

- (*Arachis hypogaea* L.) roots. *Appl. Environ. Microbiol.*, 1995, **61**, 1599–1609.
16. Khbaya, B., Neyra, M., Normand, P., Zerhari, K. and Filali-Maltouf, A. K., Genetic diversity and phylogeny of rhizobia that nodulate *Acacia* spp. in Morocco assessed by analysis of rRNA genes. *Appl. Environ. Microbiol.*, 1998, **64**, 4912–4917.
  17. de Bruijn, F. J., Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergenic consensus) sequence and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Appl. Environ. Microbiol.*, 1992, **58**, 2180–2187.
  18. Versalovic, J., Schneider, M. J., de Bruijn, F. J. and Lupski, J. R., Genomic fingerprinting of bacteria using repetitive sequence based polymerase chain reaction. *Methods Mol. Cell. Biol.*, 1994, **5**, 25–40.
  19. Evans, W. C. and Evans, D., *Trease and Evans' Pharmacognosy*, W. B. Saunders Co, UK, 1999, 14th edn.
  20. Vincent, J. M., *A Manual for the Practical Study of Root Nodule Bacteria*. IBP, Handbook No. 5, Blackwell Scientific Publication, Oxford, 1970.
  21. Somasegaran, P. and Hoben, H. J., *Methods in legume-Rhizobium technology*. NifTAL Project and MIRCEN, University of Hawaii, HI, 1985.
  22. Bazzicalupo, M. and Fani, R., The use of RAPD for generating specific DNA probes for microorganisms. In *Methods in Molecular Biology. Species Diagnostic Protocols: PCR and other Nucleic Acid Methods*. (ed. Clap, J. P.), Humana Press Inc., Totowa, NJ, 1995, pp. 155–175.
  23. Sneath, P. H. A. and Sokal, R. R., *Numerical Taxonomy, Principles and Practice of Numerical Classification*, Freeman, San Francisco, 1973.
  24. Perret, X. and Broughton, W. J., Rapid identification of *Rhizobium* strains by targeted PCR fingerprinting. *Plant Soil.*, 1998, **204**, 21–34.
  25. de Lajudie, P. *et al.*, *Allorhizobium undicola* gen. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. *Int. J. Syst. Bacteriol.*, 1998, **48**, 1277–1290.
  26. Heyndrickx, M., Vauterin, L., Vandamme, P., Kersters and De vos, P., Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns of bacterial phylogeny and taxonomy. *J. Microb. Methods*, 1996, **26**, 247–259.
  27. Kuykendall, L. D. and Saxena, B., Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. *Can. J. Microbiol.*, 1992, **38**, 506–512.
  28. Laguerre, G., van Berkum, P., Amarger, N. and Prévost, D., Genetic diversity of rhizobial symbionts isolated from legume species within the genera *Astragalus*, *Oxytropis* and *Onobrychis*. *Appl. Environ. Microbiol.*, 1997, **63**, 4748–4758.
  29. Peng, G. X., Tan, Z. Y., Wang, E. T., Reinhold-Hurek, Barbara, Chen, W. F. and Chen, W. X., Identification of isolates from soybean nodules in Xinjiang region as *Sinorhizobium xinjiangense* and genetic differentiation of *S. xinjiangense* from *Sinorhizobium fredii*. *Int. J. Syst. Evol. Microbiol.*, 2002, **52**, 457–462.
  30. Ogasawara, M., Suzuki, T., Muthoh, I., Annapurna, K., Arora, N. K., Nishimura, Y. and Maheshwari, D. K., *Sinorhizobium indiaense* sp. nov. and *Sinorhizobium abri* sp. nov. isolated from tropical legumes *Sesbania rostrata* and *Abrus precatorius*, respectively. *Symbiosis*, 2003, **34**, 53–68.
  31. Jordan, D. C., Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow growing, root-nodule bacteria from leguminous plants. *Int. J. Syst. Bacteriol.*, 1982, **32**, 136–139.
  32. Kuykendall, L. D., Saxena, B., Devine, T. E. and Udell, S. E., Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. *Can. J. Microbiol.*, 1992, **38**, 501–505.
  33. Chen, W., Wang, E., Wang, S., Li, Y., Chen, X. and Li, Y., Characteristics of *Rhizobium tianshanense* sp. nov., a moderately and slow growing root nodule bacterium isolated from an arid and saline environment in Xinjiang, People's Republic of China. *Int. J. Syst. Bacteriol.*, 1995, **45**, 153–159.
  34. Xu, L. M., Ge, C., Cui, Z., Li, J. and Fan, H., *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. *Int. J. Syst. Bacteriol.*, 1995, **45**, 706–711.
  35. Downie, A. and Young, J. P. W., The ABC of symbiosis. *Nature*, 2001, **412**, 597–598.
  36. Capela, D. *et al.*, From the cover: Analysis of the chromosome sequence of legume symbiont *Sinorhizobium meliloti* strain 1021. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 9877–9882.
  37. Laguerre, G. *et al.*, Typing of rhizobia by PCR DNA fingerprinting and PCR restriction fragment length polymorphism analysis of chromosomal and symbiotic gene regions: Application to *Rhizobium leguminosarum* and its different biovars. *Appl. Environ. Microbiol.*, 1996, **62**, 2029–2036.

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## The climbing perch (*Anabas testudineus* Bloch), a freshwater fish, prefers larger unfamiliar shoals to smaller familiar shoals

V. V. Binoy and K. John Thomas\*

Animal Behaviour and Wetland Research Laboratory, Department of Zoology, Christ College, Irinjalakuda 680 125, India

Shoaling is a behaviour pattern exhibited by several marine and freshwater fishes. However, little information is available on the social factors that form the basis of shoaling preference in tropical freshwater fishes. Here we analyse the effect of familiarity and shoal size on the preference of individuals of *Anabas testudineus*, a freshwater fish of the tropical region. Our results show that given a choice, *A. testudineus* preferred to spend more time with a familiar group compared to an unfamiliar group of equal size. The fish, however, preferred the larger group when two unfamiliar stimulus groups of different sizes were presented. The preference became significant when the ratio of stimulus groups was 1 : 1.9 and highly significant when the ratio was 1 : 4. A comparison of the

\*For correspondence. (e-mail: jkurishinkal@rediffmail.com)

**influence of familiarity and group size showed that *A. testudineus* had a bias for the larger unfamiliar group to the smaller familiar group, when the ratio of familiar versus unfamiliar stimulus shoals was 1 : 1.2 and the preference for a larger unfamiliar group was highly significant when the ratio was 1 : 4. Hence, an individual fish exhibited preference for joining a familiar shoal only when the ratio of the size of the familiar group to that of the unfamiliar group was 1 : 1. The adaptive advantage of this behaviour has been discussed here.**

FISHES of certain species associate with their conspecifics to form shoals. A group of fish that remain together for social reasons without any specific structure or form, is termed shoal<sup>1</sup>. A number of factors affecting shoal formation have been reported<sup>1</sup> in different species of marine and freshwater fishes of the temperate region. Understanding shoaling behaviour is essential for effective aquaculture practices and fisheries management. The benefits of shoaling include dilution effects, early predator detection, group defence and coordinated group manoeuvres<sup>1</sup>. All the above-mentioned factors might increase the chances of escaping from a predator and provide better opportunities for foraging under predation pressure<sup>2</sup>. However, aggregation of individuals may heighten competition for resources and the risk of transmitted parasites and diseases<sup>3</sup>. The decision to group is thus taken according to relative values of costs and benefits that accrue to individuals<sup>4,5</sup>.

The factors that determine preference for particular groups include number<sup>6,7</sup>, competitive ability<sup>8</sup> and parasitic infection status of members<sup>9,10</sup>. Familiarity is another factor that plays an important role in the formation of a shoal. Certain species of fish prefer to join a group composed of familiar conspecifics to those composed of members that they have not encountered previously<sup>11,12</sup>. The development of familiarity is time-dependent and a long-lasting phenomenon<sup>13</sup>. It has been shown that familiar groups may even re-assort after enforced mixing with non-familiar individuals<sup>14</sup>, indicating the importance of familiarity in shoal formation. However, the strength and influence of familiarity as a shoal cohesion force has not been fully investigated or understood<sup>15</sup>. Some of the advantages attributed to living with familiar individuals include maximization of shoal cohesiveness with a reduction in predation risk<sup>16</sup> or lower resource competition<sup>17</sup>. Establishment of a more stable social hierarchy in a familiar shoal may also lower levels of aggression<sup>18</sup>. In a complex habitat like tropical waters, the decision to be associated with a group contributes to the fitness of an organism in the context of high predation risk<sup>19</sup>, as well as mating/risk trade-off<sup>20</sup>. The degree to which individuals exhibit flexibility in decision-making can vary across populations and species, and such flexibility is linked to morphology, predatory regime or social structure<sup>21</sup>.

The climbing perch is a medium-sized fish inhabiting the freshwater ecosystems of India and other South-east

Asian countries. The fish exhibits obligatory air-gulping behaviour. It is omnivorous in habit and travels through ephemeral inflowing waterways, and thus often ends up on the land when the rain ceases.

To analyse the shoal size preference and the effect of familiarity on group preference, *Anabas testudineus* (standard length  $6 \pm 2$  (SE) cm) were collected from some ponds of Irinjalakuda ( $10^{\circ}25'$ ,  $10^{\circ}18'47''$  N lat. and  $76^{\circ}17'19''$ ,  $76^{\circ}12'48''$  E long.) Thrissur district, India during February–April 2002 and transferred to the laboratory. They were kept in groups of twenty in glass tanks ( $120 \times 60 \times 60$  cm) for 14 days<sup>13</sup> to make them familiar with each other. The tanks were filled with pond water up to 40 cm and provided with a sand substratum. Artificial food pellets (Marvel feeds, manufactured by Aquarium Systems, India) were given to the fishes twice, daily.

It has been observed that acclimation is one of the major factors that influence performance in preference tests (pers. obs.); the fish moved freely and showed normal behavioural patterns only after at least four trials in the experimental set-up. Hence, all test fishes were given a single trial each on four days in the experimental set-up in the absence of stimulus shoals. The data thus obtained have not been considered for analysis.

Experiments were conducted in a 70 l aquarium ( $85 \text{ cm} \times 32 \text{ cm} \times 32 \text{ cm}$ ) which was divided into three chambers – two side chambers ( $16 \text{ cm} \times 32 \text{ cm} \times 32 \text{ cm}$  each) and a central chamber ( $53 \text{ cm} \times 32 \text{ cm} \times 32 \text{ cm}$ ). The partitions were made of perforated, transparent acrylic sheets. Three sides of the aquarium were covered using black paper. The test fishes were always introduced individually into the central arena in a presentation cage made of transparent, perforated acrylic sheets ( $15 \text{ cm} \times 10 \text{ cm} \times 27 \text{ cm}$ ) with a sliding door on the top. The bottom of the presentation cage was open so that the test fishes can be released into the experimental arena by raising it. The water level in the set-up was 28 cm. A compact fluorescent lamp (11 W) was lighted on the top of the set-up. All tests were conducted in a room isolated from all other cues such as unnecessary noise, stray light, etc.

We conducted three types of choice experiments and the basic protocol was the same in all the experiments. Two stimulus shoals were introduced into the side chambers. After placing the presentation cage at the centre of the middle chamber, the test fish was introduced into it by opening the sliding door on the top. Ten minutes were given to the fish to assess the stimulus shoals on either side of the arena. In order to avoid interference due to the presence of the experimenter, the presentation cage was suspended using a string tied to a pulley and the test fish was released into the arena by lifting the cage. The time spent by the test fish near either of the stimulus shoals (within 10 cm from the side chamber; preference zone) or in the central area (non-preference zone) of the middle chamber was recorded with a stopwatch by the experimenter, sitting behind a black screen and looking through

a horizontal slit on the screen. The time spent by the test fish in the preference zone is taken as an indication of its preference for the shoal present in the adjacent chamber. The duration of each test was 6 min, after which the fishes were removed from the testing arena and put back into their home tank. No test fish was used more than once with any stimulus shoal pair and new fishes were used during in each trial. After the experiments, the fishes were released into their native ponds.

To test the influence of familiarity on shoal preference (experiment 1), two stimulus shoals, one familiar and the other unfamiliar, of equal size (1 : 1 ratio) were presented in the side chambers. The familiar shoal was composed of ten fishes from the home tank of the test fish. The unfamiliar shoal was composed of ten fishes taken from a different tank. At the end of the experiment, all fishes were returned to their respective home tanks. Fishes for the stimulus shoal were selected randomly from the home tank with replacement, so as to get a different combination of fish in each trial. We tested 42 fishes from seven different groups.

The test fish spent only a little time in the non-preference zone (<0.5% of total time), indicating a higher degree of shoaling behaviour in *A. testudineus*. When the mean values for each experiment on shoaling preference were considered, the test fish displayed a significant overall preference for shoals consisting of familiar fishes (Figure 1; Wilcoxon matched pairs, signed rank test<sup>22</sup>,  $T = 27$ ,  $N = 7$ ;  $P < 0.01$ ; Statistical programme KyPlot<sup>23</sup>). The result demonstrates that like many other fishes, *A. testudineus* also has a tendency to join familiar conspecifics<sup>15</sup>.

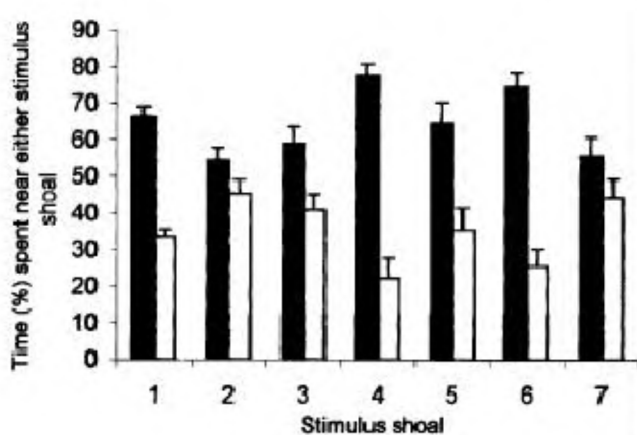
Shoal size preference was tested in another experiment (experiment 2). Here the test fishes were presented with two non-familiar stimulus shoals selected from two separate familiarization tanks, in the following numerical size combinations, i.e. 10 vs 10, 9 vs 11, 7 vs 13, and 4 vs 16 (ratio: 1 : 1, 1 : 1.2, 1 : 1.9, and 1 : 4 respectively)<sup>15</sup>. The total number of individuals in stimulus tanks was kept

constant (20) in all experiments. We tested 20 individual fishes selected from groups other than those that formed the stimulus shoals.

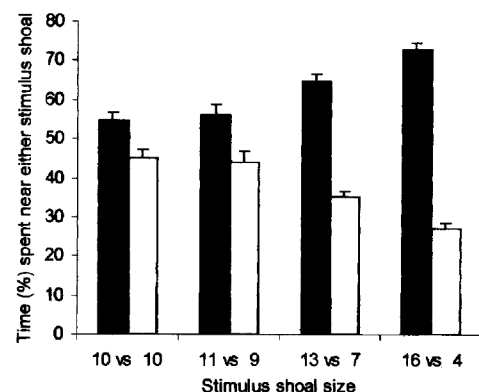
In the shoal size preference experiment (experiment 2), the test fishes preferred to remain with the larger group beyond a critical ratio. In fact, preference of the fish for larger groups, as indicated by the time spent by the test fish in the preference zone, increased with increase in shoal size (Figure 2). The preference for any group was not significant when the shoal size was in the ratio of 1 : 1 and 1 : 1.2 (10 vs 10 and 9 vs 11;  $T = 67$ ,  $N = 20$ ;  $P > 0.05$  and  $T = 58$ ,  $N = 20$ ;  $P > 0.05$  respectively). However, when the shoal size was changed from 1 : 1 to 1 : 1.9, the fish exhibited significant preference to the larger shoal (7 vs 13;  $T = 159$ ,  $N = 20$ ;  $P < 0.01$ ). A highly significant preference for the larger shoal was exhibited by the test fish when presented with two unfamiliar shoals with a size ratio of 1 : 4 (4 vs 16;  $T = 190$ ,  $N = 20$ ;  $P < 0.001$ ). This shows that the size of the shoal has a predominant effect on shoal selection in this species. The European minnow, *Phoxinus phoxinus*, also showed comparable behaviour in a similar experimental situation<sup>15</sup>.

To determine the trade-off point of familiarity against shoal size (experiment 3), the test fish was given the same stimulus combination as in experiment 2, except for the 1 : 1 ratio test for which the data were taken from the mean values of the outcome of all the seven groups in experiment 1. In contrast to the situation in experiment 2, the smaller shoal was always composed of familiar fish and the larger shoal of unfamiliar fish. We tested 20 fishes individually for each pair of shoals presented.

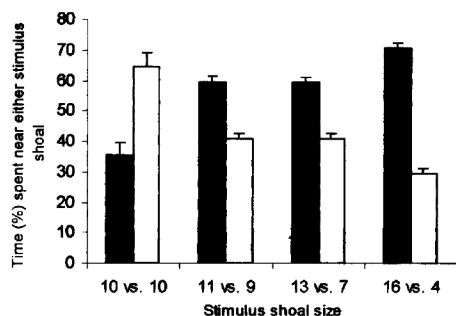
When the role of familiarity of a fish with other conspecifics of a shoal in deciding whether to remain with the familiar shoal or to desert it to join a larger unfamiliar shoal was tested (experiment 3), it was observed that the fish preferred a larger unfamiliar shoal to a smaller familiar shoal (Figure 3). It is interesting to note that a highly significant preference for an unfamiliar larger shoal to a



**Figure 1.** Preference of individual climbing perch for familiar/unfamiliar shoals. ■, Mean percentage of time + SD spent near familiar shoal; □, Mean percentage of time + SD spent near unfamiliar shoal.  $N/n = 6/42$ .



**Figure 2.** Preference of individual climbing perch for larger/smaller shoals. ■, Mean percentage of time + SD spent near larger shoal; □, Mean percentage of time + SD spent near smaller shoal.  $N/n = 20/80$ .



**Figure 3.** Preference of individual climbing perch for larger unfamiliar/smaller familiar shoals. ■, Mean percentage of time + SD spent near familiar shoal; □, Mean percentage of time + SD spent near unfamiliar shoal.  $N/n = 20/80$ .

smaller familiar shoal was shown only when the ratio of the familiar shoal to unfamiliar shoal was 1 : 4 (4 vs 16;  $T = 188$ ,  $N = 20$ ;  $P < 0.001$ ). However, the preference for an unfamiliar larger shoal could be seen even at a shoal size ratio of 1 : 1.2 and 1 : 1.9. (9 vs 11;  $T = 110$ ,  $N = 20$ ,  $P < 0.05$ ; and 7 vs 13;  $T = 110$ ,  $N = 20$ ;  $P < 0.05$ ). By contrast, *P. phoxinus* was shown to share its time equally between two shoals at a shoal size ratio of 1 : 1.9 (7 familiar vs 13 unfamiliar)<sup>15</sup>. It has been suggested that familiarity benefits are perceived as equivalent at an approximate doubling of the size of the unfamiliar shoal to that of familiar shoal<sup>15</sup>. The tendency of the climbing perch to prefer the larger unfamiliar group to smaller familiar group of 1.2 : 1 points to a heightened dependence on group-size in its shoaling behaviour.

In the natural aquatic habitat, shoal formation and disruption are likely to occur due to several environmental and anthropogenic factors. It is reported, for example, that shoaling fishes have a natural tendency to move from one group to another when they happen to encounter each other<sup>24,25</sup>. In such situations, the majority of fishes have a tendency to join the larger group<sup>4,8,26</sup>. Recently, Barber and Wright<sup>15</sup> have effectively compared the strength of preference of an individual fish for remaining with familiar shoal mates against those for other attributes of the group and found that familiarity can influence the group preference.

The present study indicates that in *A. testudineus*, acquaintance with conspecifics of a shoal can inhibit group desertion only to a limited extent. The lower limit value for desertion from a smaller familiar shoal to larger unfamiliar shoal in *A. testudineus* seems to be in the ratio of 1 : 1.2, which is lower than that shown by *P. phoxinus*<sup>15</sup>. It is reported that large size of prey groups considerably decreases the hunting success of various aquatic piscivorous predators<sup>27,28</sup>. It is therefore possible that the presence of voracious predators like *Channa punctatus* or *Channa marulius* in the natural habitat of *A. testudineus* could have influenced the shoal preference of the species. Support for this comes from the observation that a *Channa striatus* of length 16–20 cm was able to swallow an *A. testudineus* of length 4–6 cm (pers. obs.). In such a

situation, the benefits of joining a larger unfamiliar group outweighing the decision to remain with a smaller familiar group, are quite tenable.

- Pitcher, T. J. and Parrish, J. K., In *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman & Hall, London, 1993, pp. 363–349.
- Pitcher, T. J., Magurran, A. E. and Winfield, I., Fish in larger shoals find food faster. *Behav. Ecol. Sociobiol.*, 1982, **10**, 149–151.
- Alexander, R. D., The evolution of social behaviour. *Annu. Rev. Ecol. Syst.*, 1974, **5**, 325–383.
- Ashley, E. J., Kats, L. B. and Wolfe, J. W., Balancing trade-offs between risk and changing shoal size in northern red-belly dace (*Phoxinus phoxinus*). *Copeia*, 1993, **1993**, 540–542.
- Krause, J. G. and Godin, J. G. J., Shoal choice in the banded killifish (*Fundulus diaphanous*, Teleostei, Cyprinodontidae): effect of predation risk, fin-size species composition, and size of shoals. *Ethology*, 1994, **98**, 128–136.
- Reebs, S. G. and Saulnier, N., The effect of hunger on shoal choice in golden shiners (Pisces: Cyprinidae, *Notemigonus crysoleucas*). *Ethology*, 1997, **103**, 642–652.
- Lachlan, R. F., Crooks, L. and Laland, K. N., Who follows whom? Shoaling preference and social learning of foraging information in guppies. *Anim. Behav.*, 1998, **56**, 181–190.
- Metcalfe, N. B. and Thomson, B. C., Fish recognize and prefer to shoal with poor competitors. *Proc. R. Soc. London, Ser. B*, 1995, **259**, 207–210.
- Krause, J. and Godin, J. G. J., Influence of parasitism on shoal choice of the banded killifish (*Fundulus diaphanous*, Teleostei, Cyprinodontidae). *Ethology*, 1996, **102**, 40–49.
- Barber, I., Downey, L. C. and Braithwaite, V. A., Parasitism, oddity and mechanism of shoal choice. *J. Fish Biol.*, 1998, **53**, 1365–1368.
- Magurran, A. E., Seghers, B. H., Shaw, P. W. and Carvalho, G. R., *J. Fish Biol.*, 1994, **45**, 401–406.
- Griffiths, S. W. and Magurran, A. E., Schooling decision in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behav. Ecol. Sociobiol.*, 1999, **6**, 437–443.
- Griffiths, S. W. and Magurran, A. E., Familiarity in schooling fish: how long does it take to acquire? *Anim. Behav.*, 1997, **53**, 945–949.
- Barber, I. and Ruxton, G. D., Importance of stable schooling: do familiar stickleback stick together?, *Proc. R. Soc. London, Ser. B*, 2000, **267**, 151–156.
- Barber, I. and Wright, H. A., How strong are the familiarity preferences in shoaling fish? *Anim. Behav.*, 2001, **61**, 975–979.
- Chivers, D. P., Brown, G. E. and Smith, R. J. F., Familiarity and shoal composition in fat head minnows (*Pimephales promelas*): implications for anti predator behaviour. *Can. J. Zool.*, 1995, **73**, 955–960.
- Höjesjö, J., Johnsson, J. I., Petersson, E. and Järvi, T., The importance of being familiar: individual recognition and social behaviour in sea trout. *Behav. Ecol.*, 1998, **9**, 445–451.
- Johnsson, J. I., Individual recognition affects aggression and dominance relations in rainbow trout *Oncorhynchus mykiss*. *Ethology*, 1997, **103**, 267–282.
- Lima, S. L. and Dill, L. M., Behavioural decisions made under risk of predation: A review and prospectus. *Can. J. Zool.*, 1990, **68**, 619–640.
- Magurran, A. E. and Nowark, M. N., Another battle of sexes: the consequences of sexual asymmetry in mating costs and predation risk of the guppy (*Poecilia reticulata*). *Proc. R. Soc. London, Ser. B*, 1991, **246**, 31–38.
- Magurran, A. E., In ref. 1, 1996, pp. 441–477.

22. Snedecor, G. W. and Cochran, W. G., In *Statistical Methods*, Oxford & I. B. H., Calcutta, 1967, pp. 128–130.
23. KyPlot Version 2.0 beta 15 (32 bit) © 1997–2001 Koichi Yoshioka.
24. Svensson, P. A., Barber, I. and Forsgren, E., Shoaling behaviour of two spotted goby. *J. Fish Biol.*, 2000, **56**, 1477–1487.
25. Helfman, G. S., Social fidelity in fishes: the yellow perch pattern. *Anim. Behav.*, 1984, **32**, 663–672.
26. Keenleyside, M. H. A., Some aspects of the schooling behaviour of fish. *Behaviour*, 1955, **8**, 183–248.
27. Mlinski, M., An evolutionarily stable feeding strategy in sticklebacks. *Z. Tierpsychol.*, 1979, **51**, 36–40.
28. Tremblay, D. and FitzGerald, G. J., Social organisation as an anti-predator strategy in fish. *Nat. Can.*, 1979, **105**, 411–413.

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## Jurassic frogs and the evolution of amphibian endemism in the Western Ghats

**Sushil K. Dutta, Karthikeyan Vasudevan<sup>†</sup>,  
M. S. Chaitra<sup>†</sup>, Kartik Shanker<sup>#</sup> and  
Ramesh K. Aggarwal<sup>§,\*</sup>**

Department of Zoology, Utkal University, Vani Vihar,  
Bhubaneswar 751 004, India

<sup>†</sup>Wildlife Institute of India, P.O. Box 18, Dehradun 248 001, India

<sup>#</sup>Ashoka Trust for Research in Ecology and the Environment,  
659, 5th A Main Road, Hebbal, Bangalore 560 024, India

<sup>§</sup>Centre for Cellular and Molecular Biology, Uppal Road, Tarnaka,  
Hyderabad 500 007, India

**The diversity of frogs and toads (Anurans) in tropical evergreen forests has recently gained importance with reports of several new species<sup>1</sup>. We describe here a fossorial frog taxon related to the African Heleophrynidae and Seychellian Sooglossidae from the Western Ghats of India. This frog possesses a suite of unique ancient characters indicating that it is a transitional form between Archaeobatrachians and Neobatrachians. Molecular clock analysis based on the nucleotide diversity in mitochondrial 12S and 16S genes dates this frog as a Gondwana relic, which evolved 150–195 Mya during the mid-Jurassic period.**

**With this taxon, the evolution of endemism in the Western Ghats and other Gondwana breakup landmasses is now dated much before the Cretaceous–Tertiary boundary. We propose that sea level surges in the late Jurassic<sup>2</sup> isolated tablelands creating insular amphibian fauna. Reduction in area may have promoted stochastic extinctions and resulted in amphibian endemism. Our study reinforces the conservation significance of the Western Ghats as major global hotspot of biodiversity. The habitat of this endemic amphibian lineage is currently endangered due to various upcoming dam projects, which is a cause of serious conservation concern.**

THE Western Ghats mountain range in Southern India, one of the Gondwanaland breakup landmasses, is amongst the most important hotspots of biodiversity<sup>3</sup>. Recently the Western Ghats and Sri Lanka have gained attention as hotspots of amphibian diversity due to recent reports of several undescribed taxa<sup>4,5</sup>. We present here an ancient amphibian taxon, which provides new insights in our present day understanding of the endemism and distribution of fauna and flora in the Western Ghats and other Gondwanaland breakup landmasses and puts the evolution of several other anuran lineages in the middle Jurassic. A similar frog taxon has recently been described under a newly erected anuran family from Western Ghats<sup>6</sup>. In this emerging scenario, resolving the biogeography and the evolution of endemism in these landmasses becomes crucial in developing preemptive conservation strategies. Our findings imply that a large amount of the evolutionary history of amphibians is encompassed within the Western Ghats, making it an important region for conservation.

We provide here a detailed morphological and ontogenic description of the taxon based on many specimens and resolve its phylogeny using molecular data. We also put forth a hypothesis on the evolution of endemism of amphibians in the Gondwana breakup landmasses.

The study is based on three adult specimens: one male, two females and four tadpoles. Adult specimens (BNHM 4214 to 4216) are deposited in the Bombay Natural History Museum, Mumbai and tadpoles (WII 659 to 662) in the Wildlife Institute of India Museum, Dehradun. Tissue samples from the male and one female were used for genotyping. BNHM 4214, a mature male, SVL 52.8 mm was found in Sankaran Kudi ca 600 ha rainforest fragment in the Anamalais (10°14'46"N, 76°55'55"E) Tamil Nadu, by K.V. on 20 May 2001 at 1730 h. BNHM 4215 (Figure 1), a gravid female, SVL 89.9 mm was collected along with BNHM 4214; BNHM 4216, a gravid female was SVL 78.3 mm, collected from Murikkassery, Kothamangalam, Ernakulam District, Kerala by Sr. Jose Mary, Alphonsa College, Palai, Kerala. Additional specimens examined were as follows: (i) A mature male, SVL 67.0 mm, collected during July 1994 from Manimala River at Erumely, Kottayam District, Kerala. This specimen is available at

\*For correspondence. (e-mail: rameshka@ccmb.res.in)