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Identification of Individual Foothill Yellow-Legged Frogs (*Rana boylei*) using Chin Pattern Photographs: a Non-Invasive and Effective Method for Small Population Studies

The ability to identify individual animals is a valuable tool in the study of amphibian population dynamics, movement ecology, social behavior, and habitat use. Numerous methods of marking amphibians have been employed including the use of passive integrated transponder (PIT) tags, radio-transmitters, elastomers, branding, and mutilation techniques such as toe-clipping (Ferner 2007). All of these methods are invasive to amphibians to some degree and can alter their behavior, decrease recapture rates, reduce survivorship, and even cause direct mortality in some cases (Ferner 2007). One of the least invasive methods of identifying individuals is pattern recognition using photography.

Photographic pattern recognition has been utilized successfully in order to identify individuals in all major groups of amphibians including caecilians (*Dermophis mexicanus*; Wright and Minott 1999), salamanders (*Ambystoma opacum*, Gamble et al. 2008; *Pachytriton brevipes*, Lackey et al. 2006), and anurans (*Litoria genimaculata*, Kenyon et al. 2009; *Rana sierrae*, Wengert and Gabriel 2006; *Rana pipiens*, Nace et al. 1973).

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The Foothill Yellow-legged Frog (*Rana boylei*) is found in streams and rivers in California and Oregon (Stebbins 2003), and typically breeds from mid-March to early-June when high flows subside. *Rana boylei* has experienced an estimated 45–55% decline in its range, mostly occurring in the southern Sierra Nevada of California and in southern Oregon (Lind 2005), and is currently listed as a “Species of Special Concern” in California (CDFW 2009) and as a “Sensitive Species” in Oregon (ODFW 2008). Numerous potential causes for this decline have been identified including habitat destruction (Davidson et al. 2002), dam construction (Lind et al. 1996), introduced predators (Kupferberg 1997; Crayon 1998; Wiseman et al. 2005), and fungal infections (Padgett-Flohr and Hopkins 2009). Many streams and rivers where *R. boylei* occur are now impacted to various degrees by human activities, including artificial regulation of stream flows in river systems impounded by dams. Numerous relicensing efforts for federal hydroelectric dams and impoundments occurring along streams and rivers in the Sierra Nevada require monitoring of *R. boylei* populations (Kupferberg et al. 2009) and identification of individuals within these populations provides valuable ecological data relevant to the management of this species (GANDA 2008).

Rana boylei is a moderately-sized frog (3.8–8.1 cm snout–urostyle length [SUL]; Stebbins 2003) with a variable dorsal coloration that can be gray, brown, reddish, or olive. The yellow coloration on the ventral portions of the legs, from which this species gets its common name, typically extends from the lower abdomen posteriorly to the hind legs. Males are identified by their swollen and darkened thumb base or nuptial pads (Stebbins 2003). Zweifel (1955, p. 218; unpublished field notes, 1952; Fig. 1) illustrated the variation in distinct chin patterns among individuals and populations throughout this species’ range. These chin or throat patterns are created by aggregations of melanophores contrasting with a lighter, whitish background coloration (Zweifel 1955; Wheeler et al. 2005). Patterns on individuals have been observed to vary in relative contrast and may be influenced by fluctuations in temperature or water balance (Wheeler et al. 2005; Tattersal et al. 2006).

Herein, we describe a photographic method for identifying individual *R. boylei* using chin patterns from three multiple-year

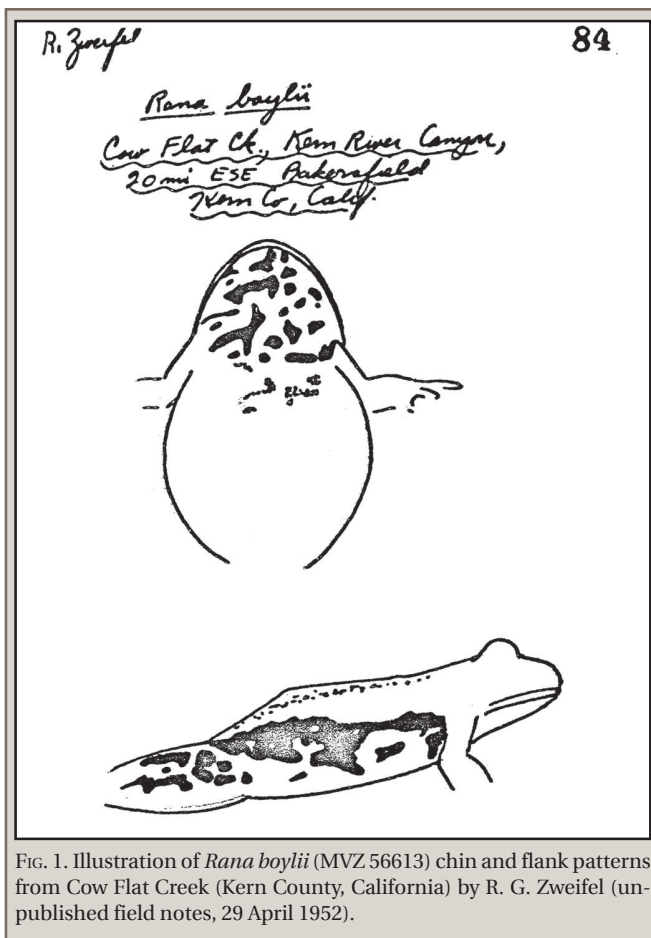


FIG. 1. Illustration of *Rana boylei* (MVZ 56613) chin and flank patterns from Cow Flat Creek (Kern County, California) by R. G. Zweifel (unpublished field notes, 29 April 1952).

studies of geographically separated Northern California populations located at Hurdygurdy Creek (a tributary to the South Fork Smith River, Del Norte County), on the North Fork Feather River (Butte County), and at Big Carson Creek and Little Carson Creek (two tributaries to Kent Lake, Marin County).

Methods.—During research examining the mating strategy and movement ecology of *R. boylei* at Hurdygurdy Creek, we conducted a pilot study to examine the potential use of chin mottling patterns to identify individuals (Wheeler et al. 2005). From 2002–2005, we captured adult and juvenile frogs, weighed (g), measured (mm), and photographed the chin pattern of each frog using a Nikon Coolpix® 950 digital camera. Frogs were held against a 10-cm² grid for scale. Female frogs greater than 50 mm SUL and males greater than 40 mm SUL were then marked with PIT (Passive Integrated Transponder) tags (Biomark® TX1400L; 12 mm, 125 kHz) inserted subcutaneously through a small, surgical incision on the posterior dorsal side, and released. Photo identification numbers were recorded along with frog locations, weight (g), SUL (mm), and breeding condition (gravid or spent). PIT tag presence was used to establish whether or not a captured frog was a new capture or recapture and tag number was used to determine individual identity. Chin photo images of recaptured frogs were later compared to previous photos of the same individuals to observe whether or not the mottling patterns were unique to individuals and if they changed over time.

A similar photographic method was subsequently utilized on a population of *R. boylei* from the North Fork Feather River (NFFR) from 2004–2009 to track individuals between tributaries

and river-breeding sites during annual monitoring efforts (GANDDA 2009). Frog chin patterns were photographed in hand after capture using an Olympus Stylus® 300 digital camera at macro setting. In 2007, PIT tags (Biomark® TXP148511B; 8.5 mm × 2.12 mm, 0.067 g, 134.2 kHz) were inserted into a subset of male (N = 2) and female (N = 44) frogs to verify that chin patterns were unique among individuals of the NFFR and did not change over time. After successfully identifying individuals with chin photographs and verifying individual patterns with PIT tags on Hurdygurdy Creek and the NFFR, photographs of chin patterns were then used at Little Carson Creek/Big Carson Creek to identify captured individuals from 2008–2010 using the same photographic methods described above, with the exception that PIT tags were not used.

Upon completion of field work, photographs were later downloaded to a database, laser-printed in black-and-white on 8.5 × 11-inch paper labeled with photo identification number, survey date, sex, and site name. In the office, chin pattern photographs were examined visually for pattern matches by arranging printouts side-by-side, and matches were confirmed by at least two biologists.

To assess accuracy rates associated with this method, we tested the ability of three naive observers (i.e., people who have not used the method, including two biologists) to identify individual frogs by comparing and matching printed photographs. To achieve this, we randomly selected a set of 150 chin photographs of 78 individual adult *R. boylei*. This included frogs (N = 36) with multiple captures (mean = 3, SD = 1, range = 2–8) within and among years from the NFFR, as well as single photos of a random selection of 42 individuals that were only captured once. The random set consisted of photographs of adult frogs only; no juveniles were included. Photographs included in the random set represented all problematic issues (e.g., blurriness, relative distance, reflectivity, relative contrast of chin patterns) that we encountered using this method. Each observer was given this set of 150 printed, black and white photographs, each marked only with a number (1–150) representing an undisclosed number of individual frogs; no duplicate photographs were included. Observers worked alone and were given no time limitations for completion. Considering each photograph as one point, observers were given one point (+1) for each correct result (match or no match) and a zero (0) for each incorrect result (false negative or false positive). An unmatched photograph was considered a false negative, a mismatched photograph considered a false positive. Where a frog with multiple captures was not matched as a full set (i.e., in two separately matched sets), one zero was given for a false negative. For example, if F7 had five matching photos (+5 points if matched correctly), but the observer matched them in separate sets of two and three, one point was taken away from the full set of points (+4 points).

To compare the accuracy of pattern matching by eye versus the accuracy of computer-based pattern recognition software, we used the program Wild-ID 1.0®, a free, downloadable software from Dartmouth College (Bolger et al. 2012). The same 150 photographs used for the accuracy test were entered into the Wild-ID® software program using the same numbering system. The program generated a range of one to 20 (maximum) potential matches for each photograph, which were visually confirmed or rejected (on screen) by a single, experienced observer. All correct program matches, matches missed (false-negatives) by the program, and matches missed by the observer were calculated for each photograph using the same point system. Errors for

the experienced observer were counted when a match was presented and the observer did not confirm the match or when the observer selected a mismatch (false positive).

Results.—A total of 1584 individual *R. boylii* (825 adult females, 756 adult males and 3 juveniles) were identified using chin pattern matching at Hurdygurdy Creek, the North Fork Feather River, and Big Carson Creek and Little Carson Creek study sites. Of these, 271 frogs (17.1%) were recaptured during at least two years. At Hurdygurdy Creek, a total of 245 adults (84 female, 161 male) were photographed and PIT-tagged; of these, 54 (22.0%) individuals were recaptured and re-photographed one to eight times (mean = 1.7, SD = 1.3) over a period of four years. Time intervals between subsequent captures ranged from one to 706 days (mean = 162.5, SD = 171.4). Chin patterns of each frog were unique and we did not detect significant differences between captures or across years.

On the NFFR, a total of 1168 adult (675 female, 490 male) and three juvenile frogs were photographed; of these, 160 (13.7%) individuals were subsequently recaptured and re-photographed, ranging from one to 24 (mean = 2.1, SD = 5.2) times over a period of six years. Time intervals between captures ranged from one to 1125 days (mean = 73.9, SD = 174.2). The three juveniles were recaptured later as adults and positively identified by their individual chin pattern. Of the 46 adult frogs that were photographed and PIT-tagged on the NFFR in 2007, 21 (45.7%) were recaptured in 2008 and seven (15.0%) were recaptured in 2009; chin patterns were unique among all PIT-tagged frogs and we did not detect significant differences between captures or across years.

At Big Carson Creek and Little Carson Creek, a total of 171 adult frogs, 66 females and 105 males, were photographed; of these, 57 (33.0%) individuals were recaptured and re-photographed, ranging from one to 19 times (mean = 3.8, SD = 3.7) over a period of three years. Time intervals between captures ranged from six to 485 days (mean = 86.3, SD = 118.6) days. Chin patterns of each frog were unique and we did not detect significant differences between captures or across years.

We found that the anterior portion of the skin covering the lower jaw, or chin, contained the most notable mottling patterns and was more easily photographed than other areas, such as the flank (see Fig. 1). Chin patterns among individuals were highly variable; some frogs possessed larger, more defined vermiculated patterns and others had markings with lesser delineation and sharpness (Fig. 2). Although the patterns themselves did not change during subsequent recaptures, the relative contrast of pigment within these patterns did vary between captures (Fig. 2), though we did not observe any consistent pattern of pigment variability over time. Relative contrast of the chin patterns appeared to change the least along the skin covering the anterior portion of the chin.

Accuracy of matching chin patterns among three naive observers averaged 92.4% (SD = 3.4%, range = 88.7–95.3%). Of 450 total records (3 observers × 150 photographs), observers incorrectly classified 34 (7.6%) photographs. These 34 errors included 28 unmatched photographs (false-negatives; “errors of omission”), and six mismatched photographs (false-positives; “errors of commission”). Twelve (35.0%) of the errors were similar between at least two of the three observers and two (10.0%) were similar among all three observers. Where errors were similar between observers, the photographs had: 1) a higher degree of matching difficulty due to densely mottled patterns, 2) variation in pigmentation contrast between captures, and/or 3) poor photograph quality. Accuracy of matching chin patterns with the

Wild-ID® program and one experienced observer was 40.7% (127 correct matches includes 42 photos with no possible match). Of 312 total possibilities within the set of 150 photographs (including 270 matches and 42 photos with no possible match), software program errors totaled 179 (57.4%) missed matches. When there were multiple possible matches (range 2–8) for a single individual, the software program generated all matches correctly in just five out of 74 (6.8%) possible instances. When viewing the software generated options on screen, the experienced observer errors totaled six (1.9%) including four false negatives and two false positive matches.

Discussion.—Chin pattern identification has proved effective in identifying individual *R. boylii* in our three separate studies. Between 2002 and 2010, 1584 individual adult and juvenile frogs have been identified using this method. Of those, 17.1% of frogs recaptured over two or more years had patterns that did not change between captures or among years, as verified by the use of PIT tags on a subset of adult frogs. An accuracy test performed by three naive observers showed a high rate of accuracy (92.4%) for the chin photograph method, much higher than a computer software photo recognition program we used as a comparison test. The computer software, verified on screen by one experienced observer, tested to a 41% accuracy rate. Our results demonstrated that this method of photographic identification of individual frogs is an effective and relatively non-invasive technique for studies of small populations of *R. boylii* using naturally occurring markings that do not appear to change over time. During this study, a small subsample of three juvenile frogs was observed with the same chin patterns when later recaptured as adults. In subsequent years since the completion of this study (2011–2014), approximately 30 additional juveniles that we recaptured as adults retained their unique chin patterns into adulthood; this information may be useful for assessing adult recruitment. One female, first captured on the NFFR in 2004 and recaptured seven times over nine years, was observed with the same chin pattern between captures and across all years throughout this study. While photographic identification worked very well in our studies, this method has limitations and may be more useful for certain objectives and may not be feasible for some species whose patterns may change over time (e.g., *Litoria genimaculata*; Kenyon et al. 2009). Photo identification may be most useful for species with very distinct patterns, for studies of small, closed populations, and for short-term studies.

There are several advantages to using a photographic identification method to identify individual frogs including: 1) it is a relatively non-invasive method (requires only capture and handling, approximately two minutes/frog), 2) unlike other marking techniques, photo-identification work adheres to the standard capture and release conditions for scientific collecting permits without additional agency authorizations, 3) it has lower equipment costs than many other methods, 4) it can be highly accurate (as was found in our study), and 5) it can provide instant, on-site recognition of individuals. On-site recognition proved beneficial during a radio-telemetry study of the NFFR population (GANDA 2008). During the course of the study, several individuals shed their transmitters. We effectively identified these individuals by using a field reference booklet of individual frog chin patterns and reattached transmitters to the same frogs, allowing for continued tracking of those individuals.

Disadvantages to consider when using such a photographic identification method are: 1) labor costs to manage and visually match photographs of large numbers of individuals can be

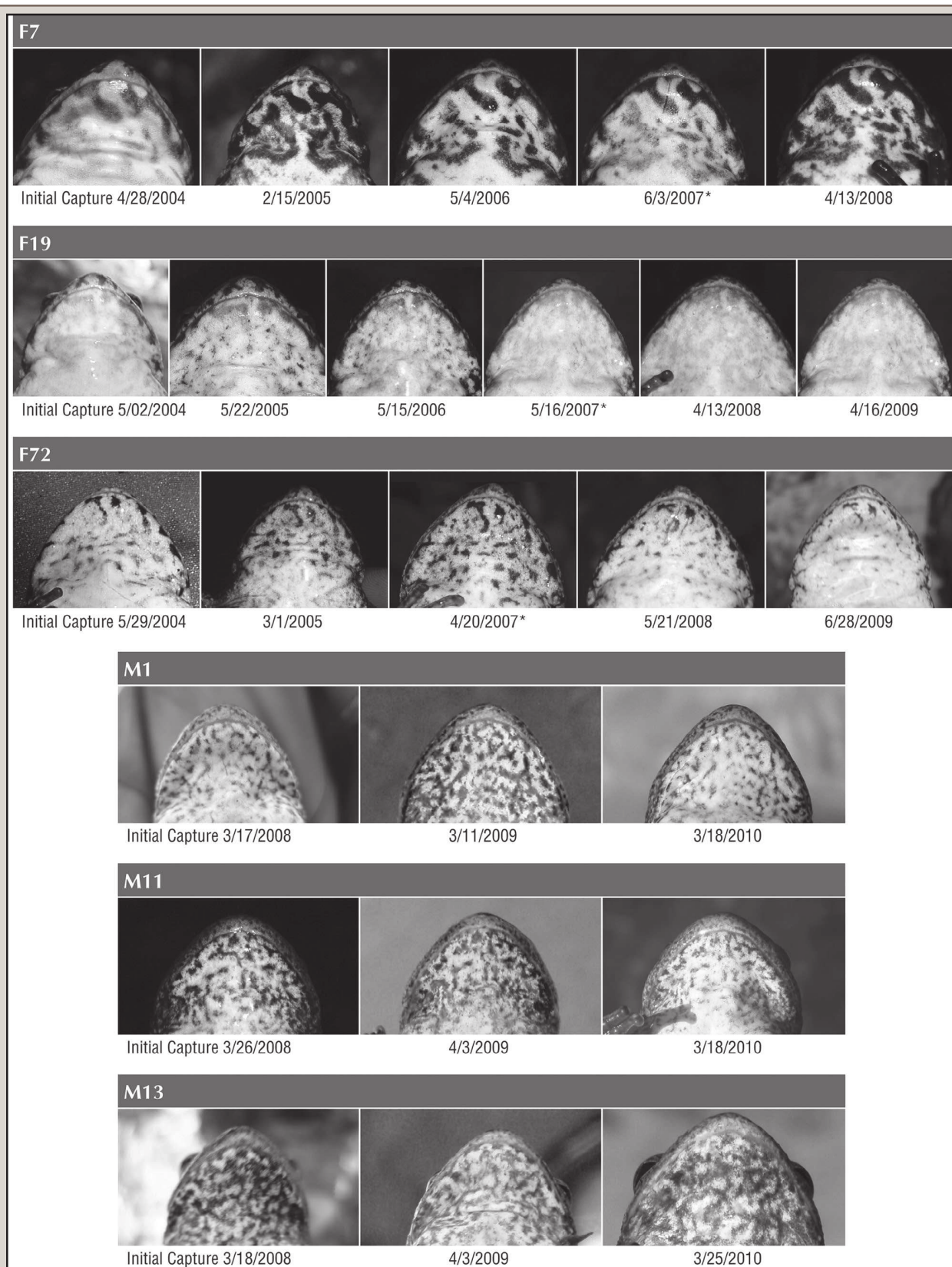


FIG. 2. Horizontal series of chin photographs of three adult female *Rana boylei* (F7, F19, and F72) recaptured on the North Fork Feather River and three adult male *R. boylei* (M1, M11 and M13) from Little Carson Creek used for individual identification. Successive capture dates of individual frogs are listed under each image. Asterisks denote the date when PIT-tags were inserted into the frogs; subsequent recaptures included verification of PIT-tag identification number.

relatively high; 2) quality of photographs (e.g., blurry photos or flash reflections) can affect photo-matching accuracy; and 3) the consequences of inaccurate matching of photos can be considerable. For example, incorrect classification of previously captured frogs as new captures (i.e., false-positives) can result in inflated population estimates (Kurashina et al. 2003; Yoshizaki et al. 2009).

We recommend several techniques to maximize effectiveness of this photo identification approach for *R. boylii*. For instance, the digital camera macro option should be used to obtain a full-frame image of the chin pattern in the field (Fig. 2), reducing pre-processing photo-editing time during data analysis. Maximum contrast in the chin pattern can be achieved by having the background as dark as possible, which can be attained by holding the frog out of direct sunlight and/or using the flash. Some problematic images (e.g., light pigmentation, slightly blurry photos) can sometimes be rectified by adjusting the contrast (possible with most photo-editing software). Flank patterns can be helpful as a secondary level of identification, although these patterns are more difficult to capture photographically than chin patterns and were not used for this study. Careful attention to photo labeling during the capture and printing processes is essential for accuracy, and separating and managing chin photographs by sex and locality (e.g., tributary, river reach, or river breeding site) maximizes efficiency when matching and entering data; however, frogs are known to migrate between breeding sites and tributaries (GANDA 2008), so care must be taken to cross-check frogs with those at other nearby study sites.

Semi-automated, computer-based pattern recognition software has been used to reduce observer error and increase efficiency in studies on other species (Gamble et al. 2008). Though computer software accuracy was found to be quite low for this study, as software improves and becomes further adapted for species-specific pattern recognition, these programs may have the potential to lower labor costs, limit errors, and allow for non-invasive, long-term monitoring of larger populations. Visual or computer-based pattern matching could be applicable to other declining western ranid species that have similar chin mottling patterns including the Sierra Nevada Yellow-legged Frog (*R. sierrae*; Wengert and Gabriel 2006), the Southern Mountain Yellow-legged Frog (*R. muscosa*), and the Cascades Frog (*R. cascadae*). Given that many native ranid species are experiencing declines, population monitoring of these species could benefit from this non-invasive method in support of demographic and ecological studies.

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