). **B.** *M. crocea* (35 skins, same region and habitat as preceding, Jan. 1993). **C.** *M. expectata* (20 skins, Massif Isalo, semiarid streambed, Jan. 1993). **D.** *M. laevigata* (6 skins, Ambodimanga, bamboo grove, Dec. 1990). **E.** *M. laevigata* (5 skins, Varary, bamboo grove, Jan. 1994). **F.** *M. laevigata* (6 skins, Nosy Mangabe, insular forest, Dec. 1993).
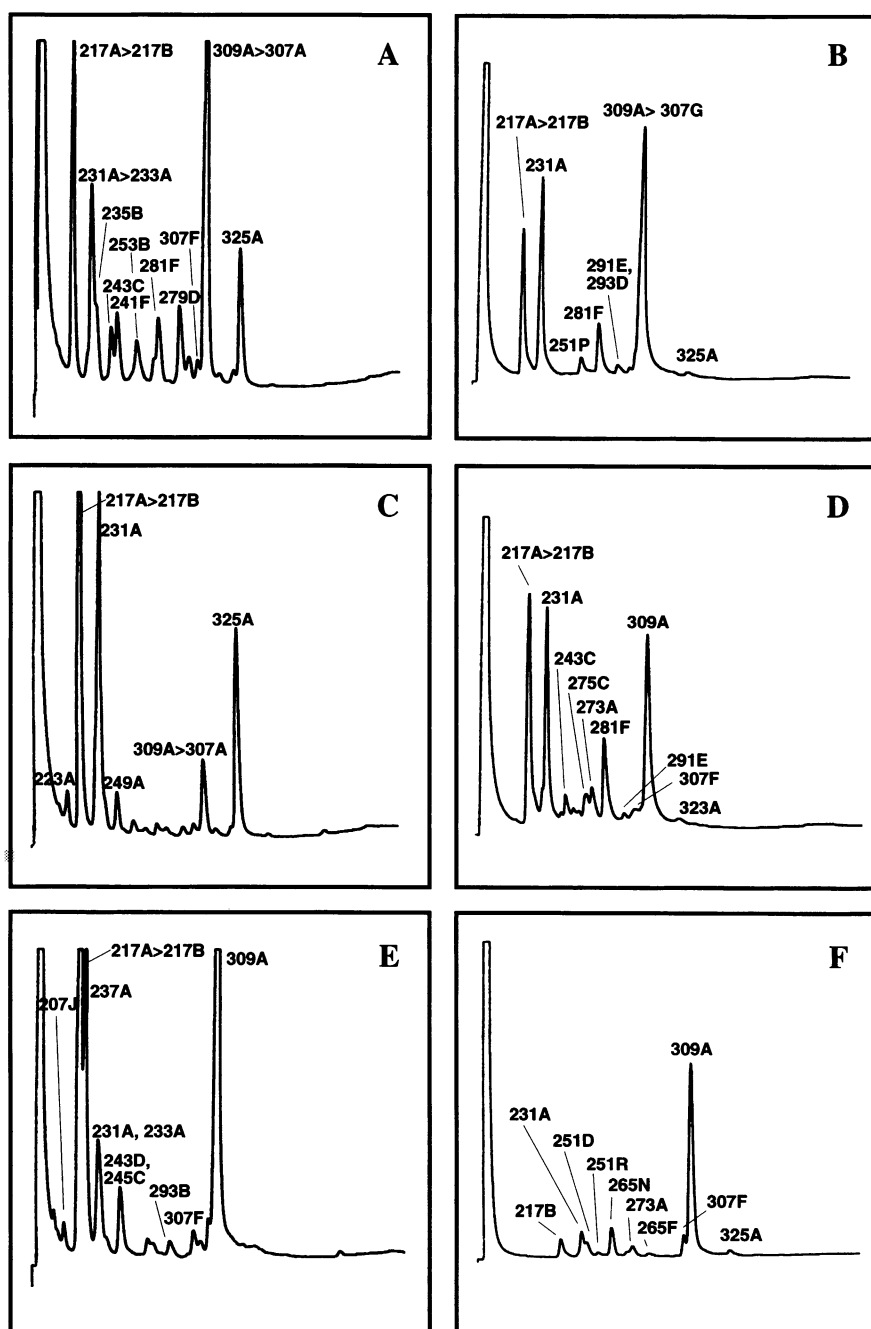
Fig. 8.　Gas chromatographic traces showing alkaloid profiles from *Mantella baroni* (*M. madagascariensis*, auctorum); conditions as in figure 6. **A.** 10 skins, approx. 14–18 km N Andasibe, stream-side forest, Nov. 1989. **B.** 6 skins, approx. 12 km SE Andasibe by road, stream-side forest, Dec. 1993 (see Garraffo et al., 1993b, fig. 2B, for a gas chromatogram of another sample of 10 skins taken at the same stream; compare samples 8B and 8B′ in appendix 2). **C.** 10 skins, 30–35 km S Moramanga, stream-side forest, Nov. 1989. **D.** 3 skins, An'Ala, stream-side forest, Dec. 1993. **E.** 10 skins, Ranomafana, stream-side forest, Nov. 1989. **F.** 17 skins, Sahavondrona, disturbed stream-side forest, Jan. 1993.
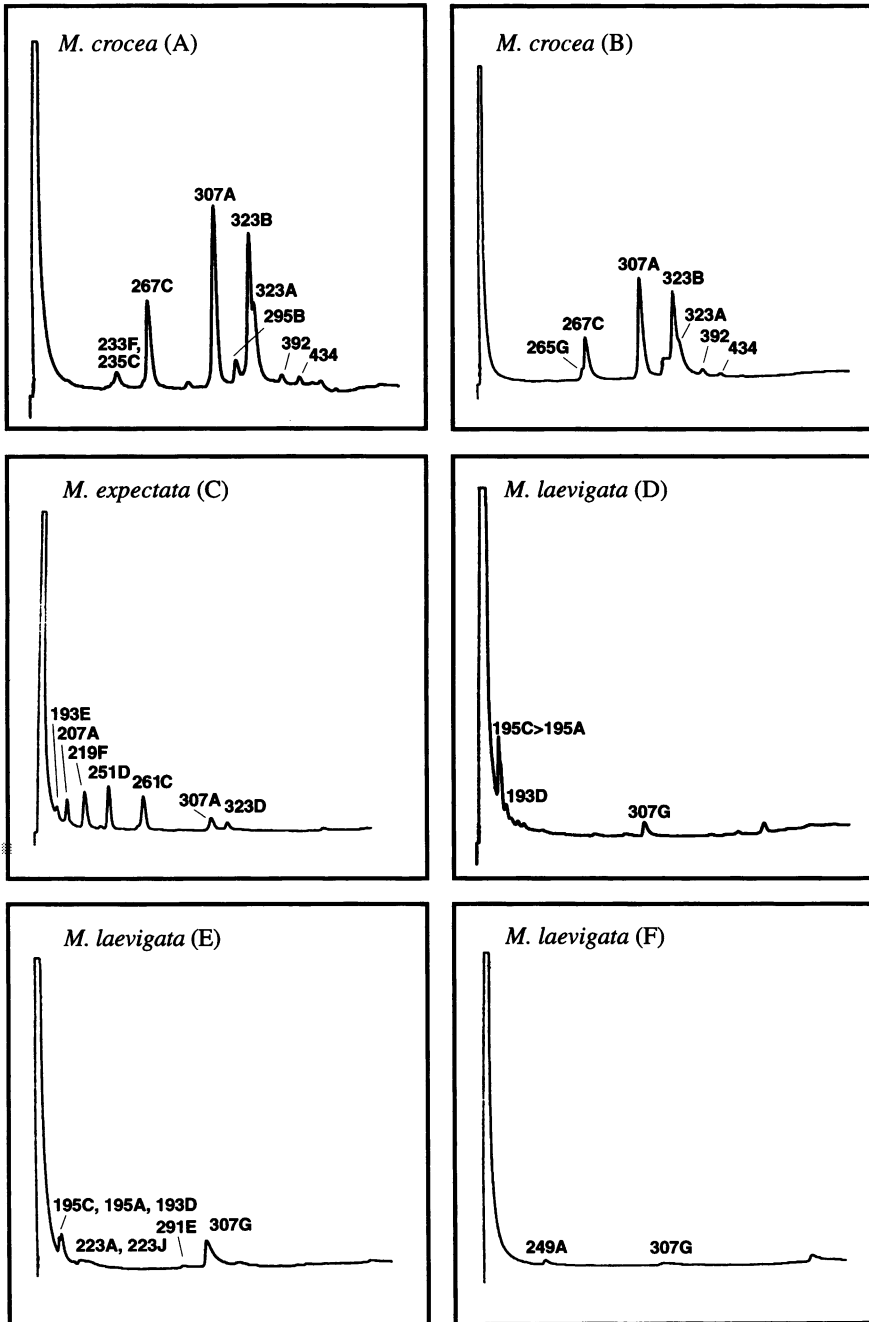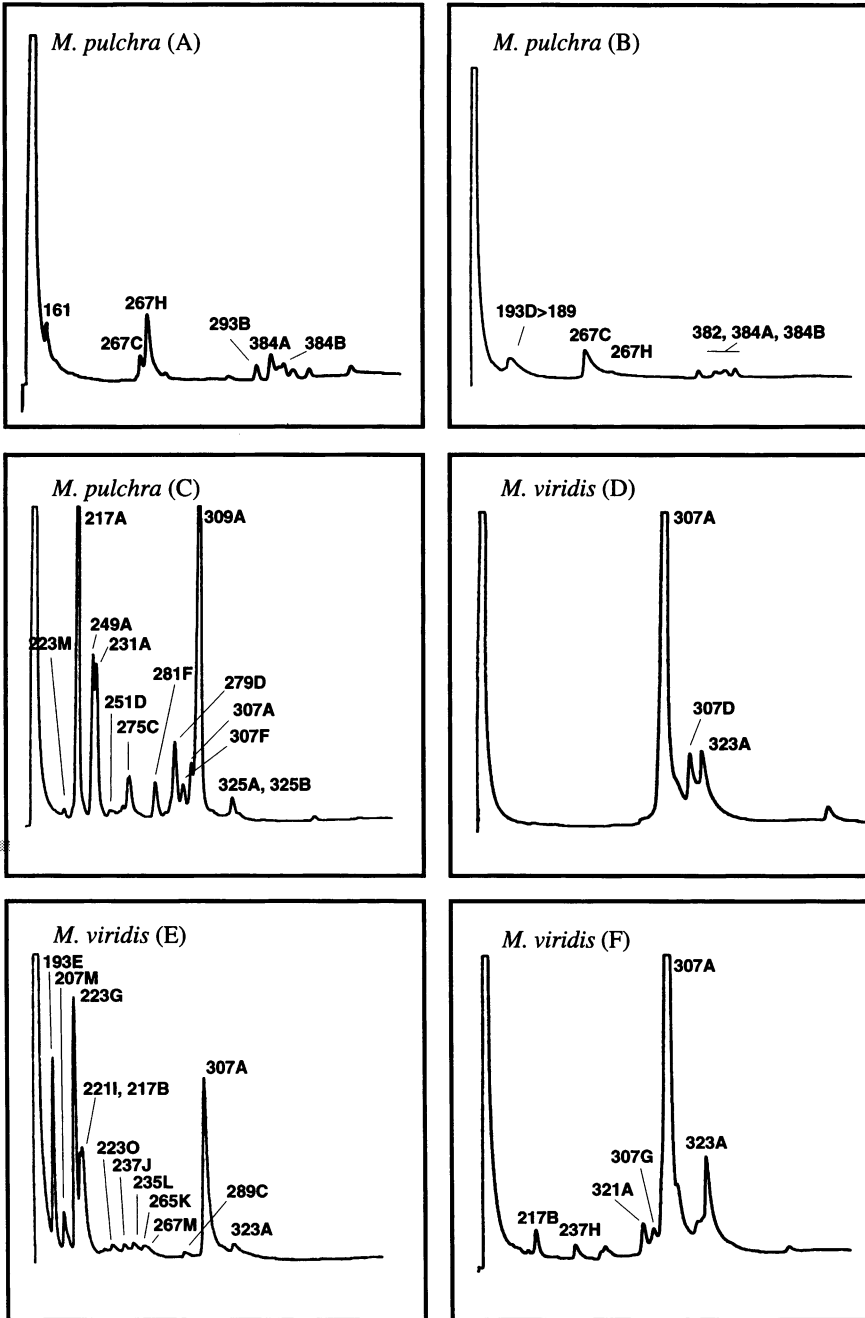
Fig. 9. Gas chromatographic traces showing alkaloid profiles from *Mantella* spp.; conditions as in figure 6. **A.** *M. pulchra*, 6 skins, near Ambavala, boggy ridge forest, Dec. 1990. **B.** *M. pulchra*, 5 skins, same locality as preceding, Jan. 1994. **C.** *M. pulchra*, 5 skins, An'Ala, boggy stream-side forest, Jan. 1993. **D.** *M. viridis*, 30 skins, Montagne des Français, semiarid streambed forest and nearby drainage areas, Jan. 1994. **E.** *M. viridis*, 5 skins, approx. 13 km S Antsiranana, semiarid streambed forest, Jan. 1994. **F.** *M. viridis*, 1 skin, "region of Antsiranana," from dealer, Nov. 1989.

is most evident on yellow frogs. We have seen red-colored specimens of unknown provenance with a black ear spot. The uniformly bright coloration seems highly derived, but the dorsum and limbs of metamorphs have dark markings according to Arnoult (1966: 938, fig. 4), possibly representative of an ancestral pattern.

Skin extracts from seven samples of *M. aurantiaca* all contained mainly pumiliotoxins and allopumiliotoxins. Levels ranged from very high to quite low (fig. 6A,B and data not shown; see also fig. 6A of Daly et al., 1984). Homopumiliotoxins also occurred, but in minor amounts. Varying, but always minor amounts of the alkaloids **235C** and **233F**, proposed to be dehydrohomopumiliotoxins (Garraffo et al., 1993b), were present. One trace alkaloid **323C** appears to represent a new subclass of pumiliotoxins and will be referred to as an "isopumiliotoxin" until its structure can be defined. No decahydroquinolines were detected. "Izidine" alkaloids occurred rarely and only as minor or trace constituents. A 5,8-disubstituted indolizidine **239C** and a putative 1,4-disubstituted quinolizidine **265L** were present in two samples obtained from commercial dealers. Two minor alkaloids **392** and an 0-acetyl derivative **434** represent a new class of alkaloids. Alkaloid **392** has an empirical formula of $C_{22}H_{36}N_2O_4$ and contains the equivalent of six rings or double bonds. It affords a mass spectral base peak at m/z 252 ($C_{14}H_{22}NO_3$).

If the skin alkaloids of *M. aurantiaca* originate from dietary sources, then the swamp forest habitat must provide arthropod prey affording large amounts of pumiliotoxins and allopumiliotoxins, only small amounts of the "izidine" alkaloids, and none of the decahydroquinolines.

Mantella baroni Boulenger, 1888
(*Mantella madagascariensis*, auctorum)
Figures 2C, 5

The name *Mantella baroni* is here resurrected for a species that, as currently recognized, occurs in upland forest over a long geographic range in eastern Madagascar. Based primarily on studies of preserved specimens, the distinctive coloration and color pattern of *M. baroni* has been confused with the patterns of other valid species named be-

tween 1882 and 1981 (*cowanii, haraldmeieri, pulchra*), leading to an erroneous belief in a species of extraordinary variability—the composite species having been known until now as *M. cowanii* (sensu Guibé, 1964, 1978) and *M. madagascariensis* (sensu Busse, 1981; Blommers-Schlösser and Blanc, 1991; Glaw and Vences, 1992a, 1994).

Guibé (1978: 84) had properly considered *Dendrobates madagascariensis* Grandidier (1872) as an "*espèce douteuse*," that is, as a *species indeterminata* or a nomen dubium—a species not identifiable from the original publication or a name not certainly applicable to any known species of frog. He based this decision on the poor condition of the syntypes and on the inadequate original description, which reads as follows (Grandidier, 1872: 10–11):

> ♂ D'un noir bleuâtre uniforme. Abdomen semé de taches d'un bleu clair; cuisses et face interne des jambes d'un beau rouge.
> ♀ D'un beau noir mat avec une tache d'un vert clair velouté à la naissance et sur l'avant de chacun des quatre membres. Abdomen semé de taches d'un bleu ciel. Face interne des jambes d'un beau rouge.
> La peau de ces Dendrobates est finement chagrinée.
> Long. du corps, O^m,022; des membres postér., O^m,032.
> Habit.: Forêt d'Ambalavatou, entre Mananzarine et Fianarantsoua.

Grandidier's emphasis on coloration of the male's thighs and, in both sexes, the concealed part of the lower leg ("thighs and inside surface of the legs a beautiful red") seems more applicable to *Mantella pulchra* than to *Mantella baroni* (cf. colors in fig. 5), but, unless new information is forthcoming, we suggest that the name *Dendrobates madagascariensis* Grandidier be left unapplied, as a nomen dubium as intended by Guibé (1978).[8]

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[8] After writing the above, we obtained the second edition of Glaw and Vences' admirable *Fieldguide*, which was published in December 1994. These authors (p. 403) designated a lectotype of *Dendrobates madagascariensis* because "the paralectotype differs largely from the lectotype and probably belongs to a different species . . . Both specimens are in a very bad state of conservation . . . Some colour patterns are present on the legs (which are separated from the body) [of the lectotype]. . . . These colour patterns partly indicate an attribution to the colour morph which in the past was attributed to *Mantella madagascariensis*, and partly also are similar to . . . *Mantella pulchra*. **No unequivocal attribution of the** *mad-*

Although the type locality of *M. baroni* is only "Madagascar," Boulenger's (1888: 106, pl. 6, fig. 2) description and illustration seem clearly applicable to the present species. Our samples, which span a north-south distance of nearly 300 km (figs. 2C, 5 left), show little variability in color pattern. We know *baroni* as a black frog with a pale, sharply defined canthal-supraocular line, lacking a labial line, a large green lateral blotch that runs onto the arm, and a green inguinal blotch extending onto the posterior flank and continuous with an elongated green blotch covering most of the upper thigh; in vivid contrast to all the green on black, the shank and foot are totally orange with black markings. The shank and foot also are orange below, but all the rest of the ventral surfaces are black with blue markings (fig. 5, left). The green markings may run to yellowish green, and there is said to be a morph with yellow markings but it was not seen by us.

Samples of skins were obtained at six localities (fig. 1), with an additional sample of unknown orgin from a commercial dealer. The habitat varied somewhat, although all specimens collected were found along streams in upland forest in an elevational range of about 800–1000 m above sea level. Collections were made along fairly rapidly flowing streams in relatively undisturbed forest at sites east of Andasibe and near Ranomafana. Other collections were made along small meandering streams in forest exhibiting varying degrees of disturbance at the sites north of Andasibe, south of Moramanga, and near An'Ala. One population was found by a large

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agascariensis **lectotype to any described form is possible** [emphasis added]; we suggest to continue using the name *madagascariensis* for the morph figured on cp. 61. The synonyms *Mantella baroni* Boulenger, 1888, and *Phrynomantis maculatus* Thominot, 1889, belong to this morph and we therefore continue considering these names as synonyms of *madagascariensis*."

Their own conclusions argue against the last action. Except in the rare case of *objective* synonyms (based on the same type specimen), junior synonyms cannot be attributed to an older name of an *unidentifiable* species. Because of the confusion associated with the name *madagascariensis*, nothing is to be gained by petitioning for its conservation.

stream in second-growth forest near Sahavondrona.

John E. Cadle (in litt., August 6, 1995) confirmed the above observations on apparent habitat preference of *M. baroni* but also emphasized that the species is sometimes found in unlikely places:

> In the Ranomafana region (Ranomafana National Park and surrounding areas), *Mantella baroni* is found in a variety of habitats. Although most specimens are found near streams, actual stream-side habitats vary; the recorded elevation in this region is about 700–1200 m. I would characterize most situations as riparian primary montane rainforest; the streams usually have a rocky (as opposed to sandy) substrate, and are generally fast-flowing white-water streams. Another streamside habitat where they were abundant in one area was dense grass (up to > 1 m tall). *Mantella baroni* was also found in swampy areas of little relief with meandering stream courses, and characterized by arborescent *Pandanus* species, many with stilt roots; these areas were often boggy and with sandy substrate. One specimen was collected in primary montane rainforest away from streams. Finally, near the village of Sahavondrona, *M. baroni* was seemingly common on a hillside with a very dry aspect and chaparral-like shrubby vegetation (very likely disturbed); although there were some streams in this area, several mantellas were found out in the open away from streams, and I was surprised to find them in this seemingly stressful environment (they were the only frogs found in that habitat).

> One thing that impresses me overall about the distribution of *Mantella baroni* in the Ranomafana area is the very local nature of populations. They are abundant in small areas, then apparently absent over wide stretches of apparently suitable habitat. This is all the more surprising given the habitat breadth for the species in the general region.

Skin extracts from all samples had moderate to high levels of alkaloids (fig. 8A–F; see also fig. 2B in Garraffo et al., 1993b). The alkaloid profiles varied among populations, but pumiliotoxins and/or allopumiliotoxins were always major alkaloids. "Izidine" alkaloids were also prominent components. The 5,8-disubstituted indolizidine **217B** and the 1,4-disubstituted quinolizidines **217A** and **231A** were present in all samples either as major or minor alkaloids. Other indolizidines and quinolizidines were also present. Homopumiliotoxins often were present as minor components. Other alkaloids occurred in one or more samples. Thus, the skin samples from relatively undisturbed forest by a meandering stream near An'Ala (fig. 8D) and by a cascading rocky stream reached by road east of Andasibe (fig. 8B) contained a major

alkaloid **281F** ($C_{17}H_{31}NO_2$) that was proposed to be a dihydropumiliotoxin (Garraffo et al., 1993b). The 8-deoxypumiliotoxins represent a new subclass of alkaloids described from dendrobatid frogs (Jain et al., 1995). The sample from east of Andasibe contained an 8-deoxypumiliotoxin **291E** ($C_{19}H_{33}NO$) and a previously undetected alkaloid **251P** ($C_{16}H_{29}NO$) of unknown structure. Alkaloid **251P** had a mass spectral base peak at m/z 136 ($C_9H_{14}N^+$) and a major fragment at m/z 122 ($C_8H_{12}N^+$). The skin sample from near Sahavondrona (fig. 8F) contained the pyrrolizidine oximes **236** and **252A**, possibly of millipede origin, and an alkaloid **281G** ($C_{17}H_{31}NO_2$) that is proposed to be an 8-deoxypumiliotoxin.

The skin sample of unknown origin (from a dealer) had high levels of allopumiliotoxins **323B** and **325A** (gas chromatograph not shown). It was lacking the quinolizidines **217A** and **231A** found in all other *M. baroni* samples. It had a previously undetected 3,5-disubstituted indolizidine **211E** ($C_{13}H_{25}NO$), a putative 1,4-disubstituted quinolizidine **265L**, and an alkaloid **392** ($C_{22}H_{36}N_2O_4$) of unknown structure.

An early report (Daly et al., 1984) on alkaloids of *M. madagascariensis* and another (Garraffo et al., 1993b) on *M.* sp., cf. *madagascariensis*, both apply to *Mantella pulchra* and are discussed under that name.

Mantella betsileo
(Grandidier, 1872)
Figure 2D

The name *Mantella betsileo* is being used for a species with an orangish (or yellowish) brown dorsum set off by black sides, with a pale labial line extending to the arm; the specimen shown (fig. 2D) was overall black ventrally (including palms and soles) with a blue line around the lower lip and small, irregular pale blue markings on the venter and under the hind limbs.

As currently recognized, Grandidier's *M. betsileo* appears to have a wide, nearly circumcoastal range (Kuchling, 1993, map), at elevations from sea level to about 500 m (Glaw and Vences, 1994: 177). However, the type locality, "Pays des Betsileos" [land of the Betsileo Tribe], is *inland* and *upland* on

the southeastern plateau—west of the eastern forest zone on the escarpment and therefore well inland from the eastern lowlands (maps in Grandidier, 1893; Labatut and Raharinarivonirina, 1969; Methuen and Hewitt, 1913; Sibree, 1880)—which causes us considerable concern and leads us to question whether the name *M. betsileo* is being correctly applied. A search for existing *Mantella* habitat along Grandidier's route would seem worthwhile. Grandidier (1893) provided a detailed route map of his 1869–1870 expedition, including travel in the Betsileo territory. A contemporary account of the territory and its limits was given by Shaw (1875).

Two of the localities reported here are inland from the northeast coast (fig. 1), but still at low elevations. Our collections were made in three quite different habitats, one from an inland clove tree forest (about 100–200 m elev.), one from a disturbed patch of inland forest and second growth around a small bamboo grove (about 100–200 m), and one from disturbed, very open coastal forest (50 m). The inland collections were in the Mananara Reserve area near Sandrakatsy. *M. betsileo* was microsympatric with the semiarboreal *M. laevigata* at the bamboo grove. *M. betsileo* was also seen in microsympatry with *M. pulchra* in nearby, relatively undisturbed forest. One specimen (AMNH 140574) of *Mantella* from that forest seemed in the field somewhat intermediate between microsympatric *M. betsileo* and *M. pulchra*; the possibility of hybridization was considered, inasmuch as the specimen had the dorsal coloring of *betsileo* and the lateral body pattern of *pulchra*. However, it had golden yellow flash marks in the groin and in the calf and lacked bright coloring on the rear of the thigh (compare hind leg colors in *pulchra*, fig. 5, right). AMNH 140574 may represent an unrecognized species, but additional collections are needed; color notes are provided at the end of appendix 1, under *Mantella* sp. Extracts of *M. pulchra* (fig. 9B), but not of *M. betsileo*, were obtained from this site.

All three skin extracts from *M. betsileo* had relatively low levels of alkaloids (fig. 6C, D, E), but alkaloid profiles differed significantly among the three populations. The inland populations (fig. 6C, D) contained a decahydroquinoline (**195A**), accompanied by un-

saturated analogs (**193D**, **189**) and apparent dimers **384A** and **384B**. The "dimers" had empirical formulas of $C_{26}H_{44}N_2$. Pumiliotoxins, allopumiliotoxins, and homopumiliotoxins were minor components of the inland populations. The inland sample from the bamboo grove had a 5,8-disubstituted indolizidine **217B**, and a 1,4-disubstituted quinolizidine **217A** as minor components (fig. 6D). A related quinolizidine **219F** was present as a minor component in the clove tree forest population (fig. 6C). An alkaloid, **195C**, was present as a major component in one inland population (the clove tree forest), but was absent in the other (bamboo grove). The structure of **195C** remains uncertain (see Garraffo et al., 1993b). Thus, the two inland populations were similar in containing the decahydroquinoline **195A** as a major component along with "dimers" **384A** and **384B**, and with pumiliotoxins, allopumiliotoxins, and "izidine" alkaloids as minor components. But the nature of the "izidine" alkaloids was quite different for the two inland populations.

In contrast, the coastal population had the decahydroquinoline **195A** as a *minor* component and the "dimers" **384A** and **384B** were not detected (fig. 6E). Pumiliotoxins, allopumiliotoxins, a homopumiliotoxin, pyrrolizidines, indolizidines, and a quinolizidine were present in the coastal population. An alkaloid **251Q** ($C_{16}H_{29}NO$) of unknown structure was present as a minor component in the coastal population and as a trace component in the inland bamboo grove population. It afforded a mass spectral base peak at m/z 122 ($C_8H_{12}N^+$).

Different sets of small arthropods available as prey in such diverse habitats might be expected to contribute to the marked differences in alkaloid profiles of *M. betsileo*.

Mantella cowanii Boulenger, 1882
Figure 3A

This very distinctive species of the east-central uplands has vivid orange-red limb markings and axillary/groin spots on a pure black body; pale labial and canthal lines are lacking; ventral surfaces are black with a dozen or so pale blue spots. Despite easy rec-

ognition of *M. cowanii*, its taxonomic status has been greatly confused, mainly because, until recently, workers have not seen living specimens, but have attempted to include the pattern of preserved specimens into presumptive variation of other species. Busse (1981) considered it a synonym of "*madagascariensis*," whereas other recent authors have used *cowanii* as a valid name (usually spelled "*cowani*") but have misapplied it to other species.

Böhme et al. (1993) cleared up some of the nomenclatural confusion and provided photographs in dorsal and ventral view of a syntype of *M. cowanii*, concluding that it might be distinct from "*madagascariensis*." The type locality is East Betsileo. Specimens recently found their way into the animal trade, reputedly from upland forest (about 1500 m elev.) near Ambositra (Andrew Clark, personal commun.). And Vences et al. (1994) have reported rediscovery of the species near both Ambatolampy and Fianarantsoa, in the same uplands as Ambositra.

Our specimens also were provided in 1993 by a commercial dealer, who gave an improbable (lowland) locality (Anosibe An'Ala) as source, for which reason we consider the specimens as lacking specific locality data. All the above localities are indicated on the map (fig. 1).

The major skin alkaloid in *M. cowanii* was pumiliotoxin **251D**, which was present at very high levels (fig. 6F). The only other *Mantella* species that had pumiliotoxin **251D** as a major alkaloid was *M. expectata*, a frog from semiarid regions of western Madagascar. In *M. cowanii* other pumiliotoxins and allopumiliotoxin **267A** were present as minor components. All alkaloids appeared to be pumiliotoxins/allopumiliotoxins and deoxypumiliotoxins. No decahydroquinolines or "izidine" alkaloids were detected even as trace components.

The predominance of pumiliotoxin/allopumiliotoxins in the skin of *M. cowanii* suggests that dietary prey available to or targeted by this species at the presumed upland forest habitat contain mainly pumiliotoxin **251D** and related alkaloids. It also suggests that arthropods containing decahydroquinolines or "izidine" alkaloids are absent from the microhabitat.

Mantella crocea
Pintak and Böhme, 1990
Figure 3B

This endemic of northeastern Madagascar is, like *M. aurantiaca*, known only from upland swamp forests (about 1000 m elev.) north of Andasibe. The coloration seems fairly constant and diagnostic (fig. 3B): A black face mask is sharply defined dorsolaterally until midbody, where it falls off obliquely to the venter; a pale labial line extends to the arm. The dorsal surfaces above the black mask and the posterior flanks are contrasting yellowish to orangish brown, ranging from nearly uniform to finely black-stippled, with or without a blackish vertebral line and sometimes also with a weak, grayish hour-glass marking on the back (AMNH 136896). The limbs are colored much like the dorsum, often with faint dark bands on the thigh and shank. The groin and rear of thigh are bright reddish orange, this color also predominating underneath the shank and, variably, on the adjacent ventral surfaces of thigh and foot. The throat and venter are black with very pale bronzy yellow or whitish markings (ventral coloration can be seen in Pintak and Böhme's 1990 description, and in Zimmerman et al., 1990 [as *Mantella* sp.]).

Four separate samples of skins of *M. crocea* were obtained with the help of local collectors. Pumiliotoxins and allopumiliotoxins were major alkaloids as they had been in another other swamp-forest *Mantella* (*aurantiaca*) from this region. Levels ranged from low to moderate (fig. 7A, B and data not shown). Furthermore, as in *M. aurantiaca*, the putative dehydrohomopumiliotoxins **235C** and **233F** were minor components and "isopumiliotoxin" **323C** and alkaloids **392** and **434** were detected. However, all samples of *M. crocea* contained a putative 5,8-disubstituted indolizidine **295B** ($C_{19}H_{33}NO_2$) as a minor component. This alkaloid, which has a mass spectral base peak at m/z 154 ($C_9H_{16}NO^+$), was not detected in any sample of *M. aurantiaca*.

The marked similarity between alkaloid profiles in *M. crocea* and *M. aurantiaca* is not unexpected if arthropod prey from similar swamp forest habitats are the source of their skin alkaloids. However, further fieldwork on the nature of the swamp forests inhabited in this region by *M. aurantiaca* and *M. crocea* are needed. It is not known if they ever occur together.

Mantella expectata
Busse and Böhme, 1992
Figure 3C

This recently described species appears to occur widely in semiarid western Madagascar, where it probably has been confused with *M. betsileo* (see Busse and Böhme, 1992: 60). The dorsum is greenish yellow to yellowish brown, sometimes with a reddish brown suffusion posteriorly, the dorsal color being sharply set off from uniformly black sides; a somewhat variable pale labial stripe extends to the arm. The limbs may be a vivid light blue (photos in Busse and Böhme, 1992; Glaw and Vences, 1992a), varying through grayish blue (fig. 3C) to light or dark brown, with blue coloring remaining on rear of thigh and concealed calf area.[9] The ventral surfaces are overall heavily marbled or spotted pale blue on black, with a tendency for a blue horseshoe shaped marking on the chin.

The type locality of *M. expectata* is 20 km SE Toliara; the frog has also been reported from near Morondava (as *Mantella* sp., Meier, 1986, color photo), with other localities being added by Glaw and Vences (1994: 177). Our collections were made in southwestern Madagascar in the Massif Isalo near Ranohira. The frog occurred in streambeds (about 800 m elev.) that were relatively dry with only standing pools of water. Vegetation along the streambed consisted mainly of grassy hillocks. There were infrequent small trees. The frogs were said to be abundant after infrequent heavy rains, but even calling males were difficult to locate under the dry conditions that pertained when collections were made in January 1993 and January 1994. Both samples were from the same streambed. This

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[9] A few brown-limbed specimens were seen in the sampled population and the limbs of captives changed from blue to blackish brown. Busse and Böhme (1992: 60) noted that this change does not affect the areas of blue on the hidden parts of the hind limbs.

drying bed led to a wet, spring-fed forested drainage area where, quite remarkably, there seemed to be relatively fewer frogs than in the open streambed.

Skin extracts of *M. expectata* contained several pumiliotoxins including **251D**, **237A** and **307A**. Levels were relatively low (fig. 7C and data not shown). One of the pumiliotoxins (**323D**) was a previously undetected member of the class and its structure is uncertain. Several "izidine" alkaloids were present including a 3,5-disubstituted indolizidine **275C**, 5,8-disubstituted indolizidines **207A** and **219F**, and a 1,4-disubstituted quinolizidine **207C**. A tricyclic alkaloid **261C** ($C_{18}H_{31}N$) of unknown structure was present. Another previously undetected alkaloid **197C** ($C_{12}H_{23}NO$) may prove to be a hydroxylated 5,8-disubstituted indolizidine. It gave a base peak at m/z 154 ($C_9H_{16}NO^+$) and a major fragment ion at m/z 96 ($C_6H_{10}N^+$).

The occurrence of pumiliotoxins/allopumiliotoxins as major alkaloids both in a frog from a swamp forest (*M. aurantiaca*) and one from a semiarid streambed (*M. expectata*) suggests that putative alkaloid-containing prey with such alkaloids can occupy a wide range of microhabitat niches.

Mantella laevigata
Methuen and Hewitt, 1913
Figure 3D

This species occurs in the northeastern coastal region and is the only *Mantella* treated in this paper that is semiarboreal in habit, the others being entirely terrestrial. The relatively large finger discs (fig. 3D), emphasized in the original description, are correlated with its scansorial abilities. Glaw and Vences (1994: 180, color photo 48) indicated the existence of a similarly colored, possibly unnamed, species (at Marojezy) that occurs sympatrically with *M. laevigata* but lacks the enlarged finger discs.

The anterior dorsal surfaces of *M. laevigata* are green or yellowish green, in marked contrast to the rest of the black body and limbs, except the rear of the thigh, which was pale blue in the specimen illustrated (fig. 3D); pale labial and canthal lines are lacking. The venter and undersides of the limbs (including palms and soles) were overall black with pale blue markings. Pale markings are weak or absent on the grayish throat and chest, which in preserved specimens are blackish brown to light brown, sometimes but not always in sharp contrast to the black belly.

Three samples of skins were obtained, two from similar inland bamboo groves (about 100 m elev.) in the Mananara reserve near Sandrakatsy and the third from forest at about 100 m on a small coastal island, Nosy Mangabe. Levels of alkaloids from all populations were relatively low (fig. 7D, E, F). All three populations contained pumiliotoxin **307G** as a minor alkaloid. The populations from the inland bamboo groves contained as minor alkaloids the decahydroquinoline **195A** and related unsaturated congeners and trace amounts of "dimers" **384A** and **384B** (fig. 7D, E). The island population did not have the decahydroquinoline class of compounds (fig. 7F). "Izidine" alkaloids were present, but limited in number. The bicyclic alkaloid **195C** of unknown structure was present as a minor component in extract from one bamboo grove population, while the 5,6,8-trisubstituted indolizidine **223A** and the 1,4-disubstituted quinolizidine **223J** were present as minor components in extract from the other bamboo grove population. A 3,5-disubstituted indolizidine **249A** was a minor component in extract from the island population.

Mantella laevigata is an arboreal species that breeds in tree holes (Glaw and Vences, 1992b). Most of the specimens seen by us were 1 to 2 meters off the ground, but a few were on the ground. If its alkaloids derive from its food, then only the inland bamboo grove sites appear to provide prey with decahydroquinoline and related alkaloids. Many alkaloids were shared between the *M. laevigata* and *M. betsileo* collected together at and around a bamboo grove, but the profiles were different (compare fig. 6D and 7E).

Mantella pulchra Parker, 1925
Figures 4A, B, 5

This species has a relatively wide distribution in eastern Madagascar, where it seems to occur from an elevation of about 200 m up to about 1000 m, becoming sympatric at some upland sites with *M. baroni*.

Mantella pulchra is a bronzy brown or

blackish frog with an ill-defined bronze can-thal-supraocular line, no labial line, a large green lateral blotch running onto the upper arm, and a smaller green inguinal blotch confluent with an elongated green blotch covering most of the upper thigh; there is a vivid red or orange flash mark[10] on the concealed part of the thigh and calf. There may be a few dark red spots underneath the thigh, but otherwise the ventral surfaces (including palms and soles) are black overall, nearly uniform or with blue markings overall, including blue edging around the lower lip. The coloration of *M. pulchra* therefore is similar to the larger *M. baroni*, but the two are immediately distinguished by hind-limb pattern—a distinct flash mark in *pulchra* vs. the uniform orange and black coloring in *baroni* (fig. 5). There is a variant in the commercial trade having blue rather than green blotches (Glaw and Vences, 1994: color fig. 63).

Busse (1981: 29) and Blommers-Schlösser and Blanc (1991) considered *pulchra* a synonym of *M.* "*madagascariensis.*" Glaw and Vences (1992a: 164–165) tentatively treated it as a color morph of "*madagascariensis,*" even though they described call differences in sympatry (at Andasibe). In the same year, Andreone (1992) confirmed call and other differences in sympatry at the same general locality (Périnet forest) and recognized that two taxa were involved—*M.* "*madagascariensis*" (*baroni*) and another taxon for which he used the name *Mantella cowani pulchra.* *M. pulchra* is recognized as a species in subsequent literature.

We here report microsympatry of *M. pulchra* and the larger *M. baroni* along a meandering forest stream near An'Ala, at an elevation of about 900 m. *Mantella baroni* occurred throughout the collection area along the stream, whereas *M. pulchra* was more

abundant in stream-side areas that were somewhat boggy. *M. pulchra* was also found about 50 m up the forested slopes in another boggy area. Another very localized population of *M. pulchra* was sampled in a boggy area of ridge forest at Ambavala, in the Mananara Reserve near Sandrakatsy, about 300 km northeast of An'Ala. Additional specimens from a dealer were said to have come from Anosibe An'Ala, some 70 km southwest of An'Ala.

Levels of alkaloids varied in three population samples of skin of *M. pulchra* (fig. 9A, B, C), being quite high in the An'Ala sample (fig. 9C) and relatively low in two samples from Ambavala (9A, B). Profiles also differed considerably in the three samples. The population of *M. pulchra* from An'Ala shared many alkaloids with the microsympatric *M. baroni*, including pumiliotoxins and allopumiliotoxins, 1,4-disubstituted quinolizidines **217A** and **231A**, and the putative deoxypumiliotoxin **291E**. It differed from microsympatric *baroni* in having an "isopumiliotoxin" **267H**, two 3,5-disubstituted pyrrolizidines **223M** and **251O**, and an alkaloid **239N** ($C_{17}H_{21}N$) of unknown structure. Skins of *M. pulchra* from a swampy ridge forest near Ambavala contained decahydroquinoline **195A** and unsaturated analogs and "dimers" **384A/384B**, pumiliotoxin **267C** and the 3,5-disubstituted pyrrolizidine **267H** (fig. 9A, B). The higher molecular-weight pumiliotoxins present as major alkaloids in the An'Ala population were absent in the Ambavala populations. The reputed "Anosibe An'Ala" sample (from a dealer) had very low levels of alkaloids (gas chromatograph not shown). The two major alkaloids in that sample were the 3,5-disubstituted pyrrolizidine **223M** and the pumiliotoxin **251D**.

Profiles in *M. pulchra* differ considerably, perhaps reflecting different habitats and diet. Based on limited observation, *M. pulchra* seems to prefer forest that is slightly swampy or boggy underfoot, in contrast to our samples of *M. baroni*, which came from streamside forest.

Two previous reports on skin alkaloids in *M. pulchra* were published under the names *Mantella madagascariensis* (in Daly et al., 1984) and *Mantella* sp., cf. *madagascariensis* (in Garraffo et al., 1993b). The first report

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[10] There is a curious differential fading in the orange or red flash marks of preserved *M. pulchra*. For example, after 1½ years in preservative, the thigh/calf mark of the specimen in figure 5 changed to yellowish white with vivid orange edging along the ventrolateral edge of the shank, and the dull reddish markings under the thighs became a bright vivid orange. This explains Parker's (1925) otherwise puzzling original description, in which the flash marking is described as a bicolor "brilliant yellow" and "bright crimson."

was based on an extract from a specimen of *M. pulchra* (then in the synonymy of *madagascariensis* sensu Busse, 1981) obtained from a commercial dealer in the early 1980s. Results from that single specimen (AMNH 114047) are not in accord with alkaloid profiles from any wild population subsequently sampled, in that it contained a set of histrionicotoxins (**283A, 285A, 285C**) and an apparent acyclic alkaloid **241B**—none of which has been detected even in trace amounts in *any* species of *Mantella* collected on four trips to Madagascar. Thus, we are cautious and believe that the presence of histrionicotoxins or acyclic alkaloids in *Mantella* must be discounted unless authenticated with data from field-collected specimens. It is conceivable that the specimen obtained from a commercial dealer in the United States had been fed with New World arthropods containing histrionicotoxins and other alkaloids.

Mantella viridis
Pintak and Böhme, 1988
Figure 4C

This frog appears to be endemic to the northern tip of Madagascar. Dorsal surfaces and flanks are greenish yellow, with or without vague grayish lines. A black face mask is sharply defined dorsolaterally to just past the arm, where it falls off obliquely to the venter (fig. 4C); a pale labial line extends to the arm. The rear of the thigh is pale blue. The ventral surfaces were overall black (including palms and soles) with pale blue markings, including a tendency for a horseshoe-shaped mark on the lower jaw. In preserved specimens, at least, the throat and chest are brown, sometimes in obvious contrast to the black belly (as in *M. laevigata*).

Samples of skins of *M. viridis* were obtained in 1994 from two sites in the region of the type locality (south of Antsiranana). One was inland, from a nearly dry streambed 13 km south of Antsiranana. The other was from a semiarid stream drainage area on Montagne des Français,[11] overlooking the

[11] A report of *M. betsileo* from Montagne des Francais (Blommers-Schlösser and Blanc, 1991: 371) could not be confirmed and may be a case of misidentification. Only *M. viridis* was found and was relatively common, more than 40 individuals being seen in about four hours of collecting.

coast. Both streambeds were forested, but both, especially the more heavily forested inland site, were disturbed by clearings.

High levels of pumiliotoxins **307A** and **323A** were present in the Montagne des Français sample (fig. 9D). In addition, an isomer of pumiliotoxin **307A** of unknown structure was present. There were trace amounts of an 8-deoxypumiliotoxin **289C** and a homopumiliotoxin **223G**. The inland sample, in contrast, contained a wide variety of alkaloids (fig. 9E). Pumiliotoxins **307A** and **323A**, the former at high levels, were present along with several other pumiliotoxins and high levels of homopumiliotoxin **223G**. There were also several 5,8-disubstituted indolizidines, three of which, namely **193E, 217B,** and **221I**, were present at moderately high levels. A previously undetected alkaloid **207M** ($C_{14}H_{25}N$) of unknown structure was present. It afforded major mass spectral fragment ions at m/z 164 and 140. An earlier (1993) sample of one skin of *M. viridis,* obtained from a dealer and stated to be from Antsiranana, had very high levels of pumiliotoxins **307A** and **323A** along with minor amounts of 5,8-disubstituted indolizidines **217B** and **237H** (fig. 9F).

Prey that are the putative source of pumiliotoxins would appear to have been present at both 1994 sites of collection of *M. viridis,* but prey containing "izidine" and other alkaloids would seem to be available only at the more forested, inland site.

SUMMARY OF ALKALOIDS

This survey of alkaloids in skin extracts of 9 of 11 species currently recognized in *Mantella* reveals the presence of about 100 compounds. The structures of many of these were known, having been previously isolated and structurally defined from New World dendrobatid frogs (Daly et al., 1993). However, about 30 alkaloids from *Mantella* represent new compounds of unknown or uncertain structure. Characterization of these alkaloids will be presented elsewhere. The major classes of alkaloids found in mantelline skin can be summarized as follows:

1. Pumiliotoxin class, which includes pumiliotoxins, allopumiliotoxins, homopumiliotoxins; the structures of most of these are known. In addition, this class contains less well-defined compounds, i.e., the putative dehydro-
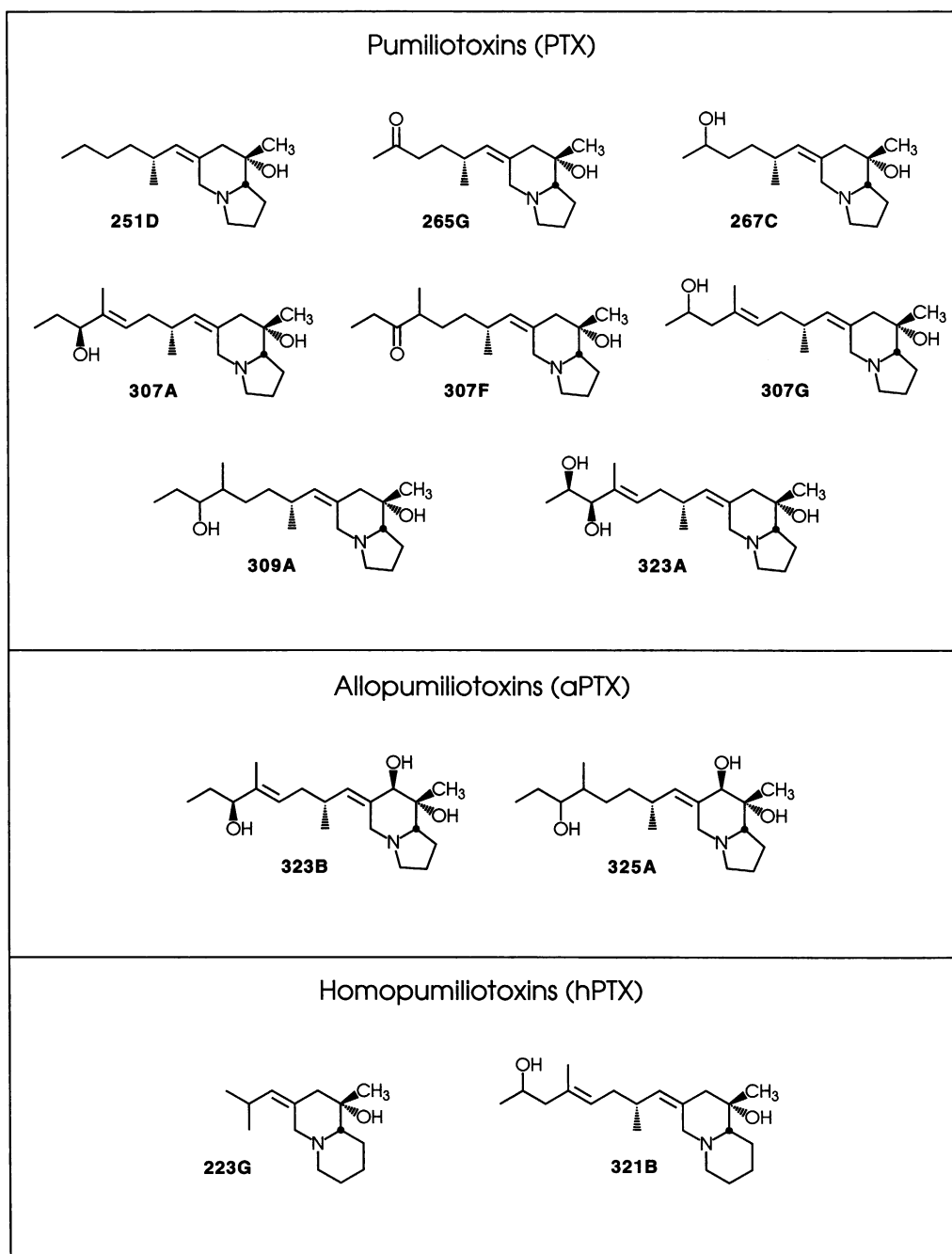
Fig. 10. Representative pumiliotoxin-class alkaloids from *Mantella* spp.

homopumiliotoxins, the 8-deoxypumiliotoxins, the 9,10-dihydropumiliotoxins, and the "iso-pumiliotoxins," most of which are as yet unique to *Mantella*. Compounds of the pumiliotoxin class occur as major alkaloids in all nine species of *Mantella* sampled.

2. Decahydroquinolines and related compounds. These occur together in several species and consist of a decahydroquinoline **195A**, which is well known from dendrobatid frogs, and, in addition, various unsaturated congeners and the apparent "dimers" **384A**

Dehydrohomopumiliotoxins (DehydrohPTX)

233F 235C

6,10-Dihydropumiliotoxins (DihydroPTX)

281F

8-Deoxypumiliotoxins (DeoxyPTX)

291E
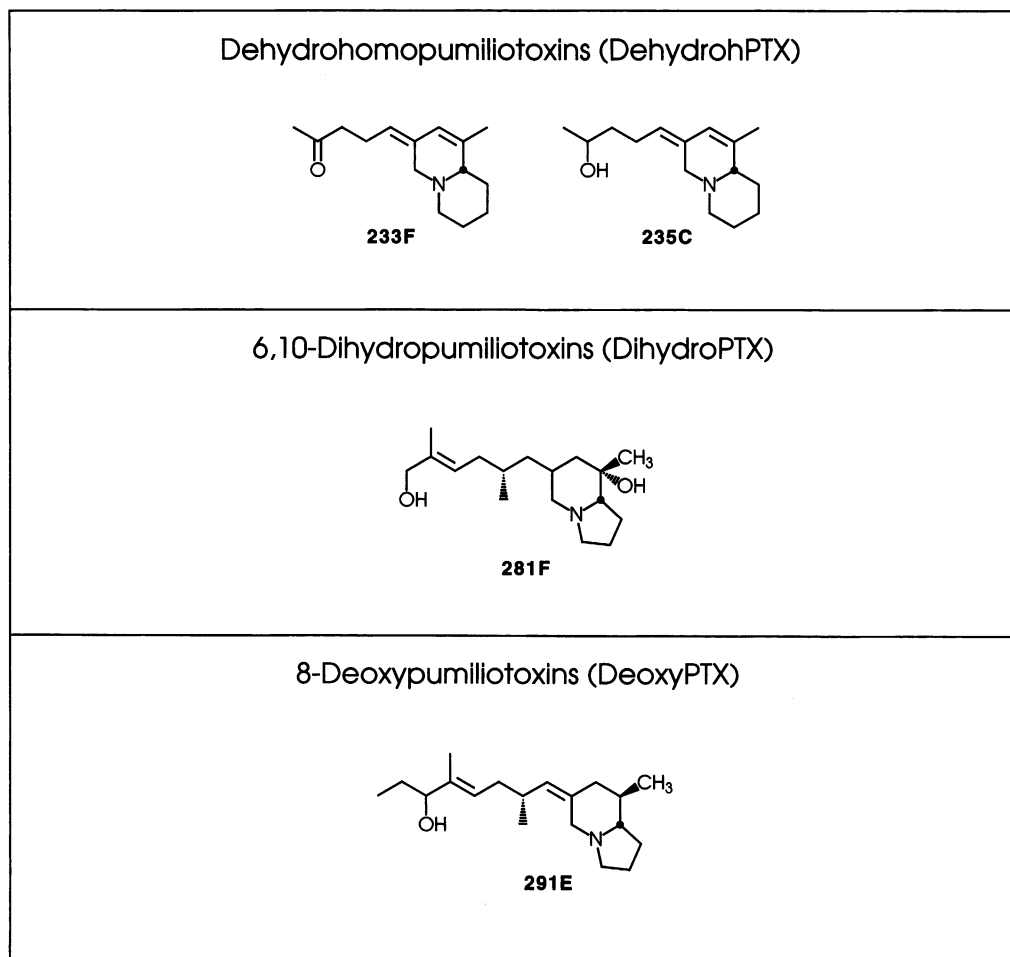
Fig. 11. Representative dehydrohomopumiliotoxins, a 9,10-dihydropumiliotoxin and an 8-deoxypumiliotoxin from *Mantella* spp. The structures are tentative.

and **384B** of unknown structure. This grouping of related compounds has been found in only three of the nine species studied (*M. betsileo, M. pulchra, M. laevigata*).

3. "Izidine" alkaloids, which consist of 3,5-disubstituted pyrrolizidines, 3,5-disubstituted indolizidines, 5,8-disubstituted indolizidines and 1,4-disubstituted quinolizidines. These occur in varying amounts and types in most populations of the nine species. In *M. baroni*, indolizidines and quinolizidines are major alkaloids often occurring at high levels.

In addition to these three general classes, various bicyclic and tricyclic alkaloids of unknown structure occur in *Mantella*. Some seem likely to represent new structural class-

es, hitherto undetected during 30 years of investigation of alkaloids from New World dendrobatid frogs. Conversely, certain "dendrobatid alkaloids," in particular the batrachotoxins, the histrionicotoxins, the gephyrotoxins, and epibatidine, were not detected in any of the recent mantelline extracts. Three histrionicotoxins were present as minor alkaloids in a single *Mantella* skin obtained a decade ago from a dealer in the United States (Daly et al., 1984). However, as concluded under *M. pulchra*, these alkaloid identifications—from a skin of a frog of unknown provenance and maintained for an unknown length of time in the New World—must be discounted unless verified from field-caught specimens.
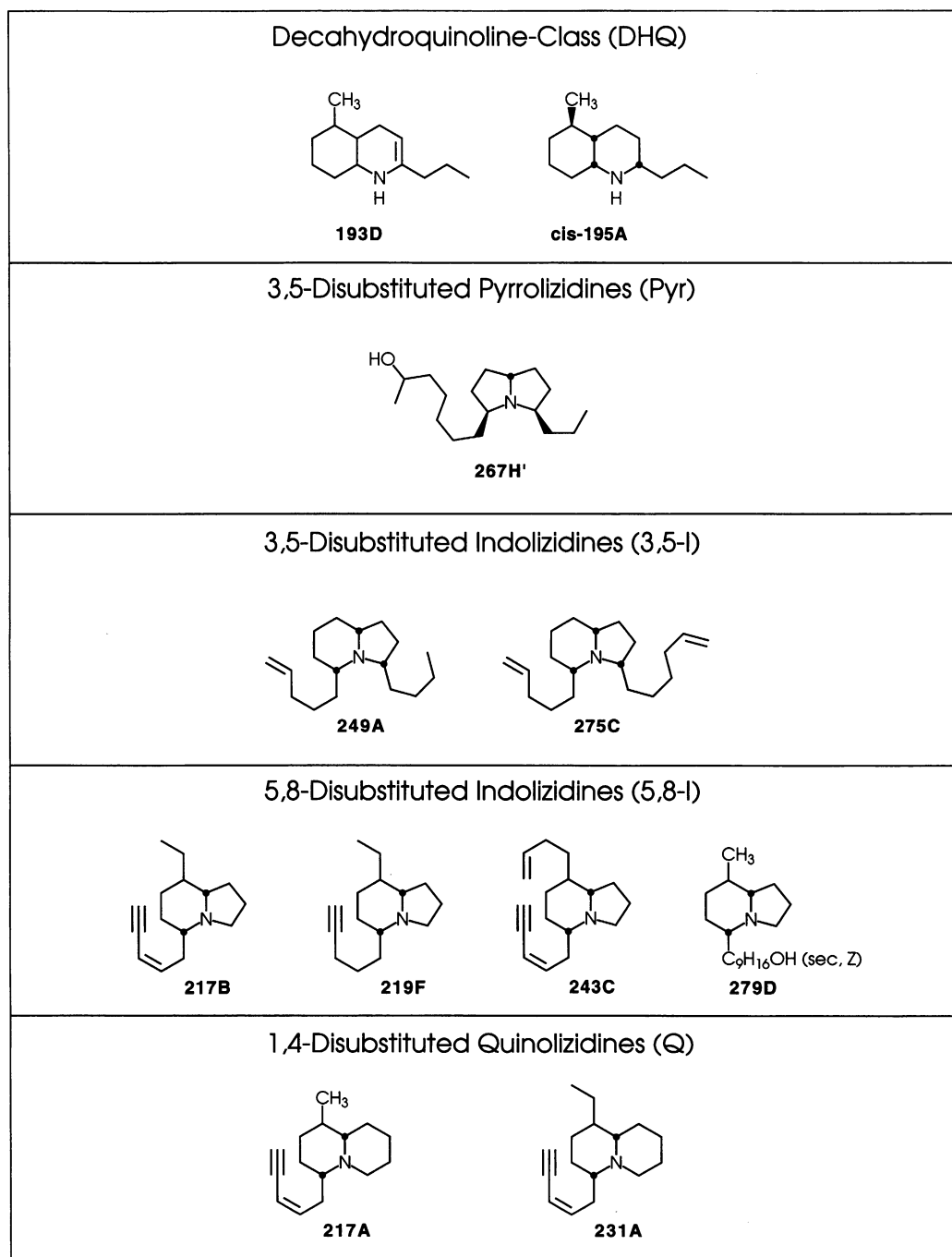
Fig. 12. Representative decahydroquinoline-class and "izidine"-class alkaloids from *Mantella* spp. The structure of the decahydroquinoline-class alkaloid **193D** is tentative.

It is of interest that an extract of *M. baroni* from near Sahavondrona contained pyrrolizidine oximes, that at least in dendrobatid frogs are suspected to have a dietary origin from small millipedes (Daly et al., 1994b). Other possible dietary sources for frog skin alkaloids are ants, some of which do contain 3,5-disubstituted pyrrolizidines and 3,5-di-

substituted indolizidines (Jones and Blum, 1983) and beetles, some which contain tricyclic coccinellines (op. cit.). The only known ant alkaloids that were detected in *Mantella* were the pyrrolizidines **223H** and **251K**. No beetle alkaloids were detected. Thus, other unknown dietary sources for most of the mantelline alkaloids must be considered. The present study demonstrating pumiliotoxin-class alkaloids as major components in alkaloid fractions from nine species of *Mantella* suggests that, if the diet is the source, prey containing these alkaloids must be widely distributed in forest habitats ranging from semiarid lowland to swampy upland.

APPENDIX 1: VOUCHER SPECIMENS

The following Madagascan specimens are vouchers only for specimens relevant to this survey of skin alkaloids in *Mantella*. Brief color descriptions are given in the text and representative specimens were photographed at the American Museum (see figs. 2–5). All specimens are alcohol preserved in the Amphibian Collection of the American Museum of Natural History. At least one to several specimens in each series are standard museum specimens, the others being carcasses of some of the frogs skinned for alkaloid extraction. The carcasses permit easy access to anatomical characters and some will be useful for cleared and stained preparations.

Mantella aurantiaca

No specific data (from dealers), AMNH 114042–114046, 123693–123695; 14-18 km N Andasibe, ca. 1000 m (18°45'S, 48°25'E), AMNH 136889–136892; region of Andasibe, AMNH 136921; near Andasibe (Périnet), AMNH 133611–133641.

Mantella baroni

14–18 km N Andasibe, ca. 1000 m (18°45'S, 48°25'E), AMNH 136888; near Andasibe (Périnet), AMNH 133668–133681; 7 km SE Andasibe, 11 km (by rd) E Andasibe, ca. 1000 m (18°58'S, 48°28'E), AMNH 136887; ca. 2 km SW An'Ala, 7-8 km SE Andasibe, ca. 1000 m (18°57'S, 48°27'E), AMNH 136901–136902; 2 km SW An'Ala, AMNH 140555–140556; 30-35 km (airline) S Moramanga, near km 45 on Moramanga-Anosibe An'Ala road, along stream ca. 2 km W of road, AMNH 133665–133666; Moramanga-Toamasi-

na road ca. 12 km (airline) SE Andasibe, along stream 0.5 km S of road, AMNH 133656–133664; 11 km WSW Ranomafana, 2 km SE Sahavondrona, ca. 1000 m (21°16'S, 47°22'E), AMNH 136898–136900; Parc Ranomafana, ca. 10 km (airline) SW Ranomafana (town on Fianarantsoa-Ifanadiana road), AMNH 133667.

Mantella betsileo

Antanambaobe, Mananara Avaratra National Park, ca. 100-200 m (ca. 16°16'S, 49°40'E), AMNH 136940–136942; Farakaraina Forest Station, ca. 10 km E Maroantsetra (on coast), 30 m (15°26'S, 49°52'E), AMNH 140566–140570; ca. 7 km SE Sandrakatsy, near village Ambavala, AMNH 140572–140573; ca. 8 km SE Sandrakatsy, near village Ambavala, Mananara Avaratra National Park, ca. 200 m (ca. 16°23'S, 49°44'E), AMNH 140571.

Mantella cowanii

No specific data (dealer claimed "Anosibe An'Ala"), AMNH 140546–140550.

Mantella crocea

Near Andasibe (Périnet), AMNH 133642–133655; 14-18 km N Andasibe, ca. 1000 m (18°45'S, 48°25'E), AMNH 136893–136897.

Mantella expectata

Massif Isalo, ca. 10 km (by rd) SW Ranohira, 500 m south [of] road, ca. 800 m (22°38'S, 45°22'E), AMNH 136922–136939, 140617.

Mantella laevigata

Ambodimanga, Mananara Avaratra National Park, ca. 100 m (ca. 16°22'S, 49°47'E), AMNH 136945–136946; Nosy Mangabe, >100 m (15°30'S, 49°46'E), AMNH 140557–140562; ca. 7 km SE Sandrakatsy, near village Ambavala, AMNH 140563–140565.

Mantella pulchra

No specific data (from dealer), AMNH 114047–114048; 2 km SW An'Ala, AMNH 140551–140552; ca. 2 km SW An'Ala, 7–8 km SE Andasibe, ca. 1000 m (18°57'S, 48°27'E), AMNH 136903–136916; ca. 8 km SE Sandrakatsy, near village Ambavala, Mananara Avaratra National Park, ca. 200 m (ca. 16°23'S, 49°44'E), AMNH 140553–140554; ca. 8 km SE Sandrakatsy, village Ambavala, Mananara Avaratra National Park, ca. 100-200 m (16°23'S, 49°44'E), AMNH 136943–136944; region of Anosibe An'Ala (from dealer) (ca. 19°26'S, 48°12'E), AMNH 136918.

Mantella viridis

No specific data (from dealer), AMNH 133682–133683; "region of Antsiranana" (from dealer, AMNH 136919–136920; 13 km S Diego Suarez (=Antsiranana) (12°12'S, 49°16'E), AMNH 140581; Montagne des Français, 8 km (by rd) southeast from Diego Suarez (=Antsiranana) (12°19'S, 49°20'E), AMNH 140575–140580.

Mantella species

About 8 km SE Sandrakatsy, near village Ambavala, Mananara Avaratra National Park, ca. 200 m (ca. 16°23'S, 49°44'E), AMNH 140574.

See text discussion under Mantella betsileo regarding this specimen. It apparently is an immature female, 21 mm SVL. Color snapshots taken before formalin fixation show an orange-brown dorsum and limbs, with green at the upper arm insertion and a large green patch covering the anterior and most of the dorsal thigh surface. Ventral surfaces were black overall, with bright blue edging around the lower lip and small, irregularly shaped bright blue spots on throat, venter, and limbs. There was a small golden yellow spot in the groin and a sharply demarcated golden yellow flash mark below the knee in the concealed part of the shank, these markings being white in preservative; the inguinal mark is small and the calf marking large, occupying the proximal 60 percent of the shank. In lateral view (not visible in the color photographs), a pale (presumably green) anterior lateral blotch is confluent with the pale limb insertions, and a similar blotch posteriorly on the flanks is confluent with the large (green) anterodorsal thigh patch.

APPENDIX 2: ALKALOIDS IDENTIFIED IN *MANTELLA* SKIN

Flame-ionization gas chromatographic profiles are shown in figures 6—9. Structures of relatively common *Mantella* alkaloids are shown in figures 10–12. Abbreviations of alkaloid classes are as follows: Pumiliotoxins (**PTX**), allopumiliotoxins (**aPTX**), homopumiliotoxins (**hPTX**), dehydrohomopumiliotoxins (**dehydrohPTX**), 6,10-dihydropumiliotoxins (**dihydroPTX**), 8-deoxypumiliotoxins (**deoxyPTX**), decahydroquinolines and related alkaloids (**DHQ**), 3,5-disubstituted pyrrolizidines (**3,5-P**), 3,5-disubstituted indolizidines (**3,5-I**), 5,8-disubstituted indolizidines (**5,8-I**), 5,6,8-trisubstituted indolizidines (**5,6,8-I**), 1,4-disubstituted quinolizidines (**Q**), 2,5-disubstituted pyrrolidines (**Pyr**), tricyclics (**Tri**), pyrrolizidine oximes (**Oximes**), unclassified (**Unclass**). Classification and structures for some alkaloids remains tentative.

Some alkaloids exist as diastereomers, i.e., PTX **307F** often occurs in *Mantella* as a mixture of diastereomeric **307F′** and **307F″** as do PTX **307G** and 3,5-P **267H** (see Garraffo et al., 1993b), but only the gross designation, i.e., **307F**, **307G**, **267H**, followed by the number of diastereomers in parentheses, is given here. An asterisk (*) means that a given sample has been previously described (in Garraffo et al., 1993b). Number designations for each population (6A, 6B, etc.) refer to figures 6–9 except for 8B′(see Garraffo et al., 1993b, fig. 2B for this sample).

Species	Population Date No. skins	Major alkaloids Minor alkaloids Trace alkaloids
M. aurantiaca	6A. N Andasibe Nov. 1989 10 skins*	PTX **323A**. PTX **267C**, **307A**, aPTX **323B**; dehydrohPTX **233F**, **235C**. PTX **305B**, **307B**; dehydrohPTX **221F**, **265F**.
	6B. N Andasibe Jan. 1993 5 skins	PTX **323A**. aPTX **323B**; dehydrohPTX **265F**; "isoPTX" **323C**; Unclass **392**, **434**. PTX **267C**, **277B**, **307G**, **309A**, hPTX **337**; dehydrohPTX **233F**, **235C**; 3,5-I **249A**; Q **217A**.
M. baroni	8A. N. Andasibe Nov. 1989 10 skins*	PTX **309A**; Q **217A**. PTX **307A**, **307F**(2), aPTX **325A**; dihydroPTX **281F**; 5,8-I **217B**; **235B**, **241F**, **243C**, **253B**, **279D**; Q **231A**, **233A**; Unclass **281F**. PTX **251D**, hPTX **249F**; dehydrohPTX **233F**, **235C**; 5,8-I **243B**, **245B**.
	8B. E Andasibe Dec. 1993 10 skins	PTX **309A**; Q **217A**, **231A**. PTX **307G**, **307F**(2); aPTX **325A**; deoxyPTX **291E**, **293D**; dihydroPTX **281F**; 5,8-I **217B**; Unclass **251P**. PTX **237A**, **251D**; hPTX **323E**, **337**; 5,8-I **241F**, **243C**, **253B**; Q **233A**.
	8B′. E Andasibe Nov. 1989 10 skins*	PTX **309A**; dihydroPTX **281F**; Q **217A**, **231A**. PTX **307A**; aPTX **325A**; 3,5-I **249A**, **275C**; 5,8-I **217B**, **241F**; Unclass **293B** hPTX **235J**; 5,8-I **243C**, **279D**; Q **273A**.
	8C. S Moramanga Nov. 1989 10 skins*	Q **217A**, **231A**. PTX **307A**, **309A**; aPTX **325A**; 3,5-I **249A**; 5,8-I **217B**; 5,6,8-I **223A**. PTX **251D**, **307F**; 3,5-I **275C**; 5,8-I **205A**, **243D**, **245C**; Unclass **251L**, **293G**.
	8D. An'Ala Dec. 1993 3 skins	PTX **309A**; Q **217A**, **231A**. PTX **307F**(2), **323A**; deoxyPTX **291E**; dihydroPTX **281F**; 3,5-I **275C**; 5,8-I **217B**, **243C**; Q **273A**. PTX **237A**, **307A**; aPTX **325A**; hPTX **323E**; deoxyPTX **293D**; 3,5-I **249A**; 5,8-I **241F**, **279D**; Unclass **275D**.
	8E. Ramomafana Nov. 1989 10 skins*	PTX **309A**; Q **217A**. PTX **237A**, **307F**(2); 5,8-I **217B**; **243D**, **245C**; Q **231A**, **233A**; Tri **207J**; Unclass **293B**. PTX **251D**; 5,8-I **203A**; Unclass **205C**.
	8F. Sahavondrana Jan. 1993 17 skins	PTX **309A**. PTX **251D**, **307F**; aPTX **325A**; hPTX **251R**, **265N**; dehydrohPTX **265F**; deoxyPTX **281G**; 5,8-I **217B**; Q **231A**, **273A**(2). PTX **237A**, **307A**; 3,5-I **271F**, **275C**; 5,8-I **241F**; Oxime **236**, **252A**.
M. betsileo	6C. Antanambaobe Dec. 1990 11 skins*	DHQ **384A**, **384B**; Unclass **195C**. PTX **307G**(2); aPTX **323B**; DHQ **195A**; 5,8-I **219F**. PTX **251D**, **307A**; aPTX **321C**, hPTX **223G**, **317**; DHQ **189**, **193D**; 5,8-I **217B**; Q **217A**, **231A**, **249C**; Tri **207J**, **235K**; Unclass **211D**.
	6D. Ambavala Jan. 1994 8 skins	DHQ **195A**; Unclass **195C**. PTX **251D**; DHQ **193D**, **384A**, **384B**; 3,5-I **247C**; 5,8-I **217B**; Q **217A**; Unclass **211D** PTX **237A**, **307G**; aPTX **323B**; hPTX **321B**, **337**; DHQ **382**, 3,5-I **205A**, **247C**; 5,8-I **251B**, **253B**; Q **249H**, Tri **235M**, **265M**, Unclass **251Q**, **269E**, **271B**.

	6E. Farakaraina Dec. 1993 12 skins	PTX **307G**; 3,5-P **223H**. PTX **251D**; hPTX **223G**; DHQ **195A**; 3,5-P **251K**; 5,8-I **219F**; Q **231A**; Unclass **195C**. PTX **237A, 267C, 293E**; aPTX **323B**; hPTX **321B, 335**; deoxyPTX **291E**; dehydrohPTX **265F**; DHQ **181D,** **189, 193D, 382, 384A, 384B**; 3,5-I **247C**; Tri **265M**; Unclass **237K, 269E**.
M. cowanii	6F. Dec. 1993 3 skins	PTX **251D**. PTX **265G, 307G**(2), aPTX 7-epi-**267A**. PTX **267C**(2), **323A**.
M. crocea	7A. N. Andasibe Nov. 1989 10 skins*	PTX **267C, 307A, 323A**, aPTX **323B**. dehydrohPTX **233F, 235C**; 5,8-I **295B**; Unclass **392, 434**. PTX **265G, 305B, 307B**; DHQ **195A**; 3,5-P **223H**(2); Q **231A**.
	7B. N. Andasibe Jan. 1993 35 skins	PTX **267C, 307A, 323A**; aPTX **323B**. PTX **265G**; Unclass **392, 434**. PTX **277B, 305B, 309A**; dehydrohPTX **233F, 235C**; "isoPTX" **267N, 323C**; 3,5-P **239O**; Unclass **293G**.
M. expectata	7C. Massif Isalo Jan. 1993 20 skins	PTX **251D**; 5,8-I **219F**; Unclass **261C**. PTX **307A**; 5,8-I **193E**; Unclass **323D**. PTX **267C**(2), **305B**; aPTX **323B**; 3,5-I **223AB, 275C**; 5,8-I **197C, 217B, 219J, 241F**; Unclass **195C, 233I, 235O**.
M. laevigata	7D. Ambodimanga Dec. 1990 6 skins*	Unclass **195C**. PTX **307G**, DHQ **193D, 195A**. hPTX **223G**; DHQ **189, 384A, 384B**; Q **207I**; Unclass **161, 211C, 211D, 223A**.
	7E. Varary Jan. 1994 5 skins	PTX **307G**. DHQ **193D, 195A**; 5,8-I **223J**; 5,6,8-I **223A**; Unclass **195C**. hPTX **321B**; deoxyPTX **291E**; DHQ **189, 382, 384A, 384B**, Unclass **275D**.
	7F. Nosy Mangabe Dec. 1993 6 skins	PTX **307G**. 3,5-I **249A**. PTX **237A, 305B**; deoxyPTX **291E**; 3,5-P **223H**; 3,5-I **247C, 275C**; 5,8-I **219F**; Q **217A**; Pyr **223N, 225H**.
M. pulchra	9A. Ambavala Dec. 1990 6 skins*	3,5-P **267H**(2). PTX **267C**; DHQ **384A, 384B**; Unclass **161, 293B**. PTX **265G**; aPTX **325A**; 3,5-P **239K**(2), **265H**(2); DHQ **189**; **193D, 195A**, Unclass **195C, 211D**.
	9B. Ambavala Jan. 1994 5 skins	PTX **267C**. PTX **265G**; DHQ **189, 193D**(2), **382, 384A, 384B**; 3,5-P **267H**. PTX **307A**; DHQ **195A**; 3,5-P **223H**; Unclass **211D, 271B, 341C, 392, 434**.

	9C. An'Ala
	Jan. 1993
	5 skins
		PTX **309A**; Q **217A**.
		PTX **251D, 307A, 307F, 325B**; aPTX **325A**; dihydroPTX **281F**; 3,5-P **223M**; 3,5-I **249A,**
		    275C; 5,8-I **279D**; Q **231A**(2).
		PTX **237A, 307G**; aPTX **323B**; hPTX **337**; deoxyPTX **291E, 293D**; "isoPTX" **267N**;
		    3,5-I **251O**; 5,8-I **245B, 249J**; Unclass **239N**.

M. viridis	9D. Montagne des Français Jan. 1994 30 skins	PTX **307A**. PTX **307D, 323A**. PTX **277B, 289C, 305D, 323F**.
	9E. S. Antsiranana Jan. 1994 5 skins	PTX **307A**; hPTX **223G**. PTX **289C, 323A**; 5,8-I **193E, 217B**(2), **221I, 237J**, Unclass **207M, 223O, 235L**(2), **265K, 267M**. PTX **209F, 305D**; hPTX **239M**; "isoPTX" **339C**; 3,5-I **223AB**; 5,8-I **231C, 245F**; Q **207I**.
	9F. Antsiranana Nov. 1989 1 skin*	PTX **307A**. PTX **307G, 321A** (artefact), **323A**; 5,8-I **217B, 237H**. PTX **265G, 309B, 307H**; hPTX **223G**; 3,5-I **223AB**; Q **217A, 231A, 233A**.

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