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STUDIES ON THE CRANIAL OSTEOLOGY
OF THE BLIND CATFISH
*Horaglanis krishnai* MENON
(PÍSCES, CLARIIDAE)

T.V. Anna MERCY* and N. Krishna PILLAI

**SUMMARY**

*Horaglanis krishnai* Menon is a blind catfish inhabiting the dug-out wells at Kottayam, Kerala, South India. Studies on the cranial osteology of the fish show that the bones on the skull are firmly articulated. The frontoparietal fontanella is very large so that the cranium virtually lacks a roof. The sphenotics and alisphenoids are hardly recognizable and the orbital bones are entirely lacking. In osteological features *H. krishnai* closely resembles *Uegitglanis zammaroni*. But in *H. krishnai* the orbital bones are further reduced or even absent. The fontanella is larger than that of any other known catfish. These two species must have evolved from the same ancestor and have taken up nearly identical ways of life. The difference between the skeletons of these two appears to be largely dependent on the relative size of the frontoparietal fontanella. Its greater development in *H. krishnai* brought about a suppression or reduction of some of the bones clearly visible in *Uegitglanis*. It would appear that the modification initiated in *Uegitglanis* gathered momentum in *Horaglanis*. These two fishes form a group distinct from clariids and bagrids but form a connecting link between the two.

**Keywords:** horaglanis krishnai, cave fishes, osteology

**Introduction**

Cavefishes attracted the attention of ichthyologists only comparatively recently. Till date, 32 species of fishes have been recorded from caves, artesian wells and subterranean waters. The discovery of the new blind clariid fish from Keala, *Horaglanis krishnai* Menon (1950) and the occurrence of a similar blind clariid fish, *Uegitglanis zammaroni* Gianferrari 1932 from the former Italian Somali land have raised points of great zoogeographical interest.

Our information about these two forms is, by no means adequate. On a perusal of available literature it was found that extensive work has been done on the osteology of ostariophysean fishes. Publications deserving special mention are those of Regan (1911), Kindred (1919), Bhimachar (1933), Gregory (1933), David

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It has been found that there is little work on blind fishes. The only important paper on this subject is by David (1935) on the osteology of *Uegitglanis zammaroni* In the present study, a detailed account of the cranial osteology of *Horaglanis krishnai* is furnished and compared with that of *Uegitglanis zammaroni* in detail.

**Materials and methods**

*Horaglanis krishnai* occurs at the bottom of the dugout wells at Kottayam, (Lat. 8°4’N and 10°21’N; Lougitude 76°13’E and 77°8’E) Kerala, South India. Specimens of *H. krishnai* were collected from the dugout wells at Kottayam. As the fishes invariably rest on the bottom they could be collected only by draining the wells. Osteological descriptions are based on Alizarin preparations of head of 20 individuals ranging in length from 2.0-2.9cm. The positions of the various bones were carefully noted from Alizarin preparations. For detailed study of the shape and size of individual bones, the bones were disarticulated from the cranium under binocular microscope. Sketches were made with the aid of camera lucida.

**Results**

The skull is twice as long as broad and steadily narrows forwards (Fig. 1&2). It is widest at the auditory region. As the bones are connected by sutures and even partially fused, the boundaries of individual bones can be made out only with difficulty. The skull has a large dorsomedian fontanella and hence the cranium lacks a roof. The fontanella is anteriorly bordered by the dermethmoid, posteriorly by the supraoccipital and laterally by the frontoparietals.

**Dermethmoid: (Figs.3 & 4)**

The dermethmoid is a median dorsal bone situated at the anterior end of the skull. It has two small frontal processes articulating with the premaxilla in front. Posteriorly, it is drawn out into two stout processes, which articulates with the anterior ends of the frontoparietals. The posterior ventral part of the dermethmoid is excavated and into this excavation fits the anterior extremity of the parasphenoid. The dermethmoid contacts the premaxilla anteriorly; the frontoparietals posteriorly; the parasphenoid
ventrally and the lateral ethmoids laterally. The posterior concavity forms the anterior boundary of the fontenella.

**Lateral ethmoids:** (Fig. 5 & 6)

The lateral ethmoids are paired bones on either side of the dermethmoid. They are irregular in shape with a posterolateral triangular process, which extends over half
Fig. 3 and 4 - The dermethmoid.

Fig. 5 and 6 - The lateral ethmoids.
the length of the premaxilla. The lateral ethmoids do not meet along the midventral line but leave a narrow space between. Into this narrow space fits the anterior end of the parasphenoid. The lateral ethmoids articulate with the orbitosphenoid posterior, dermethmoid and front parietals dorsally, premaxilla interiorly and parasphenoid ventrally.

**Frontoparietals: (fig.7)**

The bones, which laterally border the large median fontanella, are referred to as the frontoparietals. David (1936) used the same name in describing the skull of *U. zammaroni*. The frontoparietals are elongated bones contributing three fourths of the boundary of the fontanella. They are fused with the sphenotics producing a pair of complex irregular bones. The posterior ends of these bones are pointed and overlap the anterior end of the supraoccipital.

**Supraoccipital: (Figs. 8 & 9)**

This is a dors median, well-demarcated bone. Anteriorly it is broad, with two lateral wing-like expansions, but narrows backwards. There is a well-defined sharp supraoccipital spine. A small oval occipital fontanella is observed at the middle of this bone. The anterior concavity of the supraoccipital spine articulates with the neural spine of the complex vertebra. The supraoccipital spine is very prominent and occupies half the total length of the bone. The hind part of the front parietals abuts on the lateral grooves on the dorsal side of the occipital bone. The supraperiartal bone, which is the largest of the occipitals joins the frontoparietals anteriorly and the pterotics and epiotics laterally.
Exoccipital: (Fig. 10)

The exoccipitals are large, irregular bones situated ventrolaterally, on either side of the foramen magnum. There is a concavity on their anterior side. These bones form the posterior boundary of the otic capsule. Each exoccipital is connected suturally with the basioccipital ventrolaterally, prootics anteriorly and the epiotics posteriorly.

Basioccipital: (Fig. 11)

This is a single, ventromedian bone forming the posterior end of the neurocranium. Its anterior part is roughly triangular and the posterior cylindrical. At its hind end the basioccipital carries the occipital condyles, whose posterior surface is deeply con-
cave and similar to that of the centrum of the vertebra, and provides an articulating surface for the complex vertebra. The anterior end is very thin and is prolonged into a forked process, which is suturally connected to the posterior end of the parasphenoid. The basioccipital is bounded laterally by the exoccipitals, anteriorly by the prootics and parasphenoid and dorssily by the epiotics. This bone forms the ventral boundary of the foramen magnum.

**Spheuodes:**

The sphenotics are fused to the middle region of the front parietals laterally and are not demarcated as distinct bones of the roof of the cranium. Each sphenotic has two sharp ridges, giving articulation to the hyomandibular. The sphenotics extend ventrolaterally to form the posterior boundary of the optic foramen. The ventrolateral extension of the sphenotics contacts pterotics behind and the prootics and parasphenoid ventrolaterally. The lateral extension of this bone has two small foramina through which pass the trigeminal and facial nerves. The lateral ridges of the front parietals and the sphenotics are comparatively thick.

**Pterotics (Fig. 12)**

Pterotics are highly arched, paired bones forming the posterolateral parts of the cranial roof. These irregular bones take part in the formation of the auditory capsules. The dorsolateral edge of the pterotic bone has a bifid lateral pterotic process, which articulates with the post temporal and hyomandibular. The pterotics contact the prootics ventrolaterally, and the epiotics and exoccipitals posteriorly. Dorsally, the pterotics are connected to the supraoccipital.

**Epiotics: (Fig. 13)**

These paired bones form the posteriormost part of the auditory capsule and are situated on either side of the supraoccipital. Each epiotic has small spinous process, which arises from the posterior end of the outer surface and proceeds downwards.
This downward extension supports the post temporal. The epiotic has a shallow, bowl-like cavity. This bone is surrounded by the supraoccipital and the pterotic dorsally and the exoccipitals ventrally.

**Prootics:** (Fig. 14)

The prootics are large, flattened bones which lie on the posterior ventral region of the neurocranium. They have nearly semi-circular concavity to lodge the otolith. The two prootics meet along the midventral line over the parasphenoid thus completing the floor of the cranium there. From the dorsal side of the prootics proceeds forwards a splint of bone, which contacts a small process of the pterotic. Anteriorly, the prootics are connected with the parasphenoid, laterally with the pterotics and sphenotics and posteriorly with the exoccipitals and the basioccipitals.

**Parasphenoid:** (Fig. 15)

The parasphenoid is a median, ventral dagger shaped bone supporting the floor
of the whole cranium. It is comparatively very thin. Its anterior constricted part is inserted into the hollow ventral part of the dermethmoid. The parasphenoid extends from the basioccipital to the dermethmoid and is concave midventrally. It is broad in the middle and narrow at both ends. Anteriorly it is connected to the dermethmoid and lateral ethmoids, posteriorly to the basioccipital and prootics and anterolaterally to the orbitosphenoid. The alisphenoid is so intimately fused with the parasphenoid that it cannot be easily separated. The alisphenoid form the ventrolateral boundary of the optic foramen.

Orbitosphenoid: (Fig. 16)

The orbitosphenoids are a pair of well-developed bones forming the ventrolateral parts of the skull and taking part in the formation of the sidewalls of the cranial cavity. The deep notch on its hind end forms nearly half the anterior boundary of the optic foramen. The anterior portion of the sphenotics and alisphenoid forms the rest of the boundary. Each orbitosphenoid joins the frontoparietals above, the parasphenoid below and the lateral ethmoids in front. In *H. krishnai* the orbital bones are apparently absent.

**Fig. 16 - The orbitosphenoid.**

Discussion

There is indeed an abundance of literature on the osteology of fishes. But catfishes do not appear to have attracted sufficient attention. Regan (1911) classified the Ostariophysii on the basis of their cranial osteology. Bhimachar (1933) studied the osteology of eight catfishes namely, *Rita buchanani, Silundia gigantica, Plotosus canius, Wallago attu, Pangassius buchanani, Macrones aor, Arius sona, Arius sagore* and *Osteogeneosus mutilaris*. This comparative study helped the author to fix the taxonomic position of each of the species. Gregory (1933) studied the osteology of Clariidae and Bagridae and discussed the evolution of Ostariophysii. David (1935) studied clariid genera, *Clarias, Allabenchyles, Clariallabes, Channallabes, Heterobranchus, Dinopterus* and *Uegitglanis*. In this exhaustive treatise, the author
has given brief description of the skulls of all the species taken up for investigation. This work, more than any other, helped in understanding the philogeny of Clariidae. David (1936) described the skull of Uegitglanis zammaroni, the blind siluroid discovered in the former Italian Somaliland. In this work he gave a discussion of the special features of the head skeleton of Clariidae and Bagridae. He mentioned the peculiarities of the skull of Uegitglanis and came to the conclusion that it is intermediate between that of Clariidae and Bagridae.

Merriman (1940) dealt with the osteology of Galeichthys felis and Bagre marinus, two large forms of catfishes inhabiting the southern Atlantic. Nawar (1954) made detailed observations on the skull, vertebral column and fins of Clarias lazera. Joseph (1960) described the osteology of the head of the catfish Wallago attu and correlated the modifications observed with the habitat of the fish. Recently Dutta et al. (1975) made a comparative study of the cranium of Clarias batrachus and Heteropeustus fossils. Their description of the skull of Heteropeustus is not different from that given by Bhimacbar (1933).

From the above it can be seen that though there are a few publications on the osteology of catfishes, including one on a blind species (David 1936), the available information is by no means adequate. As H. krishnai is totally blind and the only work on a similar fish is that of David (1936), this alone need to be considered for a detailed comparison. The account will, therefore, be mainly on the habitat-oriented modifications in the osteology of H. krishnai.

H. krishnai shows a number of significant modifications in its skeletal system which can be directly correlated with its habit and habitat. The various bones of the skull are firmly articulated by sutures. This might be helpful in a fish, which moves about through narrow subteanean channels where the chances for the head striking against hard objects are high. However, a cephalic shield, composed of the supraorbitals, dermosphenotics and the post temporals, which is found well developed in other catfishes (Joseph, 1960) is absent in H. krishnai. But the cranial roof is similar to that of other clariids, and of Clariallabes and Channallabes in particular, which are partially blind, and Uegitglanis zammaroni, which is totally blind. The roof of the cranium of H. krishnai is reduced to the narrow frontoparietals enclosing between them large, elongate, elliptical fontanella. The frontoparietals and the sphenotics are fused together to form a complex irregular bone. In Uegitglanis (David, 1930) the frontoparietals are broad, which, along with the supraoccipital, form the dorsal surface of the cranium. The fusion of the frontoparietals with the sphenotics into a complex structure may be presumed to be a step towards increasing the rigidity of the skull.
In typical catfishes the dovetailing of the bones of the dorsal shield provides necessary strength to the roof of the cranium.

Among the catfishes, in general, the anterior halves of the frontals remain separated by wide fontanella. There is also another foramen of the supraccipital bone. The size and extent of these fontanellae vary in different species. In Bagrus (David, 1936) both fontanellae are linear and separated by a thin median parietal bone. In Bagrus the two fontanellae are similar but in all others the supraoccipital fontanella is small. In H. krishnai the anterior fontenella is exceptionally broad, exposing most of the dorsal surface of the brain. This is a unique feature of the skull of H. krishnai. The development of such a large fontanella must be considered as a result of the degeneration and disappearance of the eyes. A transparent thin smooth membrane covers the fontanella. The obvious inference is that the mid brain did not provide any evidence for this surmise, though this is the only way the presence of an elongated fontanella can be explained.

The pterotics in H. krishnai are strong, laterally arched bones of the cranial roof, taking part in the formation of auditory capsule. In both Uegitglanis and Bagrus the pterotics are laterally situated and associated with the auditory capsule. The same condition is noticed in H. krishnai also.

In H. krishnai the sphenotics can be hardly be recognized. In Uegitglanis the sphenotics form a marginal border of the cranial roof. In Clariallabes and Channallabes the sphenotics are pushed outwards by a broad process of the frontoparietals. They take practically no part in the formation of the cranial roof. It would appear that the habitat and partial or total blindness of fishes have influenced the size and position of the sphenotics.

The orbitosphenoids are well-developed bones forming the lateral wall of the anterior part of the cranium. Bhimachar (1933) observed that the presence of a well-developed orbitosphenoid is an archaic feature. But its persistence, even in highly specialized species, is probably because it helps in the attachment of the ethmoid region to the rest of the cranium (Bhimachar 1933).

In most teleosts the basisphenoid is a small median 'Y' shaped bone placed above the parasphenoid. Whether this bone is present or not in Ostariophysii is still being debated. Kindred (1919) and de Beer (1937) observed it in Ameiurus but Sagemehl (1891) and Berg (1940) did not. Bhimachar (1933) found the bone in all catfishes he studied and according to him in Wallago attu it can be seen on the ventral aspect of the parasphenoid. However, Joseph (1960), who studied Wallago attu in detail,
observed that this bone is not visible and that there is no indication that it is fused with the parasphenoid. In *H. krishnai* the basisphenoid is certainly absent. It seems that the uncertainty regarding the presence or absence of the basisphenoid indicates that among catfishes this bone has been undergoing reduction ending in its disappearance as in *H. krishnai*.

The degree of development of the supraoccipital spine is often taken as an index of evolutionary advancement. This assumption is based on the finding that in the precretaceous bony fishes the base of the cranium was nearly flat and the supraoccipital process was absent. (Woodward 1898). In *H. krishnai* the supraoccipital process is well developed. The same condition is observed in *Uegitglanis* also. In the admittedly more primitive *Silundia* and *Wallago* (Bhimachar 1933) the supraoccipital is small. Thus this bone, unlike the basisphenoid is undergoing progressive development.

The dermethmoid in *H. krishnai* is a median bone with two short anterior processes articulating with the premaxilla. It is firmly fused with the front parietals posterior. In *Uegitglanis* the bone is even, except for a short deut in the middle, forming the anteriormost part of the boundary of the fontanella. The clariids and bagrids differ particularly with regard to the bones, which form the sides of their cranium. In the degenerated clariids, *Clariallabes* and *Channallabes*, those bones are reduced but clearly recognizable (Regan 1911; David 1935). In these forms the dermosphenotics are rudimentary structures. In *Uegitglanis* it is exactly as in *Channallabes*. But in bagrids it is still more modified forming a unique bone associated with the preopercle. But the orbital bones of *Uegitglanis* correspond with those of bagrids. In latter they are modified into tubular structures, surrounding the mucus canals, which originates from the sphenotics and terminates in the maxillary after taking a curve around the eye. In *Channallabes* the orbital bones are reduced to a cartilaginous ring around the eye. In *H. krishnai* those bones are absent. This obviously is due to the total absence of the eyes.

The eye muscle canal or the myodome is supposed to be absent in siluroids. It is absent in all the catfishes studied by Bhimachar (1933). But in the primitive *Silundia* Bhimachar (1933) found a small vestige of the myodome, persisting between the prootics, the parasphenoid and basioccipital. This indicates that the myodome must have been well developed and functional in the ancestral silurids. A rudiment of the myodome was noticed in *Ameiurus* by Murrich (1884). In *H. krishnai* the myodome is absent. It can, therefore, be concluded that *H. krishnai* belongs to the more
advanced group of catfishes in which the myodome has been obliterated by the sec-
ondary simplification of this region of the skull. The secondary simplification must
have got accelerated due to the degeneration of the eyes.

The cavum cranii of *H. krishnai* extends up to the ethmoid region. Hence, the crani-
um is platybasic. The platibasic nature of the cranium of siluroids is considered to be
primitive nature (Bhimachar, 1933). In *Uegitglanis* the cavum cranii is normally
developed but highly reduced in the middle. The same is true of *Channallabes.*

Joseph (1960) came to the conclusion that the cranium of *Wallago attu* is of a primit-
ive type. Bhimachar (1933) found that, of the eight species of catfishes he studied,
six have a primitive type of skull. He suggested that the skull of *Arius* and
*Osteogeneosus* are more evolved.

In osteological features *H.krishnai* closely resembles *Uegitglanis.* But in *H. krishnai*
the orbital bones are further reduced or even absent. The fontanella is larger than that
of any other known catfish. These two species must have evolved from the same
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CLASSIFICATION OF TERRESTRIAL SUBTERRANEAN FAUNA OF VOLCANIC SUBSTRATES IN THE CANARY ISLANDS

Josè L. MARTÍN, Helga GARCÍA, Y P. OROMÍ*

SUMMARY
A system is proposed for classifying the species occurring in the hypogean environment in relation to their ecological and evolutionary characteristics. The ecological criteria utilized relate to the preferred habitat of the animals (the epigean, endogean or hypogean environment) and the evolutionary criteria specify the grade of adaptive modification in three characteristics: reduction of eyes, amount of pigmentation and extent of elongation of the appendages. The object of developing this classification is to provide a system appropriate for those regions - such as those with volcanic rocks - in which the cave faunas include elements originating in different environments, and in which the species show very variable adaptive grades, depending primarily on the antiquity of the island or other distinct geological zone, where they are found.

Keywords: cave fauna, Canary islands.

In the time since Schiödte (1849) and Schiner (1854) proposed the first classifications of cavernicolous species, great advances have been made in understanding the habitats and biology of these animals; this has revolutionised a number of old ideas.

The diversity of ecological and morphological types which occur together in caves ensures that their classification is not an easy task; nonetheless, many authors have made the attempt, some proposing new classifications (Schiner, 1854; Dudich, 1932; Chapman, 1986) and others merely trying to improve the previous ones (Barr, 1968; Vandel, 1964). Among the varied existing proposals (Table 1) the one most generally accepted at present is probably that proposed by Schiner (1854) and subsequently modified by Racovitza (1907). This classification divides cavernicolous animals into troglobites, troglophiles and trogloxenes on the basis of behaviourl characteristics, although at times these are associated with morphological peculiarities (Holsinger, 1988) and troglobites are specified as being eyeless, depigmented and with long appendages. This approach proves to be unsatisfactory in various ways,

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since there are species which lack the specified morphological characteristics and yet live permanently in caves, so that they should be considered as troglobites. In many cases such a situation permits recognition of a certain gradation in the level of adaptation of troglobites. Examples are provided by several species encountered in young volcanic islands in the Canaries (e.g. El Hierro: Oromí et al., 1991) and in Hawaii (Howarth, 1972), as well as in particular tropical karst areas in Papua New Guinea (Brignoli, 1981) and in Asia and Australasia (Chapman, 1986). Jeannel did not overlook this fact and in his classical work "Les fossiles vivants des cavernes" (1943) he distinguished between recent troglobites and relict troglobites, on the basis of the extent of development of their adaptive characteristics - more marked in the last group.

Table I - Terms for different types of species proposed in different classifications of the subterranean fauna.

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<tr>
<th>Shadow animals</th>
<th>Schiödte, 1849</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twilight animals</td>
<td>Schiödte, 1849</td>
</tr>
<tr>
<td>Animals of dark areas</td>
<td>Schiödte, 1849</td>
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<tr>
<td>Stalactite animals</td>
<td>Schiödte, 1849</td>
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<tr>
<td>Occasional cave animals</td>
<td>Schiner, 1854; Ginet and Decou, 1977</td>
</tr>
<tr>
<td>Troglophiles</td>
<td>Schiner, 1854; Racovitza, 1907; Jeannel, 1943; Hamilton-Smith, 1970</td>
</tr>
<tr>
<td>Eutroglophiles</td>
<td>Pavan, 1950</td>
</tr>
<tr>
<td>Subtroglophiles</td>
<td>Pavan, 1950</td>
</tr>
<tr>
<td>Troglobites</td>
<td>Schiner, 1854; Racovitza, 1907; Jeannel, 1943; Pavan, 1950; Hamilton-Smith, 1970; Ginet and Decou, 1977</td>
</tr>
<tr>
<td>Recent troglobites</td>
<td>Jeannel, 1943</td>
</tr>
<tr>
<td>Relict troglobites</td>
<td>Jeannel, 1943</td>
</tr>
<tr>
<td>Trogloboxenes</td>
<td>Racovitza, 1907; Jeannel, 1943</td>
</tr>
<tr>
<td>Eutrogloxenes</td>
<td>Pavan, 1950</td>
</tr>
<tr>
<td>Subtrogloxenes</td>
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<tr>
<td>Regular trogloboxenes</td>
<td>Hamilton-Smith, 1970; Ginet and Decou, 1977</td>
</tr>
<tr>
<td>Irregular trogloboxenes</td>
<td>Ginet and Decou, 1977</td>
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<tr>
<td>Accidental trogloboxenes</td>
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</tr>
<tr>
<td>Phyletic trogloboxenes</td>
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<td>Aphyletic trogloboxenes</td>
<td>Pavan, 1950</td>
</tr>
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<td>Xenocaval animals</td>
<td>Hesse, 1924</td>
</tr>
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<td>Typhocaval animals</td>
<td>Hesse, 1924</td>
</tr>
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<td>Eucaval animals</td>
<td>Hesse, 1924</td>
</tr>
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<td>Dudich, 1932</td>
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<td>Dudich, 1932</td>
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<tr>
<td>Eutroglobionts</td>
<td>Dudich, 1932</td>
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<tr>
<td>Edaphobites</td>
<td>Coiffait, 1959</td>
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<tr>
<td>Edaphophiles</td>
<td>Coiffait, 1959; Ginet and Decou, 1977</td>
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<tr>
<td>Edaphoxenes</td>
<td>Coiffait, 1959; Ginet and Decou, 1977</td>
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<td>Chapman, 1986</td>
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<td>Chapman, 1986</td>
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<td>Parasites</td>
<td>Ginet and Decou, 1977</td>
</tr>
<tr>
<td>Guanobites</td>
<td>Ginet and Decou, 1977</td>
</tr>
<tr>
<td>Cryptozoic animals</td>
<td>Peck, 1990</td>
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</table>
Originally troglobites were defined as inhabitants of caves, with the implication that they lived only inside the caves themselves. But long ago Racovitza (1907) realised that their habitat was really more extensive when he wrote “... j’incline à penser que beaucoup de cavernicoles ont leur habitat normal dans les fentes, et non dans les grottes ...”. Subsequently authors such as Jeannel (1943) and Ginet & Decou (1977) came to the same conclusion. In any case almost all the specialists implied that terrestrial troglobites occurred only in karst environments (Vandel, 1964), in spite of the fact that since the end of the 1930s terrestrial troglobites were known from Japanese caves (see Torii, 1960).

Discoveries made in recent decades have influenced current concepts relating to the habitat of troglobites. Studies in the Galapagos (Leleup 1965), Japan (Torii 1960 and Ueno 1960), Hawaii (Howarth 1972) and the Canaries (Español & Ribes 1983; Hernández, et al. 1986) demonstrated the existence of a multitude of troglobites in volcanic regions, and the investigations of Juberthie and his collaborators at the end of the 1970s (Juberthie, et al. 1980) led to the discovery of new troglobites in continental non-calcareous zones.

Both authors who consider the superficial and deep subsoil as distinct environments and those who consider them as different parts of the same environment, treat the most highly adapted species that live in them as troglobites; this implies an intrinsic contradiction since the word troglobite refers literally to life in caves rather than to life in cracks. Nonetheless, the term troglobite can remain valid as soon as we specify that caves are merely large cracks. If we discard the anthropocentric viewpoint on the concept of a cave and accept that a cave is no more than a crack of large size, for tiny subterranean animals a crack is effectively a cave. Following this reasoning, the term troglobite recovers - at least etymologically - exactly the same meaning which it was given by Schiner in 1854 and remains valid for referring to subterranean species that live in cracks in the underground environment.

Many of the difficulties in applying the traditional classifications universally, result from the “atypical” characteristics of the underground environment of certain regions. This is particularly true in the case of volcanic terrain in tropical and subtropical zones, which differs in significant ways from the karst environment of the temperate zones. Volcanic activity gives rise to caves very close to the surface - where roots can penetrate them and other forms of external energy input can occur - and to a great variety of types of shallow, interconnected, underground environments which considerably broaden and diversify the habitat of the troglobites (Oromí et al., 1986). On the other hand in warm and humid climates there is often less difference between conditions above and below ground, with the result that the limits of the epigean and
hypogean environments are less distinct.

The need for a precise terminology when comparing the faunas of the separate islands of the Canary Archipelago has led us to develop a new classification which, without abandoning the classical one of Schiner-Racovitza, will be more useful for our purpose. In order to avoid mixing up aspects that are not exactly correlated, such as form and habitat, we have developed two systems of classification for the species concerned, defining respectively their evolutionary characteristics (morphology) and their ecological characteristics (habitat).

**Ecological classification (habitat) of the species**

In general, species that live on the surface of the soil are called epigean, those that live within it endogean and those that live under it hypogean. But not all the species live exclusively in one of these three environments; they may make use of several of them, although always living primarily in one.

When a species has the majority of its individuals in a particular environment, and furthermore is capable of reproducing and completing the whole of its life cycle there, we say that it is “characteristic” of that environment. We can therefore refer to “epigeobites” and “endogeobites” and “troglobites” as species characteristic respectively of the epigean, endogean and hypogean environments. The terms “epigeobite” and “endogeobite” combine the name of the environment in which the animals live with the termination “-bite”; we have used the term “troglobite” in preference to the perhaps more precise term hypogeobite, because it is more established among biospeleologists and because in reality the hypogean environment constitutes a world of cracks as mentioned above. One can apply similar reasoning with respect to troglophiles and trogloxenes.

| Table 2. System of classification of animal species based on their habitats. |
|-----------------------------------|-----------------------------------|-----------------------------------|
| **Epigeon Environments**          | **Endogean Environments**         | **Hypogean Environments**         |
| Epigeobite                        | Endogeobite                       | Troglobite                        |
| Epigeophile                       | Endogeophile                      | Troglophile                       |
| Epigeoxene                        | Endogeoxene                       | Trogloxene                        |

By combining the name of a specific environment with the ending “-phile” (which means “lover of”) we get new terms which correspond to particular types of
animals, “epigeophiles” “endogeophiles” and “troglophiles”. These have significant populations respectively in epigean, endogean and subterranean environments, in which they can reproduce and complete the whole of their biological cycle, but nonetheless have the majority of their individuals in one of the other two types of environment.

Finally, if we do the same with the termination “-xene” (which means “foreigner”), we get “epigeoxenes”, “endogeoxenes” and “trogloxenes”, which are animals occurring respectively in the epigean, endogean and subterranean environments, but generally in a casual manner and without being able to complete their whole biological cycle in it. Furthermore the majority of their individuals are never in the environment to which the term refers, but in one of the others. Some authors distinguish between facultative trogloxenes and accidental trogloxenes, depending on the cause of their presence in the hypogean environment (Barr 1968).

Following this classification, a single species can be considered “-bite” in one environment, “-phiIe” in another and “-xene” in a third. All the combinations between these categories are shown in one of the columns of Table 2, applying the constraints that one takes the categories in order and that a species which is “-bite” in one environment cannot also be “-bite” in another.

When the biological cycle of an arthropod includes stages that take place in different environments its classification can become difficult. In these cases we take as a fundamental basis the habitat of the adult. One can see several relevant examples in the fauna of the Canaries.

For instance, beetles of the family Rhizophagidae can be found in epigean environments, but also in endogean and hypogean ones, provided that the food plant of the larvae is present. Their life cycle has a larval phase in the endogean environment and an adult phase outside, during which the adult reproduces. Their presence in the hypogean environment can be considered accidental, since these are animals which may go underground when attracted by the presence of baited traps. This can be confirmed by the fact that in caves it is very difficult to see living adults, although they are relatively abundant in pitfall traps. It is therefore appropriate to classify this species as an epigeobite-endogeophile-trogloxene.

Another example is provided by Diptera of the family Phoridae, especially in the genus *Megaselia*, whose larvae normally develop in accumulations of rotting organic material. They are extremely abundant in the endogean environment, although they also appear in epigean and hypogean environments. When the adults emerge they
move to the surface to reproduce, but they are also able to get down into the subsoil; they do this even where there is soil at the surface if there is an appropriate place for them to lay their eggs. Phorids are relatively frequent in some caves, where they represent an important inward flow of energy. This group of species can thus be considered as epigeobites-endogeophiles-troglophiles.

Some difficulties with the proposed system of classification also arise with animals which spend some stage of their life in litter, such as many sprintails. There are epigeobite, endogeobite and troglobite sprintails; the first are almost always on the surface and the last underground, but the endogeobite species frequently turn up in litter. This happens partly because litter is in some ways an ecotone between surface and soil environments. In fact litter can be considered as the deepest layer of the surface environment, and it supports many species which live there and have nothing to do with the soil; this category includes many isopods, chilopods, thysanurans etc; these animals are therefore epigeobites.

Ants and gastropods are other groups which sometimes penetrate the soil and accidentally occur in caves. They are, however, epigeobites and not endogeobites, since they normally feed and reproduce on the surface.

There are other groups of animals which are not covered by this classification because their lifestyle is not directly linked to one of the environments considered. This is the case with parasites, inhabitants of guano and carrion feeders, whose presence in a particular place depends in the first case on where they find their host, in the second on the existence of an accumulation of guano and in the third on the presence of a corpse.

**Evolutionary classification (morphology)**

Along with the terminology based on the particular environments which make up the habitat of a species, it is useful to have available another based on its morphology. Those species that are most highly adapted to subterranean life are normally eyeless, lacking in pigmentation and with long appendages (Barr 1968; Culver 1982; Ginet & Decou 1977; Vandel 1964); although these are the most common morphological specialisations, one sometimes encounters others such as reduction of wings, special development of certain sense organs and enlarged abdomens etc., for reasons that are not always entirely clear.

Reduction of eyes and pigmentation are seen most commonly and there is no
doubt that these trends contribute to better adaptation for subterranean life; elonga-
tion of the appendages, however, is less common and there is some controversy as to
whether it is really characteristic of subterranean forms. It is generally accepted that
the most highly adapted species show some allometry in the development of their
appendages - especially the antennae - and this is linked to an increase in the num-
ber (or size) of the sense organs. Although there is not much relevant literature, argu-
ments have been presented both in favour and against these ideas. Culver (1982)
reviewed the subject and concluded that the most parsimonious explanation for the
allometric tendency was that various cave populations undergo substantial elongation
of their appendages in conditions where energy sources are limited, so that there is
strong selection favouring the enhancement of mechanisms for foraging and detect-
ing mates in an environment where food shortage precludes the existence of dense
populations (see Culver et al. 1990).

It is clear that not all species with morphological adaptations for subterranean
life are modified to the same extent. One can find species which, although they live
more or less exclusively underground, show scarcely any loss of pigmentation,
reduction of eyes or elongation of appendages. The existence of a variety of adaptive
grades has been noted by authors from the time of Jeannel (1943) up to the present
(Christiansen 1961; Peck 1973; Martin et al 1989). It is possible that the lack of con-
sensus that adaptive evolution to subterranean life necessarily results in a type of
morphology with elongated appendages, results from inappropriate comparisons
among troglobites in different grades of specialisation. For example, a troglobite with
little modification which lives in a eutrophic tropical cave, may have spent more time
in the underground environment than a highly modified troglobite in an oligotrophic
cave of the temperate zone (Mitchell 1969). The two examples are not comparable,
since food shortage does not apply such strong selection pressure in the first as in the
second case. It is thus essential, if one is to determine whether there is really a con-
sistent direction of evolution, to make comparisons in the same region, in the same
type of cave and, if possible, using species of the same group (genus).

In an attempt to produce a general evolutionary classification we here consider
only three characters (or group of characters): the development of eyes, the extent of
pigmentation and the enlargement of appendages. On this basis, following the ideas
of Christiansen (1962), we have established the following morphological types:

• Hypogaeomorph: eyeless species, strikingly depigmented and with elongated appen-
dages (especially the antennae).
Epigeomorph: species with eyes and body pigmentation well developed and appendages normal.

Endogeomorphs: eyeless species, strikingly depigmented and with short appendages.

Ambimorph: species intermediate between the epigeomorph type and one of the other two.

The strongest candidates for the hypogeomorph category are troglobites, although occasionally epigean or endogean species conform with one of the requirements for this category. The best candidates for the epigeomorphic category are epigeobites; rarely, endogeobites and troglobites may belong to this morphological category, but the normal situation is that they are hypogeomorphs, ambimorphs or endogeomorphs.

The classification that we propose presents several difficulties, especially because there are epigean species which lack eyes (for instance polydesmid diplopods) or pigmentation (many species which live in dark situations) or whose appendages are elongated even in the absence of any special selective pressure (for example spiders of the family Pholcidae or Heteroptera of the subfamily Emesinae). There are also highly variable species in which pigmentation can be present or absent in different populations, as in the case of the spider *Nesticus cellulanus* (Clerck) in the Iberian peninsula (Ribera, 1979). There are also species within which the extent of development of eyes ranges from forms in which they are almost entirely lacking to those in which they are fully developed, as happens in the spider *Agraecina canariensis* Wunderlich in the Canaries (Wunderlich, 1991). The species concerned are usually those which live partly in subterranean environments and partly in dark situations on the surface. Such cases are relatively rare, however, and have little effect on comparison of whole faunas. We are therefore of the opinion that, in spite of the difficulties and imperfection of the proposed classification, its application will help to distinguish general levels of adaptation among subterranean faunas of different volcanic islands.

According to the evolutionary state of each of the three characters analysed (eyes, pigmentation and appendages) it is possible to assign a code to each species (Table 3) made up of a combination of three letters specifying the state of the characters. In this way a hypogeomorph is given a code adl and an ambimorph will have some combination containing at least one letter from the pairs a/r (eye development), d/m (pigmentation) or n/s (length of appendages).
Table 3. Typical evolutionary states for the characters specified.

<table>
<thead>
<tr>
<th>Eye development</th>
<th>Pigmentation</th>
<th>Length of appendages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent (a)</td>
<td>Depigmented (d)</td>
<td>Long (l)</td>
</tr>
<tr>
<td>Reduced (r)</td>
<td>Medium pigmentation (m)</td>
<td>Normal (n)</td>
</tr>
<tr>
<td>Normal (n)</td>
<td>Pigmented (p)</td>
<td>Short (s)</td>
</tr>
</tbody>
</table>

The inferred evolutionary polarity for each character in troglobites and endogeobites is shown in Table 4. It is assumed that in the original epigeomorphic form the appendages were of intermediate size between those of an endogeon congener and a hypogean one, the eyes completely developed and functional, and the melanic concentration in the integument high.

Table 4. Evolutionary polarity for the characters “eyes”, “pigment” and “appendages” in two groups of subterranean species

<table>
<thead>
<tr>
<th></th>
<th>Troglobite</th>
<th>Endogeobite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>n→r→a</td>
<td>n→r→a</td>
</tr>
<tr>
<td>Pigment</td>
<td>p→m→d</td>
<td>p→m→d</td>
</tr>
<tr>
<td>Appendages</td>
<td>n→l</td>
<td>n→s</td>
</tr>
</tbody>
</table>

Combining the ecological and evolutionary classifications and taking polarity into account, we obtain the hypothetical evolutionary pathway presented in Figure 1. A subterranean community of recent origin will consist primarily of species such as accidental and facultative trogloxenes, with little relevant adaptation; as time progresses, the first troglophiles will appear and eventually troglobites. The latter will initially be ambimorphs and over time will become transformed into hypogemo-morphs.

The proposed classification is not intended to take the place of traditional ones which are much simpler and more useful when referring to subterranean animals and their general ecological requirements. It could, however, be useful when making comparative analyses between subterranean faunas of distinct areas or regions, and may facilitate understanding of the patterns of evolution and colonisation followed by faunas of particular regions, for instance of the Canary Islands.
Fig 1. Hypothetical evolutionary sequence for each lineage which colonises the subterranean environment.

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Evolutionary Biology, Maryland, vol. II: 380-395.


THE DISTRIBUTION OF PLANTS IN SCOSKA CAVE, NORTH YORKSHIRE, AND THEIR RELATIONSHIP TO LIGHT INTENSITY

Allan PENTECOST and Zhang ZHAOHUI*

SUMMARY

The flora of a small limestone cave was investigated. A total of 59 species was recorded (4 algae, 3 lichens, 47 bryophytes, 4 ferns, 1 angiosperm) making it bryologically the richest cave in Britain and one of the richest in Europe. All but nine of the species had been recorded from other European caves. Species-richness declined irregularly from the entrance (relative irradiance with respect to open sky 12%) to 34m depth (rel. irradiance 0.004%). Bryophytes were found at 0-16m depth where relative irradiance declined to 0.2% and only algae were encountered at 34m depth. While irradiance, which declined exponentially, was the major factor controlling plant distribution, substratum characteristics and surface moisture were also important.

Keywords: caves, flora, bryophytes, light.

Introduction

Remarkably little has been published on the flora of British caves, despite the popularity of caving as a British sport. While cave surveys have been numerous, there has been little effort to systematically examine caves for their flora. This contrasts with continental Europe where the cave flora is much better known, e.g. Maheu (1906), Lammermayer (1916) and Dobat (1966 ;1970; 1998a,b). In Britain, Mason-Williams & Benson Evans (1958, 1967), Mason-Williams (1962; 1966) and Cubbon (1970; 1976) provide lists of bacteria, algae, bryophytes and angiosperms from several South Wales caves including some information on substratum pH, light and humidity. Dalby (1966) investigated the growth of a moss, *Eucladium verticillatum*.

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in an English mine and found interesting adaptations to light, while Zhang & Pentecost (1999) published a short list of cave bryophytes from Yorkshire. Some British cave algae have also been noted by Claus (1967) and Carter (1971), but apart from this, there are only isolated references to the British cave flora in more general works such as Hill et al., (1994).

The largest area of limestone caves in Britain is to be found in the Craven district of North Yorkshire. Surprisingly, this area is also one of the least well known for its cave flora. The senior author recognised Scoska Cave as a good site for the investigation of plant threshold communities some years ago. The entrance is 70m above the River Skirfare on a NE-facing steep scarp below Scoska Moor in Littondale (Nat. Grid Ref. 34/915724, alt. 285 m). It is the source of a small stream, Gildersbank Sike. Scoska is noted for its moderately wide (3-4m) and straight entrance passage which follows a horizontal bedding plane developed in Dinantian Great Scar Limestone. Light is able to visibly penetrate at least 40 m into the passage and is gradually attenuated by the simple geometry, an unusual occurrence in British caves. The roof is about 2m high following a higher bedding plane (Fig. la).

We report here on the flora of the cave and its relationships with light intensity, with particular reference to biodiversity (as species-richness).

Methods

We examined the cave on two dates: 1.4.2000 and 15.7.2000. In addition to collecting small samples of cave flora at regular intervals from the entrance, cave dimensions were measured together with microclimate; air temperature, relative and absolute humidity and irradiance. Humidity was measured with a hand-held psychrometer and irradiance with a Licor 185 B quantum meter, the sensor of which was placed directly toward the cave entrance. Absolute and relative photosynthetically available radiation (PAR) measurements were obtained by comparison with the open, unobstructed sky. Critical plant groups were identified using standard British floras. Bryophytes were particularly conspicuous but we also examined lichens, ferns, spermatophytes and in less detail, the algae. Lichen, bryophyte and pteridophyte nomenclature follows Purvis et al., (1992), Blockeel & Long (1998) and Stace (1991) respectively.
Fig. 1. Morphometric and climatic data for the entrance passage of Scoska Cave, North Yorkshire.

a) Cave profile showing rock-strewn passage and flat cave roof.
b) Photosynthetically available radiation (PAR) in Scoska Cave (lx). Closed circles April 2000; open circles July 2000. Note logarithmic scale for PAR.
c) Air temperature, °C (closed circles) and % relative humidity (crosses).
Result

1 Cave substrata

At the entrance, blocks of limestone cover the floor for a distance of about 10 m within (Fig. 1a). Thereafter a brown cave earth is exposed in places with scattered limestone blocks. The cave walls are steep, largely devoid of crevices and mostly dry with a little speleothem development about 30-40 m from the entrance. Sikes Beck breaks out from the floor 24 m within the cave but soon disappears under rocks, reappearing at the entrance. Its discharge was low and about 11/sec when visited. The water had pH 8.3, Ca 1.55 mM/l, Mg 0.02 mM/l, Na 0.11 mM/l, SO$_4$ 0.09 mM/l and alkalinity 2.3 mEq/l.

2 Microclimate

Climatic data are shown in Fig. 1. Irradiance (Fig. 1b) becomes attenuated approximately logarithmically from the entrance to a depth of about 40m. Beyond this the passage turns to the left and light is abruptly diminished. No plants were observed beyond this point. The decline in intensity followed the exponential Bouguer-Lambert Law surprisingly well (regression Anova p<0.001) and provided a mean ‘extinction coefficient’ of 0.24 m$^{-1}$. Absolute light intensity was higher during April than July as the April measurements were made on a brighter day. Light levels at the entrance were 12% and 11% of the open sky during the April and July visits respectively. For the entrance measurements the sensor was directed horizontally out of the cave toward hillsides and trees and not the sky above. Absolute light levels in this cave would be expected to be maximum just before leaf expansion (April) since deciduous trees partly obstruct the entrance.

Temperature and humidity profiles were obtained in July and are shown in Fig. 1c. Air temperature underwent a linear decline from the entrance to a depth of 20m where it stabilised at around 9 °C. In contrast the relative humidity rose from about 80% at the entrance to 95% at 15 m depth. Deeper in the cave this high humidity was maintained. The air showed little movement and was probably stagnant as the cave system has only the one entrance. The water vapour pressure remained approximately constant, ranging from 11.3-11.9 mb (mean 11.4mb).

3 Flora

A total of 59 taxa were recorded consisting of four algae, three lichens, 47 bryophytes, four ferns and one flowering plant. The flora was dominated by bryophytes in terms of species-richness and cover (Table 1). Bryophytes grew only on the lower walls and floor of the cave and not the roof which was probably too dry.
<table>
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<th>authority</th>
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<th>4-6m</th>
<th>6-8m</th>
<th>8-10m</th>
<th>10-12m</th>
<th>12-15m</th>
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<th>20-40m</th>
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<td>E</td>
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<td>(Ercegovic) Geitler</td>
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<td>Plagiommium undulatum (Hedw.) Kop.</td>
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B: Recorded from other British caves; E, other European caves.
The total species richness peaked at 0-4m and 10-15m from the entrance where 16 species were recorded. Beyond 15m species richness declined rapidly and beyond 20m only two plant species, both algae, were recorded. Common threshold bryophytes which were absent from the interior included Eucladium verticillatum, Gymnostomum aeruginosum and Palustriella commutata var. commutata. These grew on moist walls of the cave where there was a small seepage, depositing small amounts of travertine. At the threshold, 0-4m from the entrance, irradiance was 5-10% of the open sky (hereafter defined as the relative irradiance or RI). Many species penetrated further. For example, frequent bryophytes in the region 6-10m with RI 1-2% were Eurhynchium pulilum, Fissidens adianthoides and Pseudotaxiphyllum (Isopterygium) elegans. Deeper still were Amblystegium serpens, Fissidens cristatus and Thamnobryum alopecurum. The last species was the most frequently encountered bryophyte in the cave and also occurred at the threshold. Progressing further into the cave we found Orthothecium infricatulum, Pseudotaxiphyllum elegans, Rhynochostegiella teesdalei and Platydictya confervoides (15.9m, RI 0.23%). Bryophytes penetrating to the greatest depth were Fissidens cristatus and Thamnium alopecurum (both to 16.2m with RI 0.20%). Liverworts were much less common with large thallose species abundant only at the moist threshold. Within the cave conditions were probably too dry to support luxuriant liverwort growth, but a few species such as Conocephalum conicum and Metzgeria conjugata penetrated to 12m. Four ferns were noted only 12m from the entrance but none was common. Three species of leprose lichens were conspicuous on threshold walls where they formed diffuse yellow and white patches. Lepraria incana was the commonest and found to a depth of 17m.

Only a few algae were collected. Sparse populations of the cyanobacteria Gloeocapsa and Schizothrix were recovered from damp speleothem at depths of 34m and 27m respectively and no algae were visually evident beyond 34m. Light levels were extremely low at these points, with RI values of approximately 0.004% and 0.014% respectively. An unidentified coccoid green alga was associated with the Gloeocapsa.

Discussion

Our measurements of irradiance in Soska Cave demonstrate an exponential decline in light with distance from the entrance. This is unusual and caused by the straight tube-like form of the passage and would also be expected in artificial excavations such as mine adits. Other caves show for part of their length a similar relationship (Dobat, 1998a) but presumably only where cave geometry permits. In
Skoska, air temperature fell to about 9.5 °C within the cave which is close, though slightly higher than the predicted mean air temperature at the site (8.5 °C). Ground air temperature is usually close to mean air temperature in Britain though minor differences often occur as discussed by Wigley & Brown (1976). Relative humidity was high which is to be expected for a cave with a moist floor and stream running through it. The humidity profile is remarkably similar to that of the Scheunenhöhle, Germany (Dobat, 1998a).

With a total of 59 plant species in five phyla the flora of this cave must be considered both rich and diverse. This is particularly surprising considering its small size. However, comparable species richness has been reported for several caves in Germany and France (Maheu, 1906; Lämmermeyer, 1912; Dobat, 1998b). In our site the richest area was at the threshold where 16 species were recorded over a length of 2m. Further into the cave species richness declined irregularly. This is partly the result of the rocky nature of the floor at the cave entrance, which gave way to clay within. The wet clay supported several species of fern, a flowering plant and several bryophytes. This must be one reason why the 6-8m and 10-12m sections had a species-richness approaching the threshold. Richness declined rapidly once the RI fell below 0.5% (Table 1). A similar decline has been found to occur at increasing distances from artificial cave lighting (Dobat, 1998b). Among bryophytes the moss Thamnobryum alopecurum penetrated the to greatest depth within Skoska Cave. In artificial ‘Lampenfloras’, Dobat (1998b) found that Fissidens species could tolerate an irradiance of 80-90 lux, which corresponds to the depth where Thamnobryum was found in Skoska. We also found Thamnobryum abundant in Sleets Cave nearby and it is one of the best-known cave mosses in Britain and Europe. In South Wales it has also found in low light regimes (Mason Williams & Benson-Evans, 1958) and it is common in shady British woodlands and ravines (Hill et al., 1994). The fern flora of this cave according to Chapman (1993) is typical of British cave thresholds and the same species are encountered in caves of the adjacent continent. Only one flowering plant, Chrysosplenium oppositifolium was recorded. In South Wales, it was the most frequently recorded flowering plant in the caves investigated by Mason-Williams & Benson-Evans (1958), where it occurred at light intensities of 18-24 lux, much lower than the intensities recorded at Skoska.

We did not pay much attention to the algae of Skoska but a thorough investigation would probably yield many species judging from previous studies (e.g. Claus 1962; Carter, 1971). It was clear that some algae tolerate lower irradiances than the bryophytes and vascular plants. Coccoid cyanobacteria such as Gloeocapsa are known to occur at low irradiances in other caves (Cox et al., 1971). Some cyanobac-
bacteria can grow heterotrophically and their occurrence at such low levels may be explained by this. However their absence from the darkest recesses and a tendency for them to occur only on parts of speleothem facing the entrance of Soska suggest they are in fact growing autotrophically. We know that algae occurred through most of the 35m section of cave investigated since lichens grew to a depth of 17m and contained the symbiotic green algae Chlorella and Stichococcus. Little is known of the ecology of lichens in caves but Jaros (1964) found that Lepraria nivalis occurred in relative light intensities ranging from 93-0.5% in a Hungarian cave. In Soska this species was only found close to the entrance and exposed to an RI of 5-10%.

Among the plants recorded at Soska, nine have not been previously recorded from caves as far as we are aware, and all were bryophytes. Of these, six did not penetrate further than 8m from the entrance and the remaining three, Hypnum resupinatum, Orthothecium intricatum, and Weissia cf. personnii penetrated regions where the RI fell to about 0.4%. Most of the cave bryophytes are typical of limestone and base-rich waters such as those of Gildersbank Sike. Many of the species encountered grew on sheltered cliffs nearby. A few such as Dichodontium pellucidum grew only on clay soil demonstrating the importance of suitable substrata in caves for colonisation.

Light was clearly the most important factor controlling plant distribution as many other studies have demonstrated (see review of Dobat, 1998a). However, the irregular decline in species-richness points to other important factors, namely substratum type and moisture. Skoska Cave has few roof seepages in the illuminated zone and the bare limestone surface can only be wetted by condensation. While condensation undoubtedly occurs near the cave entrance due to temperature and humidity change, this lack of moisture places a stress on many hygrophilous bryophytes which would only find sufficient moisture on or near the cave floor where moisture can be gained through capillarity. The occurrence of large limestone blocks prevents this near the entrance and largely explains the variation in species-richness. Water relations and substratum type are therefore important factors for distribution and biodiversity.

Acknowledgements

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References


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BIOGENIC SPELEOTHEMS: AN OVERVIEW

Paolo FORTI

ABSTRACT

The idea that speleothems may be somehow influenced by living organisms is rather old, but specific studies have only started in the last few decades and presently there are only a couple of systematic papers on this topic. The role of micro-organisms is perhaps the best investigated even if it is not fully understood, while studies over upper organisms and speleothems in a cavern environment are scarce and details are not always given on the involved genetic mechanisms.

The aim of the present paper is to give an updated overview on these topics in order to enhance the interest of the scientific community. In fact the complex biochemical reactions involved in the development of the different cave deposits, though still not well understood, clearly have an interest and an importance far exceeding the simple speleogenetic interest.

Keywords: Chemical deposits in caves, micro-organisms, plants, upper animals

RIASSUNTO

L’idea che la formazione di depositi chimici secondari in grotta possa essere in qualche modo influenzata da organismi viventi è abbastanza antica, ma solo da poche diecine di anni sono partiti studi specifici sull’argomento e a tutt’oggi esistono solamente un paio di lavori generali in questo campo.

Il ruolo giocato dai microrganismi è sicuramente quello meglio studiato anche se si è ancora ben longi dall’averlo definito in maniera puntuale; al contrario il ruolo giocato dagli organismi superiori nell’evoluzione dei depositi chimici secondari di grotta è ancora molto poco investigato e nei pochi lavori presenti in bibliografia raramente vengono forniti dettagli sui meccanismi genetici.

Lo scopo del presente lavoro è quello di dare una panoramica aggiornata su questi argomenti anche allo scopo di aumentare l’interesse della comunità scientifica su questi temi. In fatti le complesse reazioni biochimiche che controllano l’evoluzione dei differenti speleotemi, la maggior parte delle quali sono ancora del tutto non studiate, hanno certamente un interesse e un’importanza che tralasca di gran lungo il semplice studi speleogenetico.

Parole chiave: Depositi chimici in grotta, microorganismi, piante, animali superiori

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I - Introduction

The idea that the development of the secondary chemical deposits in caves may be somehow influenced by living organisms is rather old (SHAW, 1997): the shape and the internal structure of some speleothems (stalactites, stalagmites, coralloids...) suggested to the early visitors of caves the possibility that they grow as plants even though the current idea was that minerals (and therefore also the speleothems) were living organisms but at a lower level with respect to plants or animals until the second half of XVII century.

In the second half of the XVII century a scientist put forth the idea that speleothems are true "rock plants" (BEAUMONT, 1676), the development of which is very similar to that of the normal trees.

The theory was later perfected in 1704 by J. P. TOURNEFORD who wrote:

"...That certain rocks nourish themselves in the same way as plants. Perhaps they reproduce also in the same way..... that there are seeds which gradually swell up and develop the regular structure which is perhaps hidden beneath their surface... Thus the congelations grow up from seeds."

These are the reasons why in the XVIII century some of the most common types of speleothems were often represented just as part of a tree: with stalactites as roots, stalagmites and columns as trunk, helictites as leaves or flowers (Fig. 1).

Since the second part of XVIII century the progress in chemical studies allowed the detection of the main mechanisms by which calcite and other minerals deposit in caves and consequently any possible biogenic interaction in speleothem evolution was rejected for over one century.

Fig. 1 - The speleothems of the Antiparos cave (Greece) represented as living plants in an engraving of the XVIII century
But the increase of scientific observation inside caves which characterised the XX century allowed the opportunity to reconsider all the matter and therefore in the last 50 years the possibility that living organisms (mainly microorganisms) may somehow control the development of chemical deposits in caves was once again taken into consideration.

Today the fact that living organisms may influence, and in reality often control the external shape and/or the chemical composition of the speleothems is generally accepted. Nevertheless systematic studies on this topic have never been done, except for a few dealing with microbiology (SASOWSKY & PALMER, 1994; NORTHUP et al., 1997).

Research in this specific field started only fifty years ago, but their development progressively enhanced the role played by micro-organisms in the genesis and the evolution of secondary cave minerals. Therefore at present someone even doubts that caves may host speleothems developed without the active and/or passive control by living organisms.

The role of micro-organisms is perhaps the best investigated, at least for some of the most important reactions, even if it is far from being fully understood. Studies over upper organisms and spelothems in caves are scarce and normally refer only on the occurrence of biologically controlled chemical deposits saying nothing on the involved genetic mechanisms.

The aim of the present paper is to give an updated overview on these topics in order to enhance the interest of the scientific community in the study of the biogenic speleothems.

II - The role of micro-organisms

Presently, it is well established that microorganisms can directly cause biomineralization through enzymes, or they can produce substances that lead to the precipitation of minerals (e.g. by changing the pH in their surrounding) or they may become the privileged support to nucleation.

The microbial processes in caves often involves redox reactions. The microbial players are varied: aerobic (chemolithotrophs) microorganisms, which obtain energy directly from the oxidation of inorganic compound, but also anaerobic (heterotrophs) organisms which obtain energy from the oxidation of organic matter and reduce inorganic compounds. Best examples of such processes are given by the “sulfur cycle”, where both sulfur-oxidizing and sulfate reducing bacteria are involved (fig. 2).
1 - The “sulfur cycle”

The microbial reactions of the “sulfur cycle” are perhaps the best studied and have been proved to cause the development of a lot of cave minerals: native sulphur, gypsum and iron oxides-hydroxides are the most common speleothems developed by them, but plenty of others (Tab. I) have been reported in literature (HILL & FORTI 1997; FORTI 1989, 1996). Sometimes the large amount of organic matter produced in the “sulfur cycle” allows for the evolution of speleothems (pseudo-stalactites) consisting of single organic mat (mucus), which are normally called “mucolites” (Fig. 3).

Apart those related to the “sulfur cycle”, many other kinds of biomineralization can occur in the cave environment: the most important of which are:

- The Salpeter evolution
- The Phosphate deposition
- The Guano digestion
### Tabl. 1 - Minerals directly related to Sulphur Cycle

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Genetic mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Sulphur</td>
<td>anaerobic reduction of sulphates</td>
</tr>
<tr>
<td></td>
<td>aerobic oxidation of sulphides</td>
</tr>
<tr>
<td>2 Gypsum</td>
<td>direct reaction of H2SO4</td>
</tr>
<tr>
<td></td>
<td>over limestone or dolostone</td>
</tr>
<tr>
<td>3 Calcite</td>
<td>incongruent dissolution of sulphates</td>
</tr>
<tr>
<td>4 Fe-Mn oxyhydroxides</td>
<td>final products of the sulphide oxidation</td>
</tr>
<tr>
<td></td>
<td>Goethite, Hematite, Magnetite, Lepidocrocite Braunite, Pyrolusite, Romanechite,</td>
</tr>
<tr>
<td></td>
<td>Hausmannite...</td>
</tr>
<tr>
<td>5 Metallic Sulphates</td>
<td>final products of the sulphide oxidation in presence of metallic ions</td>
</tr>
<tr>
<td></td>
<td>Barite, Anglesite, Brochantite, Fibroferrite, Jarosite, Halotrichite Melanterite,</td>
</tr>
<tr>
<td></td>
<td>Rozenite, Zaherite...</td>
</tr>
<tr>
<td>6 Metallic Carbonates</td>
<td>final products of the sulphide oxidation or mobilization of sulphates in carbonate</td>
</tr>
<tr>
<td></td>
<td>environment</td>
</tr>
<tr>
<td>7 Metallic Phosphates</td>
<td>final products of the sulphide oxidation in presence of guano and/or bones</td>
</tr>
<tr>
<td></td>
<td>Hopeite, Parahopeite, Tarbuttite, Scholzite, Tinticite, Sampleite...</td>
</tr>
<tr>
<td>8 Opal</td>
<td>deposition induced by pH lowering during the sulphide oxidation</td>
</tr>
<tr>
<td>9 Sulphides</td>
<td>anaerobic reduction of sulphates</td>
</tr>
<tr>
<td>10 Silicates</td>
<td>deposition in strongly acidic environment induced by sulphide oxidation</td>
</tr>
<tr>
<td></td>
<td>Allophane, Endellite...</td>
</tr>
</tbody>
</table>

Fig. 3 - A mucolite in the sulphide rich part of the Frasassi Karst System (Italy)
2-The salpeter evolution

Actually it is well known that the deposition of saltpeter (nitrocalcite) and all the other cave nitrates, is driven by nitrifying bacteria, but in the early times of cave science, there were several supposed origins for nitrates in caves. Among them the most curious referred to the uplifting of nitric gas from deep inside the earth (ZIMMERMANN, 1789), or postulated the deposition by electric currents (GIOVENE, 1819). Only in 1839 the Danish Lund put forth the hypothesis that:

"...Salpeter earth (in caves) derives from surface organic matter..."

Anyway this theory took over 150 years to be demonstrated: in fact only in 1981 C. HILL established that:

".... nitrates leached from the surface soils, transported into caves by seeping waters, are deposited by the aid of the nitrogen bacteria Nitrobacter..."

Moreover it is presently proved that the same mechanism may also leach nitrates from guano, rat droppings, urine and deposit them into cave earth. Therefore it is only in very peculiar cave environments (like the volcanic caves) that salpeter may have an inorganic origin related to weathering of basaltic rock (HILL & ELLER, 1977).

3-The phosphate deposition

The sources for $\text{PO}_4^{3-}$ ions to produce minerals in caves are normally represented by bones and/or guano deposits inside the cave. The reaction between phosphoric acid cave walls, clay and sand in the floor and/or other minerals dispersed in the hosting rock is absolutely an inorganic process, but the transformation of organic phosphorous into $\text{PO}_4^{3-}$ ions seems to be always, or at least often, driven by the micro-organisms ruling the complex mineralization (mainly oxidation) processes of the organic mat inside a cave. Therefore probably almost all of the known cave phosphates are at least partially biogenic products. Anyway no specific study on this topic has been done until now.
4-The guano digestion

Mineralization of guano is a complex mix of different reactions, many of which are surely biologically driven. The previously described, related to the sulphur cycle, the saltpeter and phosphate evolution are among them and surely the most important: all of them occurring inside guano deposits. Anyway micro-organisms may reasonably control many other processes, like those causing the deposition of

- Halite
- Gypsum
- Iron and manganese oxides-hydroxides

Until present no specific study on the eventual biologically driven guano reactions has been done. Anyway recently, in a different context (without guano) it was possible to demonstrate that the iron oxides-hydroxides depositing inside Odyssey Cave, Bugonia, Australia are surely a biogenic mineralization: here the bacteria *Lepothrics* spp and *Gallionella* sp were proved to precipitate ferrihydrite with characteristic morphologies (CONTOS 2001, CONTOS et Al. 2001).

5-Biogenic speleothems in silica-Rich cave environments

The presence of high silica content in the cave wall and/or sediments may allow the development of peculiar micro-organisms which may in turn give rise to biogenic mineralizations. In some volcanic caves of Japan (KASHIMA et Al., 1989) the development of several silica coralloids and helictites have been found to be strictly related to the presence of colonies of diatoms (genus *Melosira*). In fact these speleothems consist mainly of skeletons of such organisms that are cemented by a few of silica. Their presence is strictly confined in the first part of the caves where a little of the external light can still reach the colonies of *Melosira*, because they need the energy supplied by the light to live. The light control is evident non only by the fact that these speleothems develop only in the threshold zone but also by their shape, which is always pointing towards the cave entrance. Beside this proven occurrence of biologically controlled speleothems in silica rich cave environment, there are several other cases in which a biogenic origin seems to be highly probable.
Filamentous organic structures have been reported in opal coralloids from quartzite caves in Venezuela (URBANI 1996, ONAC et al. 2001) and in opal-sulphur speleothems in a gypsum cave of Sicily (Forti & Rossi, 1987) (Fig. 4). Moreover in many of the lava tubes of the Pico Island (Azores) weathering of basaltic rock caused the evolution of a widespread amorphous silica moonmilk (Fig.5), extremely rich in organic matter (over 20%, unpublished data by the author), thus suggesting that the weathering process is probably driven by micro-organisms. In the same island there are gigantic opal flosststones (up to 5-6 meter long and over 1 m thick) inside the Argar do Carballo volcanic cave, which seem to derive from the diagenesis of the previously described silica moonmilk and, therefore, should be considered biogenic speleothems.

Fig.4 – Santa Ninfa gypsum cave (Sicily): an opal-sulphur flowstone developed due to the biological oxidizing reaction of the sulphur cycle.

Fig. 5- Amorphous silica moonmilk from a lava tube of the Pico island (Açores): the speleothem is very rich in organic matter thus suggesting some biogenic mechanisms active in its evolution.
6-Biogenic Carbonate Speleothems

It is presently well accepted that by far the large majority of carbonate speleothems developing in a cave environment are absolutely inorganic, anyway at least some of these deposits undergo biological control. Fungi, Algae and bacteria have all been implicated in the precipitation of carbonate dripstones but until recently it was not proved if these micro-organisms helped actively (by driving the chemical process) or passively (serving as crystallization nucleus) in the deposition, or if they where just accidentally buried there, which seems to be the rather more common occurrence.

Perhaps the best example of these processes are represented by the gigantic stromatolitic stalactites developing worldwide just at the entrance of many caves mainly in the tropical area. The algae colonies responsible for their development needs strong light to survive and therefore such formations are present only up to the threshold zone. Over a score of several tens of years, a extensive documentation of the microbial precipitation of calcium carbonate exists in non cave carbonate/travertine literature (EHRLICH 1996), their direct involvement in cave carbonate deposition have only been demonstrated very recently (CONTOS et Al. 2001a,b; CONTOS 2001). In her experiments CONTOS proved the active role played by bacteria in precipitating calcite crystals, and also experimentally evidenced that micro-organisms in Nullarbor caves (Australia) control also the shape of the generated calcite crystals, which are far different from those of the inorganic precipitated ones.

Fungal hyphae may act as nuclei for crystallization and a site for attachment for crystals. Algae can trigger the precipitation of calcium carbonate from solution, and may subsequently trap and bind the particles to carbonate speleothems. This may occur as the algae change the microclimate by respiring carbon dioxide and consequently causing the CaCO\textsubscript{3} to precipitate. Anyway due to their photosynthetic nature, except in show-cave environments, algae will only contribute to carbonate deposition at the entrance and twilight region of the caves, being responsible for the evolution of the well known stromatolithic stalactites, which may sometimes reach even gigantic dimension in the tropical areas. Finally bacteria which utilize carbon dioxide (like Thotthrix in the sulphur cycle) have been proved to cause accelerated carbonate speleothem growth.

Among carbonate speleothem types, moonmilk is that which most frequently seems to be originated through microbiological reactions. In fact the two most common mechanisms for the evolution of moonmilk (HILL & FORTI, 1997) are:

1 - Biochemical corrosion of the bedrock by organic acid produced by microorganisms (Arthrobacter, Flavobacterium, Pseudomonas)
2 - Active precipitation of moonmilk by bacteria (*Macromonas Bipunctata*)
Finally it is important to note that microorganisms should be fundamental also in the
deposition of moonmilk made by different minerals: not only in the case of the amorphous silica already cited, but also when the moonmilk is made by gypsum, amorphous silica, etc. (FORTI, 2000) even if no specific studies have been done on this topic at present.

**III - The role of plants and animals in the evolution of speleothems**

If the actual knowledge on the role played by microorganisms in speleothem evolution are surely not exhaustive but fairly good, the situation is completely different when considering the effect of plants and animals over the secondary chemical deposits in caves. In fact, in these two fields the research is extremely scarce and the available few papers always deal with spot observations without any attempt to consider the topic from a general point of view.

Presently it’s well accepted and enough documented throughout the world that plants (mainly roots) and remnants of animals (spider nets, bones etc…) may passively improve the development of speleothems by enhancing capillary migration of waters to places where evaporation may occur.

Anyway, in most cases, an active involvement of plants and animals in the evolution of speleothems is still speculative.

**1-The influence of roots on speleothem growth**

The roots apparatus is the single portion of a tree which may somehow interact with speleothem evolution.
The interaction may affect both the morphology of the speleothem (passive effect) and its chemical composition (active effect).

Anyway, plants cannot normally directly control the mechanisms of chemical deposition deep into the caves, because they need light to survive and their roots can hardly reach depths of several tens of meter. On the contrary in the show caves, where light is artificially supplied, plants often become not only a element of disfigurement but also they may lead to a halt in the calcium carbonate deposition or even cause the corrosion of speleothems, due to the acid secretion of their roots.

A peculiar lithogenetic (active) effect induced by plants was described in the gypsum area of Bologna (FORTI, 1983): with the life activity of plants causing a concentrated local increase of CO$_2$ close to the roots apparatus that allows the development of the incongruent dissolution of gypsum (Fig. 6) which leads to the evolution of car-
bonate speleothems to form layers inside gypsum flowers or even thin, pure calcite, flowstones (Fig. 7).

The morphological (passive) effects induced by roots over speleothems are much more frequent and evident: in fact when roots enter cave voids their surface may become a preferential area for the flow of the seeping water and, if the suitable environmental conditions (diffusion of CO$_2$ but also evaporation) exist, for calcium carbonate deposition. This mechanism causes the evolution of peculiar stalactites and columns, with a tilted often anastomized shape, over which several pseudo-helictites

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Fig. 6 - The incongruent dissolution of gypsum: when a CO$_2$ rich water flows over gypsum the saturation point with respect to CaCO$_3$ is reached quicker thus the deposition of calcite causes an additional solubilization of gypsum.

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Fig. 7- Calcite-gypsum flowers over gypsum rock near Bologna (Italy): the deposition of calcite is caused by the localized increase of CO$_2$ close to the roots.
grow (Fig. 8): these speleothems, growing over roots, have been observed with the same characteristics all over the world and are normally called “rootsicles” (HILL & FORTI 1997).

Finally, in the wet tropical environment, the root apparatus of large trees may become the main driving factor for the evolution of those peculiar speleothem, called “Showerhead”, which were firstly described inside Brazilian caves (LINO, 1989) and then observed in many other tropical areas. Showerheads are cone-shaped, stalactitic speleothems from which a steady or intermittent shower of water can emerge: in many cases they develop along large fissures widened by the presence of roots which in turns are partially or totally transformed into rootsicles.

2-The influence of animals on speleothem growth

When considering the influence of animals on the chemical cave deposits, it must be clear that lithogenetic phenomena like corals or others biogenic structures growing inside sea caves cannot be considered speleothems, because a cavern environment is normally not fundamental for their genesis and/or evolution. Therefore on the basis of the existing literature it should be stated that the influence of animals over speleothems is extremely scarce. In reality it is highly probable that even upper living organisms should be able to influence specific speleothems inside peculiar cave environments, therefore the lack of papers on this topic would be the consequence of the scarcity of specific observations instead of the rarity of the event. Most of the existing papers deals with marine caves in which the biogenic calcite overgrowth on pre-existing continental (inorganic) speleothems (Fig. 9) is induced by serpulids (ANTONIOLI et Al., 2001).
Anyway this kind of overgrowth rarely gives rise to specific new speleothems, normally just enlarging the pre-existing speleothem, the morphology of which results reasonably unaffected.

Sometimes, if the environmental conditions are suitable, the biogenic deposition induced by *sepulids* may allow for the development of single biogenic formations, the most characteristic of which are presently the “biogenic trays” observed inside the Lu Lampiune cave in Apulia (ONORATO *et al*., 2001).

The speleothems inside this cave consist of big (up to 2 meters long and 40 cm in diameter) clearly deflected stalactites: in fact their tip always points towards the dominant water flow inside the cave (Fig. 10): the resulting growth direction changes from subhorizontal close to the cave entrance to rather vertical in the cave bottom.

The shape of these speleothems is not conical but flattened with the major axis being 3-4 time greater than the smaller one and with the tip larger than the base.

The analysis of the internal structure evidenced the absence of even a small pre-exist-

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**Fig. 9** - *Sepulids overgrowth on submerged stalactites (photo by Fabrizio Antonioli)*

**Fig. 10** - *The biogenic trays of Lu Lampiune cave, Apulia, Italy (photo Raffaele Onorato, C.S.S. Apogon)*
ing continental stalactite (Fig. 11), being totally developed due to a biogenic deposition in the marine environment.

Morphologically they are extremely similar to the gypsum trays described inside the caves of New Mexico (CALAFORRA & FORTI, 1994). In both these occurrences the deposition is controlled by the same agent (they develop against the flow direction), the fluid being the only difference (water in the Lu Lampiune cave and air in the New Mexico caves) (Fig. 12).

Practically the direction of evolution of these biogenic stalactites is controlled by the serpulids, which must filter the water to obtain the necessary

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**Lu Lampiune Cave**

- biogenic trays
- maximum water flow
- rock

**Fig. 12** - Schematic sections of Lu Lampiune cave where the water flow control over the shape and the direction of the biogenic trays is evident.

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**Fig. 11** - Longitudinal section of a trays from Lu Lampiune cave (left: internal part; right: external surface): all its structure is clearly biogenic.
trophic support, and therefore tend to direct just upstream the water flow. The apical part is flattened and enlarged with respect to the basal section of the stalactites for the same reason: in fact the trophic support is maximum at the apex rapidly decreasing toward the base, thus allowing the enlargement of the tip and avoiding the radial growth of the other part of the trays.

Until now a rather large single biogenic flowstone made by upper living organisms has been described from a continental karst system in the southern part of Italy (POLUZZI & MINGUZZI, 1998).

The Vallone Cufalo gypsum cave (Verzino, Italy) is an active sinkhole with a river flowing inside. It hosts a rather large flowstone consisting of a gently terraced calcite crust up to 50 cm thick, 4-5 m wide and some tens of meters long, covering the cave floor along the subterranea stream (Fig. 13). Its complex genesis has been referred to the large community of larvae of a troglobic insect (Tricoptera Wormaldia), once living inside the cave over wide anthropogenic organic matter (olive oil factory waste discharged every year into the cave). Their life processes caused the production of large amounts of CO$_2$ which in turn reacted with the water saturated in gypsum thus causing the deposition of calcite just around the worms. The final morphology of the flowstone was the result of a combined action of larvae living activities and kinetic energy of the flowing water: in fact until the larvae were alive the tubes were bended upstream to catch as much fresh water as possible (Fig. 14), but
after their death &/or evolution the further deposition of CaCO₃ was controlled by the kinetic energy of the water transforming the upper part in a normal flowstone evolving downstream.

It is hard to believe that the Vallone Cufalo would be the only cave in the world in which the environmental conditions are suitable for an active upper living organism control over speleothems, therefore it seems reasonable that chemical deposits should develop due to the presence of animals in many other caves.

**IV - Final Remarks**

This short, and surely not exhaustive, overview on the biotic influence over the genesis and the evolution of speleothems clearly put in evidence the very important role played by living organisms, mainly micro-organisms, over the lithogenetic cave processes (speleothems and cave minerals evolution). The complex biochemical reactions involved in the development of the different deposits, though still not completely understood, clearly have a more interest and importance far exceeding the simple speleogenetic interest.

Normally they are low-enthalpy reactions and their knowledge is fundamental to improve our understanding of the natural mechanisms by which even orebodies of economic interest are formed and then mobilized. But the study of biologically driven speleothems is also fundamental to enhance the knowledge over peculiar environments like the chemioautotrophic ones, which are presently not well known despite their scientific interest. It is therefore reasonable to expect an increase in the co-operation between biologists and geochemists in the near future in order to obtain a fast improvement in the study of these phenomena.

In conclusion it can now be stated that:

*Speleothems definitely does not develop via vegetative growth...*  
*But without biogenic control caves should be very poorly decorated!!!*

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STUDIES ON CERTAIN ASPECTS OF BEHAVIOUR
IN THE BLIND CATFISH *Horaglanis krishnai*
MENON

Anna MERCY, T.V*, N.K. PILLAI & N.K. BALASUBRAMANIAN**

ABSTRACT

*Horaglanis krishnai* is a blind catfish inhabiting the dug-out wells at Kottayam, Kerala. This fish has great zoogeographical importance as a similar blind clariid, *Uegitiglanis zammaroni* is found only in the artesian wells of the former Italian Somaliland. Studies on certain behavioural aspects of the fish in captive conditions showed that, this fish even though is blind, exhibited a high degree of thigmotactism. Locomotion, comfort behaviour, feeding and light sensitivity of the fish were studied under laboratory conditions. Though the fish is totally blind and histological study did not reveal the presence of any light sensitive structures, the fish is found to be sensitive to light stimulus. It is a predator. Under laboratory conditions it unerringly snapped up food organisms. This is obviously facilitated by the high degree of development of the tactile and olfactory sense organs.

This study form part of the Ph.D thesis of the first author

Keywords: horaglanis krishnai, cave fishes, behaviour.

Introduction

The study of comparative behaviour in animals has now developed to such an extent that it can be said to have grown beyond the confines of biology. In the narrow sense, behaviour is the sum total of what an animal does or "the whole complex of observable or measurable, recordable activities of the living animal"(Verplanc, 1957). During the last two decades fish behaviour has been studied fairly extensively by field observations and under laboratory conditions (Breder, 1934, ’35, ’36; Noble and Curtis 1939; Greenberg 1947; Arosen 1945; Nymon 1953; Tinbergen, 1951, 53; Keenleyside 1955; Clarke 1970 and Kortmulder 1972).

*Horaglanis krishnai* is a blind clariid inhabiting the dug-out wells of Kottayam, Kerala State, India. Cavefish’s attracted the attention of ichthyologists only comparatively recently. Till date, 32 species of fishes have been recorded from caves, artesian wells and subterranean waters. The discovery of the new blind clariid fish from

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Kerala, *Horaglanis krishnai* Menon and the occurrence of a similar blind clariid fish, *Uegitlanis zammaroni* Gienferrai from Italian Somaliland have raised points of great zoogeographical interest. Our information about these two forms is, by no means adequate. Breder and Gresser (1941) made extensive studies on the blind characins. But no information is available on the behaviour of *H. krishnai* in captivity. *Horaglanis krishnai* has a peculiar habitat and hence only certain aspects of its behaviour could be investigated.

**MATERIALS AND METHODS**

*Horaglanis krishnai* occurs at the bottom of the dugout wells at Kottayam, (Lat. 8°4'N and 10°21'N; Longitude 76°13'E and 77°38'E) Kerala, South India. Specimens of *H. krishnai* were collected from the dug-out wells at Kottayam. As the fishes invariably rest on the bottom they could be collected only by draining the wells. This is a tedious and time-consuming exercise. Because of this difficulty, collections were made mostly during the summer (January to April), as the water level in the wells is low then. The wells were drained by drawing water out with buckets. During the rainy season (June to September) the water level is high and hence the wells can be drained only by pumping water out using an electric motor. Whatever method is employed the wells can never be completely emptied as water will be gushing in through springs. Hence as soon as the bottom mud becomes visible, one of the workers would enter the well and vigorously agitate the small quantity of water present. The bottom sediments along with the water will be sent up in buckets in a semifluid state and emptied at a suitable place near the well. As the fishes are pink in colour and move about vigorously in the mud. Then they can be easily picked up and transferred to a vessel containing clear well-water. The maximum number of fishes I could collect from one well was fifteen. This may be because it is virtually impossible to drain these wells completely.

The fishes were transferred to polythene bags filled with water saturated with oxygen and transported to the laboratory at Trivandrum, 150 km away. The fishes invariably reached the laboratory, apparently in good condition, but during the course of two or three days, started dying. Hence, as a trial, subsequent collections of fishes were kept in aquarium tanks filled with well-water and having a bottom of silt transported from Kottayam along with the fishes. This appears to provide a natural environment. Mortality was considerably lower. The food given was mainly *Chironomus* larvae. The aquaria holding the fishes were kept in semi-dark place. I succeeded in keeping a few fishes healthy under these conditions for nearly two years. However, handling
these fishes for experimental purpose was found to result in mortality of at least a few fishes. For behavioral studies, only fishes well-acclimatised to the laboratory conditions were used. The temperature of the water ranged between 27° and 28°C. Water was replenished to compensate for the loss by evaporation. For all experiments, glass aquaria of size 60 x 25 x 25 cm³ were used. Observations were generally made 3-4 times a day and all experiments were continued for more than 24 hours. Each experiment was duplicated and the average of the results (expressed as percentage) was calculated. The containers were kept in a secluded place where the fishes were subjected to least disturbance. Feeding the fish and changing the water were done invariably at 5 P.M. and after that no readings were taken on that day.

RESULTS
The following aspects of the general behaviour of the fish were studied.
1. Locomotion
2. Comfort behaviour
3. Feeding
4. Light sensitivity

1. Locomotion

The locomotion of *H. krishnai* is comparable to that of other clariid fishes. It can be described as serpentine, anguilliform, or eel-like. The undulating movement of the body is primarily in the horizontal plane. Vertical upward or downward movements are very rare. As the fish moves forwards the tail is wagged providing the initial momentum. Transverse waves of contraction of the myomers travel from the anterior to the posterior end. The amplitude of these waves of contraction increases sharply where the trunk approaches the tail. Observations under laboratory conditions, obviously somewhat unnatural, showed that these blind fishes perform a variety of movements. When they are first introduced into the tank, or in the presence of light, they swim about continuously; some sort of aimless wandering. Once the fishes get acclimatised to the artificial conditions in the tanks or when they are provided with some sort of shade, they often remain quiescent. At such times they swim away only if disturbed in one way or another or when they are engaged in taking food.
2. Comfort behaviour.

As shown by Schiche (1921), catfishes in general, are strongly thigmotactic. *H. krishnai*, in spite of its peculiar habitat, is no exception. They invariably rest, with as many points of the body as possible in contact with the substratum. *H. krishnai* virtually lies on the bottom. This habit appears to have played an important part in developing a tendency to aggregate. These fishes appear to be eager to collect together in some shade, lie beneath leaves or stones or among pebbles. When hurried together like that, they darted away in all directions if suddenly disturbed. Formation of aggregations can be easily observed by making a group of fishes disperse by disturbing them. In 10-15 minutes after the disturbance, two or three individuals slowly approach the original site or another suitable site nearby. Individual fishes swim about at random and sooner or later manage to join their companions. Almost all the fishes come together in about an hour. The struggle to occupy the original site is so irresistible that late comers wriggle into the meager space available among those that remain in a bunch. Even after this, there are constant adjustments by individuals to such an extent that the members of an aggregation are never at complete rest. The aggregations are always formed beneath a stone, a leaf or some such hiding place. The least disturbance would make them scatter. But soon the above procedure will be repeated. Thigmotactic behaviour of a high degree is evident in this fish.

3. Feeding

The examination of the stomach contents of specimens soon after they were collected from the wells revealed the presence of only insects, insect larvae or their severed parts. Diptera and Coleoptera predominated. Fishes kept in aquaria were given *Chironomus* larvae and *Tubifex* worms. Though a few fishes occasionally ate bites of tubifex worms, generally they did not relish them. On the other hand, when *Chironomus* larvae were placed near the place where they lay hiding, they invariably pounced on the prey. As soon as the prey comes near the fishes hiding under the cover, and provided it is within striking distance, the fish partially emerges from hiding, seizes the prey in its mouth and retreats into the hide. Under laboratory conditions they were never seen feeding on dead matter or detritus. The jaws of *H. krishnai* are provided with several rows of incurved sharp teeth which help in holding the prey firmly preventing its escape. The prey is caught and taken in whole. *H. krishnai* can hence be described as a predator though a small edition of it. In spite of being blind the fish unerringly
catches food and I have never seen any fish failing to do this. Obviously both olfactory and tactile senses help in this.

4. Light sensitivity

As already stated, *H. krishnai* shows a strong tendency to hide under stones, leaves etc. Whenever a beam of light is directed on to them, they move away from the beam of light and gather at the darkest portions of the aquarium. When they were placed in a glass tank with half of its sides shaded by pasting black paper, they always collect at this portion. They never go to the lighted area. Every fish shows this preference. No matter from which direction the light come, they swim *en masse* to the darkest part. The fishes are strongly photonegative.

Other behavioral aspects

A series of simple experiments were conducted to study the behaviour of *H. krishnai*. Though these experiments were conducted wholly under artificial conditions it can be assumed that the results are, to a large extent, similar to those under natural conditions. It may be pointed out that this fish cannot be observed under natural conditions as it inhabits subterranean waters. The behaviour of the fish in an aquarium, with different kinds of substrate, and in relation to phototropism was investigated.

Behaviour in an aquarium

An aquarium 60 x 25 x 25 cm$^3$ was set up with a bottom of river sand to a height of 4 cm and water column 20 cm. Aquatic plants were planted irregularly in the bottom sand. Four large, nearly spherical, stones of identical size were placed on the sand bed, equidistant from one another and from the sides of the tank so that all the stones received the same degree of illumination. A few fishes were introduced into the centre of the aquarium from a small container, which was placed previously in the aquarium. The fishes were released by opening the container with the help of a string-arrangement operated from a distance. As soon as the fishes were released, they swarm all over the tank and remained scattered on the bottom at random. A few hid themselves amidst the weeds while others lay fully exposed. But gradually, during the course of an hour, every fish was found beneath the stones.

From the next day onwards, the frequencies of behaviour in this habitat were observed at definite intervals. The percentage of frequency in preferred sites is given
in Table 1. The results definitely showed that *H. krishnai* preferred to remain hiding under the stones. Moreover, even when more than one identical stones were available the fishes congregated under a single stone showing a pronounced gregarious tendency. When disturbed, the fishes scattered and quite often grouped themselves under a different stone. The size of the stone, the actual position of the stone, or the actual distance between the stone and the site at which the fishes were released, had no significant effect on the behaviour of the fish. Hence it can be concluded that, subject to the availability of some sort of cover, the fishes exhibit a strong gregarious tendency and react socially to each other.

### Behaviour in an aquarium with different substrata

To investigate whether the nature of the substratum influences the behaviour of the fishes, another experiment was conducted. The bottom of a glass aquarium was partitioned into five compartments with wooden strips, 4 cm in height. The five compartments were filled with mud, coarse river sand, gravel, pebbles and large stones respectively, to a height of 4 cm so that the bottom had an even surface flush with the partitions. It was ensured that the whole tank received the same intensity of illumination. Fishes were then introduced as in the previous experiment and left undisturbed for about an hour.

The number of fishes in each experiment was counted periodically during the succeeding four days. The percentage of the number of fishes which took shelter in the different compartments was then calculated. It can be seen from the data presented

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<th>Sl. No of observations</th>
<th>Plants</th>
<th>Stones</th>
<th>Sand</th>
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<td>11</td>
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<td>100</td>
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</tbody>
</table>

*average of three observations*
in Table 2 that the number of fishes which preferred a substratum of pebbles is significantly higher than that which selected the substratum of stones. Even when the positions of the different compartments were interchanged the results were the same. Thus the fishes showed a definite preference for a pebbly bottom.

**Tab. 2 - Behaviour of Horaglanis krishnai in relation to different types of substratum**

<table>
<thead>
<tr>
<th>Sl.No of observations</th>
<th><em>Percentage of fishes on</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sand</td>
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<td>11</td>
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<tr>
<td>12</td>
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</tbody>
</table>

*denoted by percentage occurrence of fish on different substrata.

**Behaviour in relation to phototropism**

How the fishes respond to the intensity of illumination was studied in a third experiment. For this, a glass aquarium with a bottom of fine river sand was set up. Half the area of the sides of the tank was covered with black paper. The fishes were then introduced as before. After 24 hours the positions occupied by the fishes were recorded. Except for occasional sojourns into the illuminated area the fishes remained in the shaded area. Illumination has a strong influence on the behaviour of the fish. They are negatively phototropic.

**Correlation between blindness and general behaviour**

To understand the effect of the absence of eyes on the general behaviour of the fish, the following experiment was conducted which is mainly based on that of Breder (1941). Tanks of size 60 x 25 x 25 cm³, having an illuminated part and a shaded part were set up. The tanks were arranged in such a way that one half of the area was illuminated with a 60 watt bulb placed 3 ft. away and the other half was shaded with
black cover placed close to the water surface. The idea was that by recording the positions of the fishes at definite intervals their response to illumination could be studied in a quantitative manner. In the case of a randomly wandering fish the number of observations obtained over a given area on the bottom should then be directly proportional to the whole area, if no preference was evident.

Tanks of the above mentioned size were half filled with water. One half of the tank was covered and the other was exposed. The entire side was covered with black paper. Fishes were then introduced. The position of the fish in the illuminated half was noted at five seconds interval. The illuminated area was changed and observations were continued to eliminate extraneous influences if any. However, the fishes appeared to be influenced solely by illumination in taking up any particular position. The experiment was repeated with 2, 4, 5, 6, and 7 fishes at a time and each experiment was repeated six times. The details of the experiments are tabulated in (Table 3). The results were statistically analysed.

### Analysis and Interpretation

The numbers of observations in each experiment with light and dark regions were pooled together. 't' test was applied to find out whether the number in the light/dark region deviated significantly from half or not. The test was applied to observations on one side only as this would apply to the other half also. Denoting \( P_1 \) = proportion of observations in the dark region to the total and \( P = 1/2 \), then 't' will be given by

\[
t = \frac{P_1 - P}{\sqrt{1/2 \times 1/2 \times 1/n}}
\]

where 'n' is the total number of observations in the light and dark regions. 't' value greater than 2.0 would enable us to conclude that the proportion observed differed significantly from 1/2, indicating a definite preference to one of the two regions. The 't' value were calculated and are given in Table 4 which shows that the first two halves are not significant as they are less than 2.0. All the other values are greater than 2.0 and are significant. The proportion observed in the dark region is (Table 4) greater the light. This clearly shows that the fish definitely prefers the dark region. On the other hand, when only one or two fishes were introduced at a time, no pronounced preference, either for the dark or for the light portion, was evident, presumably because the fishes continued to move in a random fashion. When a larger number was introduced they formed a group which *en masse* showed pronounced aversion for direct illumination. To compare the proportions observed, when the number
**STUDIES ON CERTAIN ASPECTS OF BEHAVIOUR IN THE BLIND CATFISH *Horaglanis krishna* MENON**

Tab. 3 - The results of the experiment conducted to study the response of the fish to light

<table>
<thead>
<tr>
<th>No of Expts.</th>
<th>No. of fish</th>
<th>Total observations</th>
<th>Ob. in light</th>
<th>Ob. in dark</th>
<th>Darkness area</th>
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<td>100</td>
<td>50%</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>50%</td>
</tr>
</tbody>
</table>
Tab. 4 - 't' values comparing proportion observed in the dark area for significance from 0.5.

<table>
<thead>
<tr>
<th>No. of Expts.</th>
<th>No. of fish examined</th>
<th>Proportion</th>
<th>'t' values</th>
<th>Inference</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.5207</td>
<td>1.0402</td>
<td>Not significant</td>
<td>P &lt; 0.25</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.5247</td>
<td>1.3646</td>
<td>Not significant</td>
<td>P &lt; 0.25</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.8787</td>
<td>29.1327</td>
<td>Significant</td>
<td>P &lt; .01</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0.9525</td>
<td>25.5640</td>
<td>Significant</td>
<td>P &lt; .01</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0.9848</td>
<td>27.3126</td>
<td>Significant</td>
<td>P &lt; .01</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>1.0000</td>
<td>28.2486</td>
<td>Significant</td>
<td>P &lt; .01</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>1.0000</td>
<td>28.2486</td>
<td>Significant</td>
<td>P &lt; .01</td>
</tr>
</tbody>
</table>

of fish is increased, 't' will be given by

\[
t = \frac{P_1 - P_2}{\sqrt{Pq (n_1 + n_2) / n_1 n_2}}
\]

Where, \( P = \frac{n_1 P_1 + n_2 P_2}{n_1 + n_2} \)

q = 1-P and \( P_1 \) is the proportion observed in the experiment and \( P_2 \) in that of another; \( n_1 \) and \( n_2 \) are the total number of observations in two experiments respectively; 't' values calculated for each experiment are given in table 5. These values show that when we compare the proportion between each experiment, all the values are significant. With the increase in the number of fishes there is a gradual increase in the proportion observed in the dark region Table 5, which reaches the highest level when seven fishes were used. This reveals that density of fishes has an apparently marked influence on its behaviour.

**DISCUSSION**

Though *H.krishnai* apparently lacks any organ capable of receiving visual stimuli, they do react to the presence of light. Breder and Gresser (1941) found that the blind characins are negatively phototropic. Breder and Nigrelli (1935,1938) suggested that
Tab. 5 - *‘t’ values for comparing the proportion observed in the dark region between experiments.

<table>
<thead>
<tr>
<th>No. of Expt.</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>3.8787</td>
<td>18.1068</td>
<td>19.0933</td>
<td>20.9964</td>
<td>22.0819</td>
<td>22.0819</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>18.3087</td>
<td>19.2356</td>
<td>21.1182</td>
<td>22.1482</td>
<td>22.1482</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.7209</td>
<td>8.6918</td>
<td>10.2623</td>
<td>10.2623</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6957</td>
<td>6.2418</td>
<td>6.2418</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.4917</td>
<td>3.4917</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0*</td>
</tr>
</tbody>
</table>

* not significant All other values are significant at 1% level (P < 0.01)

Schooling is dependent solely on vision. This appears to be the reason why the blind characins do not show a schooling tendency as suggested by Breder and Gresser (1941). As already stated, *H. krishnai* does not appear to have any light receptor organ but the fish shows a gregarious tendency which is akin to schooling. This must, therefore, be induced by some factor other than the sense of sight.

The life of fishes is influenced by several kinds of olfactory stimulants: both repellants and attractants (von frisch, 1941). It has been suggested that attractant odour, more than vision, helps to keep members of cyprinid species together. Hemmings (1966) observed that attractant odour plays an important role in forming and maintaining schools of fish, especially during night. Byrant and Atema (1980) while experimenting on *Ictalurus nebulosus* found that the body odour has decisive influence in keeping members together. They also found that the odour is dependent on the kind of food taken. Groups of individuals, feeding on different kinds of food, fail to keep together at least for a definite interval of time. In the present study, the schooling of *H. krishnai* might be influenced by the high development of olfactory and tactile sense organs.

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DEPOSITION OF CALCIUM CARBONATE IN KARST CAVES: ROLE OF BACTERIA IN STIFFE’S CAVE

Claudia ERCOLE*, Paola CACCHIO*, Giorgio CAPPUCCIO**, Aldo LEPIDI*

ABSTRACT
Bacteria make a significant contribution to the accumulation of carbonate in several natural habitats where large amounts of carbonates are deposited. However, the role played by microbial communities in speleothem formation (stalactites, stalagmites etc.) in caves is still unclear. In bacteria carbonate is formed by autotrophic pathways, which deplete CO₂ from the environment, and by heterotrophic pathways, leading to active or passive precipitation. We isolated cultivable heterotrophic microbial strains, able to induce CaCO₃ precipitation in vitro, from samples taken from speleothems in the galleries of Stiffe’s cave, L’Aquila, Italy. We found a large number of bacteria in the calcite formations (1 x 10⁴ to 5 x 10⁹ cells g⁻¹). Microscopic examination, in laboratory conditions at different temperatures, showed that most of the isolates were able to form calcium carbonate microcrystals. The most crystalline precipitates were observed at 32°C. No precipitation was detected in un-inoculated controls media or in media that had been inoculated with autoclaved bacterial cells. X-ray diffraction (XRD) analysis showed that most of the carbonate crystals produced were calcite. Bacillus strains were the most common calcifying isolates collected from Stiffe’s Cave. Analysis of carbonate-solubilization capability revealed that the non-calcifying bacteria were carbonate solubilizers.

Keywords: bacteria, calcium carbonate precipitation, karst cave.

Introduction
Geomicrobiology examines the role of micro-organisms in a wide range of geological processes, such as rock dissolution, rock formation, the transformation of soils and sediments, genesis, and the degradation of minerals and fossil-fuels (Ben Omar et al., 1997; Ehrlich, 1990).

This discipline is closely related to other scientific disciplines, such as microbial-
ecology and biogeochemistry (Ben Omar et al., 1997).
The microorganisms in the biosphere, including in caves can aid the concentration, dispersion, and fractionation of matter.
For example, microorganisms can promote the dissolution of poorly soluble minerals, such as CaCO$_3$, iron and manganese hydroxides, to soluble compounds. They can also act upon mixtures of inorganic compounds, selectively concentrating or diluting them. Finally, microorganisms can accumulate inorganic materials via processes such as intracellular deposition, adsorption and cellular fixation, and extracellular precipitation of insoluble compounds (Ben Omar et al., 1997). The field of biomineralization is devoted to the study of microbes that can act as concentrating agents (Lowenstam & Weiner, 1989).

**Biomineralization**

Biomineralization is the process by which organisms form minerals, by creating the physical and chemical conditions necessary for mineral formation and growth. Some aspects of biomineralization overlap with some aspects of geomicrobiology and of other scientific disciplines.

Organisms that can precipitate minerals are present in all taxonomic groups, from Bacteria to Chordata, even though the processes involved can be very different. The most abundant cation in most known biominerals is calcium and the most abundant anions are carbonates.

Microbial communities also play an important role in the formation of some minerals deposits. Microbial metabolism is highly versatile and is characterised by the frequent release of metabolic products, such as organic acids, sugars and enzymes. These products can change the physico-chemical environment, (e.g. pH), they can also catalyse redox reactions, which directly or indirectly change the redox state of metals, favouring deposition and dissolution. For example, the most abundant Mn- and Fe-containing minerals are Mn-dioxides and ferric oxides, respectively. These minerals are formed by the oxidation of Mn or divalent-Fe in the presence of free O$_2$ or highly oxidised compounds - such as nitrates and sulphates - by chemolithotrophic microorganisms.

The type of mineral produced, however, is more dependent on the environmental conditions in which the micro-organism is living than on the biological processes involved in its formation. Thus, the same bacterial species can produce different minerals in different environmental conditions.

A relationship exists between the microbial activities of the S cycle and elementary S deposits in both marine and terrestrial environments (Northup & Lavoie, 2001). One of the most relevant and well-known examples of mineralization driven by bac-
teria is the precipitation of CaCO\(_3\) (Rivadeneyra et al., 1993).

**Biotic and abiotic precipitation of CaCO\(_3\)**

In natural conditions, the precipitation of CaCO\(_3\) can be considered to be the result of a series of chemical and biochemical processes. CaCO\(_3\) can exist in three different polymorphic forms: calcite, aragonite and vaterite. Calcite is the most stable form and is thus the most common form of carbonate on the Earth’s surface, where it is the most common constituent of sedimentary rocks.

Equilibrium exists between insoluble (carbonate) and soluble (bicarbonate) forms in water:

\[
\text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{Ca(HCO}_3\text{)}_2
\]

The depletion of CO\(_2\) from water favours the deposition of carbonate.

Abiotic chemical precipitation can occur due to a decrease in the partial CO\(_2\) pressure, the shaking or stirring of the water, an increase in temperature, or a decrease in hydrostatic pressure.

However, the contribution of biological systems to limestone precipitation (biotic precipitation) is due to:

- the production of internal and external skeletons by eukaryotes (shells, skeletons).
- the production of carbonate by autotrophic and heterotrophic bacteria and by fungi (Le Metayer-Levrel et al., 1999).

**Role of bacteria in the precipitation of carbonate minerals**

CaCO\(_3\) precipitation is one of the most relevant examples of bacterial mineralization and can be traced back to the Precambrian period.

Both autotrophic and heterotrophic bacteria, including sulphur, photosynthetic-, and nitrogen-fixing bacteria, which hydrolyse urea and cellulose, are involved in CaCO\(_3\) precipitation. Boquet et al. (1973) stated that, in favourable conditions, most of these bacteria could produce calcite crystals. The mechanisms, by which bacteria precipitate carbonate, however, are still unclear. Metayer-Levrel et al. (1999) and Castanier et al. (2000) showed that the precipitation of CaCO\(_3\) by micro-organisms can occur via several different pathways.

In autotrophic bacteria three major pathways are involved: methanogenesis and oxygenic and anoxygenic photosynthesis. All three pathways involve the use of CO\(_2\).

In heterotrophic bacteria, several pathways from the nitrogen and sulphur cycles are involved in the precipitation of carbonate. For example the ammonia produced during the nitrogen cycle leads to an increase in pH, which then shifts the carbonate-bicarbonate equilibrium towards carbonate production.
Micro-organisms can also act as crystallisation nuclei for the formation of CaCO₃ crystals. Rivadeneyra et al. (1996) demonstrated that Ca ions bind to microbial cell surfaces (possibly to capsular structures). When these surfaces become saturated with Ca ions, the ions start to aggregate, thus initiating the first crystallisation nucleus. Crystals then grow from these structures.

A CASE STUDY: STIFFE’S CAVE

Isolation of calcifying bacteria from speleothems

The following types of sample were collected from speleothems in Stiffe’s Cave, a limestone cave located near L’Aquila (central Italy) that is open to the general public:

- Soda straw stalactites from an area located close to a pathway that is used by visitors (sample A)
- Stratified tubular stalactites from an area located far from a pathway that is used by visitors (sample B)
- Stratified tubular stalactites from an area located far from a pathway that is used by visitors (sample C)
- Flowstones (sample D)

Speleothems samples were collected in sterile conditions and kept in sterile tubes at 4°C until analyses. For the microbiological analyses, 1g of the sample was crushed by use of mortar and pestle and then suspended in 9mL of sterile saline solution (1x10⁻¹ dilution). Ten-fold serial dilution was made until 1x10⁻⁹ dilutions. BAM (Boquet et al., 1973) plates were inoculated with the diluted samples in triplicate and incubated at 32°C for two weeks. Individual colonies were selected and purified by repeated subculture on solid B-4M.

Precipitation of calcium carbonate by micro-organisms

We tested the ability of selected microbial strains to precipitate calcium carbonate on both liquid and solid B-4M.

B-4M plates were inoculated with strains that were able to precipitate calcium carbonate and incubated at 4, 22 and 32°C in aerobic conditions. All experiments were carried out in triplicate. The controls consisted of uninoculated medium and medium inoculated with bacterial cells that had been autoclaved at 120°C for 20 min. Plates were routinely examined under a light microscope every day for 25 days (Leitz Biomed) for the presence of crystals.

The morphology and size of the crystals and micro-organisms were analysed by scanning electron microscopy (SEM- Philips XL30CP).
The mineral composition of the precipitates was characterised by power X-ray diffraction (XRD). Crystalline phases were identified by use of the ICDD database (JCPDS). Two purification procedures were used, depending on concentration of crystals.

Cultured solid medium was dried at 22 or 32°C. The agar medium was cut into 10x30x0.5 mm blocks, and those richest in crystallites were fixed into adhesive tape. This tape was placed in the middle of a diffractometer in a "U" shaped sample holder to minimise background signals. Samples with a low crystallite content were collected as described by Rivadeneyra et al. (1994). Washed crystals were air-dried at 37°C and held by a glass sampler for X-ray measurements.

**Cyto-morphological and biochemical characterisation**

Calcifying bacterial strains were identified by phenotypic tests. We used a light microscope to examine the size and morphology of the cells. The cells were stained according to the Shaeffer-Fulton method and the presence of endospores in the cells was observed by phase-contrast microscopy or by light microscopy. Bacterial cells were Gram stained (bioMérieux, Marcy-l’Etoile/France).

The morphological changes that occur during the growth cycle were monitored for some bacterial strains grown on specific media (Jones & Keddie, 1992). Conventional tests (Bergey’s Manual, 1986) and the API 50 CH and 20 E test kits (bioMérieux) were used to characterise the biochemical nature of the cells. The nitrate- and sulphate-reducing capacity of the cells (Cappuccino & Sherman, 1987) and their ability to oxidise ammonia to nitrite (Pochon & Tardieux, 1962) were also assayed.

**Results and discussion**

This preliminary study involved the isolation and characterisation of all cultivable micro-organisms and of calcifying bacteria from a carbonate concretion in Stiffe’s cave. The amount of bacteria isolated varied enormously between the different samples (Table 1). Bacteria might have been scarce in some areas due to their presence having a trivial origin or due to other reasons (e.g. percolation, contamination due to the presence of Chiroptera, other cave-dwelling animals and visitors). The existence of millions or billions of cells per gram, such as in samples C and D, require suitable local conditions for growth. The samples that contained the most bacteria also tended to contain a higher proportion of calcifying strains. Furthermore, these samples contained fewer different microbial species. All of these findings confirm the permanent nature of these bacterial communities.
Table 1 – Density of bacteria in the speleothem samples: Soda straw stalactite situated near to a pathway used by tourists (sample A), Stratified tubular stalactites located some distance away from a pathway used by tourists (samples B and C), Mamelons (sample D).

<table>
<thead>
<tr>
<th>SPELEOTHEMS</th>
<th>WALL THICKNESS (mm)</th>
<th>MICROBIAL DENSITY (CFU/g)</th>
<th>CALCIFYING STRAINS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>0.5</td>
<td>$4 \times 10^3$</td>
<td>11</td>
</tr>
<tr>
<td>Sample B</td>
<td>0.3-0.6</td>
<td>$4 \times 10^5$</td>
<td>54</td>
</tr>
<tr>
<td>Sample C</td>
<td>0.4-1.1</td>
<td>$4 \times 10^6$</td>
<td>63</td>
</tr>
<tr>
<td>Sample D</td>
<td></td>
<td>$5 \times 10^9$</td>
<td>91</td>
</tr>
</tbody>
</table>

We then concentrated on the non-mycelia bacteria, particularly the 22 calcifying strains isolated from flowstones. We evaluated the ability of these strains to produce carbonate precipitates in vitro at three different temperatures (4°C, 22°C and 32°C). We also analysed the cyto-morphological and biochemical characteristics of these strains.

The precipitation kinetics showed that the calcifying bacteria needed 7 days to precipitate carbonates at 32°C, 15 days at 22°C, and 25 days at 4°C (Fig. 1). Thus high...
temperatures decrease the time necessary for the initiation of the precipitation process, which in turn increases the precipitation rate. No precipitation was observed on any of the control plates. X-ray diffraction measurements showed that the bacteria mainly precipitate calcite and sometimes precipitate a mixture of calcite and vaterite. XRD analysis also showed that the carbonate formations from which the micro-organisms were isolated are formed from pure calcite (Fig. 2). We checked the calcifying ability of the isolated bacteria on a solid and in a liquid culture. Figures 3, 4, 5 and 6 show calcite crystals adhered to the glass surface and precipitated from *Bacillus sphaericus* (Fig.3) and *Bacillus firmus* (Figs 4,5,6) after 5 months at 32°C. The morphology and sizes of the calcite crystals were correlated with the species involved in the calcification process. Bacterial prints are clearly visible on the surface of the crystal in Fig. 4.

Biochemical characterisation of the calcifying bacteria showed that many strains were able to reduce nitrates and sulphates, and that some strains were also able to oxidise ammonium ions. The isolated bacteria were all able to ferment and/or to oxidise several sugars and their by-products.

Such metabolic behaviours could be related with the depuration of water percolating in hypogean cavities. Furthermore, the redox processes primed by micro-organism present in the percolation water may modify the amount of dissolved CO₂, which would modify the chemical-physical conditions and induce carbonate precipitation.
Fig. 3, 4, 5, 6 SEM of calcite crystals precipitated from Bacillus sphaericus (Fig. 3) and from Bacillus firmus (Figs. 4, 5, 6). The imprints of the bacteria are clearly visible on the crystal surface in Fig. 5. In Fig. 6 microbial cells can be seen around and near to the crystals.

Thus, bacteria are “indirectly” involved in the precipitation of carbonate inside caves. However, it is not clear whether they have a “direct” role in the formation of calcium carbonate.

We identified the calcifying bacteria according to their cyto-morphological characteristics and biochemical profiles as described by Bergey (1986). The micro-organisms isolated from the formations were quite homogeneous: 77% of the calcifying bacteria belonged to the Bacillus genus, which consists of heterotrophic spore-forming bacteria able to survive in adverse environmental conditions. The remainder strains belonged to the Arthrobacter genus (Fig. 7). Members of the Arthrobacter genus, most of which are epigean, aerobic, chemoeterotrophic bacterial, have an important role in the mineralization of organic matter. The genus Arthrobacter is extremely common in soil and in both epigean and hypogean sediments (Gounot, 1967). They are able to survive in such harsh environments because of their capaci-
ty to utilise a wide range of nutrients and their ability to adapt to low temperatures (Cacciari & Lippi, 1986). This may be correlated with the ability of many *Arthrobacter* to secrete a biopolymer, in which they often become trapped. This biopolymer acts as a water and nutrient reserve by limiting exchanges with the external environment. (Hobbie & Fletcher, 1988).

**Concluding remarks**

We found a large number of calcifying bacteria associated with speleothems from Stiffe’s Cave. However, it is difficult, on the basis of the results obtained, to define clearly the role played by bacterial communities in the formation of carbonate formations. The analysis of microbial communities from different karst caves and different speleothems from a single cave may help us to elucidate this crucial point.

In conclusion, our results show that:
- Most of the isolates from Stiffe’s Cave induced CaCO$_3$ precipitation in vitro (no crystals were found in the controls). The temperature effect suggested that these bacteria are actively involved in the precipitation process. The precipitated crystals were mostly calcite.
- Most of the calcifying bacteria belong to the *Bacillus* and *Arthrobacter* genera. The nutritional and ecological characteristics of these genera are typical of bacteria from waters percolating through the soil surface and deeper layers.
- Calcite deposition does not seem to be restricted to a small numbers of bacteria in complex microbial populations. Most of the bacteria found in caves induce calcite precipitation, showing that there is a correlation between carbonate deposition and
microbial global balances.
- XRD analyses did not reveal elements such as Fe, Mn in speleothems samples or in the precipitates obtained in laboratory conditions. We still do not know if any relationships exist between the redox metabolisms of these elements and carbonate deposition.

It will be useful to verify whether calcifying bacteria dissolve these elements, thus justifying their absence from the biogenic sediments.

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LE METAYER-LEVREL G., CASTANIER S., ORIAL G., LOUBIÈRE J.F., PERTHUISOT


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2. Papers should be written preferably in English. Other allowed languages are French, German, Italian and Spanish. Authors using a language not their own are urgently requested to have their manuscripts checked for linguistic correctness before submission. SI system should be used. Dates should be in the form “5 February 1975”.

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5. Bibliographical references should be listed in alphabetical order at the end of the paper. References should be in the following forms:


6. References should be cited in the text in parentheses, e.g. “(Jones, 1961)” except when the author’s name is part of the sentence, e.g. “Jones (1961) has shown that...”. When reference is made more than once to the same author and year, a, b, c, etc. should be added to date in text and reference list.

7. Each table should be reported on a separate sheet. Tables should be numbered in Arabic numerals e.g. “Table 1”, etc. Should a table not be an original, the exact reference should be quoted. Tables should be supplied with headings and kept as simple as possible.

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